NCCT
NATIONAL CENTER FOR COMPETENCY TESTING

Phlebotomy Order of Draw

COURSE DESCRIPTION

To assure the accuracy of patient test results, evacuated tubes must be filled in a particular order. Contamination of the blood by the needle entering a stopper or by contact with additives in the tubes can cause erroneous patient test results, leading to improper diagnosis and treatment of the patient. This CE course will review the composition of blood, types of blood specimens, evacuated blood collection tubes, preanalytical variables, laboratory test directories, and the order of draw.

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COURSE TITLE: Phlebotomy Order of Draw

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Intended Audience: Phlebotomy technicians, medical assistants, and patient care techs who perform phlebotomy. This course is also appropriate for any healthcare worker in possession of a valid NCCT credential.

Objectives: Upon completion of this continuing education course, the professional should be able to:

1. Identify substances found in plasma.
2. Name the types of formed elements found in blood.
3. Describe the three types of blood specimens analyzed in the laboratory.
4. Identify the factor that determines what type of blood specimen is used for analysis.
5. Name anticoagulants and other additives found in evacuated blood collection tubes.
6. For each tube type listed in the course, correlate the stopper/shield color with the additive in the tube.
7. Describe preanalytical variables that can affect the accuracy and precision of laboratory test results.
8. Describe information included in a laboratory test directory.
9. Name the organization responsible for recommending an order of draw.
10. List evacuated tubes in the correct order of draw by both additive and stopper/shield color.
11. Describe the importance of following the order of draw.
12. Describe the use of black stopper/shield tubes, the BD CPT tube™, and the BD PAXgene© RNA system tube.
13. Identify the skin puncture (capillary) order of collection.
14. Use a sample test directory to identify specimen requirements and place tubes in the correct order of draw.

INTRODUCTION

It may seem unbelievable that collecting blood into evacuated tubes in the wrong order can affect test results. However, many studies demonstrate that additives in one type of tube can contaminate another type of tube and lead to inaccurate patient test results.

To understand fully the order of draw, the composition of blood, methodology of laboratory testing, types of evacuated blood collection tubes, preanalytical variables, and laboratory test directories must first be reviewed.

THE COMPOSITION OF BLOOD

Blood is a mixture of fluid and cells. It flows throughout the circulatory system delivering nutrients, oxygen, and other substances to the cells, while removing waste products away from the cells for elimination. The fluid portion of blood is called plasma, and the cellular portion is referred to as formed elements. The average adult has five liters (5.2 quarts) of blood, of which approximately 55% is plasma and approximately 45% is formed elements.

PLASMA

Normal plasma is a clear, pale yellow fluid that is nearly 90% water and 10% dissolved substances. The composition of the dissolved substances includes the following:

- Proteins such as albumin, which is made in the liver and functions to help regulate osmotic pressure (the tendency of blood to attract water); antibodies that combat infection; and fibrinogen, which is also made in the liver and helps with the blood clotting process.
- Energy supplying nutrients including carbohydrates such as glucose and lipids (fats) such as triglycerides and cholesterol.
- Minerals such as sodium, potassium, calcium, and magnesium. Sodium helps maintain fluid balance, pH, and calcium and potassium balance necessary for normal heart function. Potassium is essential for normal muscle activity and the conduction of nerve impulses. Calcium is needed for proper bone and teeth formation, nerve conduction, and muscle contraction; it is also essential for the blood clotting process.
- Gases such as oxygen, carbon dioxide, and nitrogen.
- Other substances including vitamins, hormones, and waste products of metabolism such as blood urea nitrogen, creatinine, and uric acid.

FORMED ELEMENTS

Erythrocytes

Erythrocytes, also called red blood cells (RBC), are the most abundant cells in the blood.

- Their main function is to carry oxygen from the lungs to the cells of the body. They also carry carbon dioxide from the cells of the body back to the lungs to be exhaled.

- Red blood cells are produced in the bone marrow and, following a maturation process, move into the blood circulation where they have a life span of approximately 120 days.
- The main component of red blood cells is hemoglobin, which is used to transport oxygen and carbon dioxide. Hemoglobin also gives red blood cells their red color.
- When viewed under a microscope, red blood cells are anuclear (have no nucleus) and are biconcave disks (indented on both sides somewhat like a bagel).
- Red blood cells stay within the vascular system.

White Blood Cells

White blood cells (WBC) are also called leukocytes.

- White blood cells are made in the bone marrow and lymphatic tissue. Following a maturation process, they move into the blood circulation.
- White blood cells function to destroy pathogens like bacteria and viruses.
- Five types of mature white blood cells are found in the peripheral blood: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. When a drop of blood is smeared on a glass slide, treated with a stain, and looked at under a microscope, the five types of cells have different appearances. They are identified by their size, the shape of the nucleus, and the presence or absence of granules in the cytoplasm.
- White blood cells work both inside and outside of the vascular system. Their life span varies by type, some living only days and others for years.

Platelets

Platelets are sometimes called thrombocytes.

- Platelets are pieces of a large cell called a megakaryocyte found in the bone marrow.
- Pieces of the cell break off and move into the vascular system.
- Platelets are the smallest of the formed elements.
- Platelets are essential to the blood clotting process.
- Their life span is approximately 10 days.

TYPES OF BLOOD SPECIMENS

WHOLE BLOOD

Some laboratory tests can only be performed on whole blood. For these tests, the formed elements and the fluid plasma component of blood cannot be allowed to separate. This specimen is called whole blood. To obtain a whole blood specimen, the blood must be collected in a tube that contains an anticoagulant. The sample then does not clot and, before testing, the sample can be well mixed to equally distribute the formed elements and the plasma. Following are examples of laboratory tests that can be performed only on whole blood.

- Complete blood cell count (CBC)
- Erythrocyte sedimentation rate (ESR)

- Hemoglobin electrophoresis

SERUM

Blood that has been removed from the body will coagulate (clot) in about 20 minutes. The clot consists of the blood cells trapped in a mesh of fibrin, a product produced during the coagulation process. The remaining portion of the blood specimen is fluid and is called serum. Serum can be separated from the clot by centrifugation (spinning the blood specimen in an instrument called a centrifuge). Normal serum is a clear, pale yellow fluid with the same composition as plasma **except** it does not contain fibrinogen. Fibrinogen, found in circulating blood, is not found in serum because it is used up in the formation of the clot. Some laboratory tests can be performed only on serum. Following are some examples.

- Serum protein electrophoresis
- Hepatitis B antibody

PLASMA

To obtain plasma in a blood sample, the blood must be prevented from clotting. This is accomplished by drawing the blood into a tube that contains a substance called an anticoagulant. Several different types of anticoagulants exist and they employ differing mechanisms to interfere with blood's normal clotting process.

The red blood cells of a blood sample collected in a tube with an anticoagulant can be separated from the plasma by centrifugation. Centrifugation forces the red cells to the bottom of the collection tube. The clear, pale yellow fluid on top of the red cells is the plasma. Unlike serum, plasma **contains** fibrinogen. Some laboratory tests can be performed only on plasma. Following are some examples.

- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- D-Dimer

NOTE:

Many laboratory tests, especially chemistry tests, can be performed on either plasma or serum. Plasma is often the preferred specimen for the following reasons.

- To obtain serum, the specimen takes at least 20 minutes for a specimen to clot followed by a 10-minute centrifugation to obtain the serum to test. On the contrary, to obtain plasma, anticoagulated specimens can be centrifuged immediately upon arrival in the laboratory and can be ready to test as soon as the centrifugation is completed. Therefore, most STAT tests are often performed on plasma, as there is no need to wait for the blood to clot. This means the specimen can be analyzed quicker making patient tests results available more rapidly, which may be helpful in emergencies.

- In serum specimens, if not allowed to clot for a sufficient time and/or centrifugation of a clotted specimen is rushed, the serum may contain strings of fibrin. The fibrin is

sticky and it can present problems with analysis of the serum by adhering to parts of laboratory instrumentation and test tubes.

LABORATORY INSTRUMENTATION

There are many methods and techniques used to measure the quantity of substances (analytes) in blood specimens. Automated instrumentation is used to detect and quantify most laboratory analytes. The use of automation increases the efficiency of the laboratory, as well as provides mechanisms to increase the accuracy and precision of laboratory test results.

Many makes and models of laboratory analyzers are available for purchase. Some are designed for doctor's offices, others for large laboratories. One size does not fit all – each laboratory must evaluate and purchase the analyzers that meet their specific needs. Following are some of the manufacturers of laboratory analyzers.

- Abbott
- Baxter
- Bayer
- Beckman Coulter
- Becton Dickinson
- Behring Diagnostics
- bioMerieux
- Ciba-Corning
- Dade Behring
- DuPont
- International Laboratories
- Olympus
- Radiometer
- Roche
- Sysmex
- Technicon

Due to the complexity of laboratory test methods, there is no one analyzer that performs all testing. Most laboratories have specific analyzers for hematology tests, coagulation tests, chemistry tests, immunology tests, urinalysis tests, etc.

Almost all laboratory instrumentation is interfaced with computers to assist with reporting of the test results and maintenance of the instrument. The analytic methods used by laboratory instrumentation include spectrophotometry, fluorometry, turbidimetry, nephelometry, electrophoresis, immunoassays, electrical impedance, flow cytometry, molecular diagnostics, and more.

These analytic methods are beyond the scope of this CE course. However, the analytic method is what determines the type of blood specimen used for the testing, as well as the volume of blood required. Therefore, each laboratory determines the recommended specimen type based on the methodology used for that specific test. For example, one laboratory may require serum from a red stopper evacuated tube for electrolyte testing; another laboratory may require plasma from a green stopper evacuated tube. The specimen types described in this CE course are the ones most commonly used.

Reference ranges (normal values) also differ from laboratory to laboratory. Each laboratory determines their reference range using their instrumentation and patient population.

EVACUATED BLOOD COLLECTION TUBES

Blood collection tubes come in a variety of types. The glass or plastic tubes have an assortment of color stopper / plastic shield covers. The color-coded stoppers indicate

the type of additive in the tube. Additives include anticoagulants that prevent the blood from clotting, chemicals to make the blood clot quicker, preservatives, or other chemicals with specific uses. For consistency, all evacuated tube manufacturers use the same color codes.

Glass evacuated tubes have a rubber stopper; plastic evacuated tubes have a plastic shield over a rubber stopper. For safety purposes, plastic tubes should be used whenever possible. However, some laboratory tests require the collection of blood into glass tubes.

Evacuated tubes are designed to draw in a set volume of blood. Some tubes appear to be the same size but draw smaller quantities of blood, as there is less vacuum in the tube. These smaller draw tubes are useful when collecting blood on infants, children, the elderly, or "hard-to-stick" patients.

Tubes containing additives must be gently inverted (i.e., not shaken) immediately after collection to assure that blood quickly comes into sufficient contact with the additive. Failure to adequately mix the blood specimen with the anticoagulant will produce clots, which render a specimen unacceptable for testing or inaccurate patient test results.

The following substances are types of anticoagulants. Their presence in tubes prevents the blood from clotting.

- K_2EDTA (potassium ethylenediamine tetra-acetic acid)
- Na_2EDTA (sodium ethylenediamine tetra-acetic acid)
- Sodium citrate
- Sodium heparin
- Lithium heparin
- Potassium oxalate
- ACD (acid citrate dextrose)
- SPS (sodium polyanethol sulfonate)
- CTAD (citrate, theophylline, adenosine, dipyridamole)

The following substances are other types of additives in tubes that are not anticoagulants.

- Thrombin (helps the blood clot quicker)
- Sodium fluoride (prevents glucose in the blood from decreasing in quantity)
- Gel (during centrifugation, moves up in the tube to form a barrier between red cells and serum/plasma)

The chart on the following page summarizes information on tube types.

Evacuated Tube Types

STOPPER / SHIELD COLOR	ADDITIVE	NUMBER OF INVERSIONS AT TIME OF COLLECTION	COMMON LABORATORY USE
• Gray stopper • Gray shield	(anticoagulants) • Potassium oxalate/sodium fluoride or • Sodium fluoride or • Sodium fluoride/Na_2EDTA	8 8 8	Glucose test requiring plasma Lactic acid test requiring plasma
• Green & gray stopper • Light green shield	Lithium heparin & gel for plasma separation (anticoagulant)	8 8	Chemistry tests requiring plasma
• Green stopper • Green shield	Sodium or lithium heparin (anticoagulant)	8 8	Chemistry tests requiring plasma
• Lavender stopper • Lavender shield	(anticoagulants) • Liquid Na_2EDTA (glass) • Spray coated Na_2EDTA (plastic)	8 8	Whole blood hematology determinations, viral antigen culture of blood
• Light blue stopper***** • Light blue shield***** • Clear shield over light blue stopper*****	(anticoagulants) • 0.105 M sodium citrate (glass) or • 0.129 M sodium citrate (3.8%) or • CTAD	3 - 4 3 – 4 3-4	• Sodium citrate: routine coagulation studies • CTAD: platelet function assays, routine coagulations studies
• Orange shield (No glass tube)	• Thrombin or • Thrombin & gel for serum separation	8 5 - 6	STAT tests in chemistry requiring serum
• Pink stopper • Pink shield	K_2EDTA (anticoagulant)	8	Blood bank testing; has a special label for required patient information per AABB (American Association of Blood Banks) for crossmatch testing
• Red & gray stopper • Gold shield	Clot activator & gel for serum separation	5	Chemistry tests requiring serum
• Red & light gray • Clear shield over red stopper	None	0	Use as a discard tube
• Red stopper • Red shield	• Silicon coated (glass tube) • Silicon coated & clot activator (plastic tube)	0 5	Chemistry and serology tests requiring serum
• Royal blue shield	• Clot activator or • Na_2EDTA (anticoagulant)	8 8	Trace metals/elements, toxicology, & nutritional determinations for serum or plasma
• Tan shield	K_2EDTA (anticoagulant)	8	Lead testing
• White shield	K_2EDTA with gel for plasma separation (anticoagulant)	8	Molecular diagnostic tests such as PCR; BD's trade name for this tube is PPT™ (plasma preparation tube)
• Yellow stopper*****	(anticoagulants) • SPS (sodium polyanethol sulfonate) or • ACD (acid citrate dextrose) Solution A or • ACD (acid citrate dextrose) Solution B	8 8 8	• SPS = blood cultures, acid fast bacteria • ACD = HLA phenotyping for transplant, DNA, & paternity test

NOTE: Maximum volume of draw for each size of tube is generally indicated by a line (the same color as the rubber stopper/shield) found at the top of the label when holding the tube upright. Filling to 50% of capacity is usually sufficient for testing.

*****Those marked with an asterisk indicate that **fill volume is critical**. Tubes must be filled to 100% of capacity to assure accurate laboratory test results.

PHLEBOTOMY PREANALYTICAL VARIABLES

Many variables can affect the accuracy and precision of laboratory test results. Laboratories must be aware of these variables in order to minimize them, as the diagnosis and treatment of patients can be impacted. These variables are divided into preanalytical, analytical, and post-analytical.

- Preanalytical variables include specimen collection, transport, and processing
- Analytical variables include testing
- Post-analytical variables include results transmission, interpretation, follow-up, and retesting

Preanalytical Variables

The following summarizes preanalytical errors in specimen collection that can affect laboratory test results and/or cause injury to the patient. This list is not all-inclusive.

- Patient identification errors: These identification errors occur when the incorrect patient drawn, incorrect patient labels affixed to tubes, tubes not labeled at time of collection, tubes labeled by someone other than the individual who collected the patient.

- Patient complications: These include drawing non-fasting patients for fasting lab tests, patient allergies to alcohol / iodine used to prepare venipuncture site, fainting, etc.

- Vein selection: The basilic vein should be last choice as puncture may injure the median nerve causing damage.

- Site selection: Avoid sites with IV, on side of a mastectomy, edema, hematoma, burns, and scarring as test results can be affected or injury caused to the patient.

- Tourniquet: Hemoconcentration, which may affect test results, can occur if the tourniquet is left on for more than one minute.

- Cleansing of venipuncture site: Alcohol must be allowed to dry to assure any bacteria present have been killed. Additional cleansing of site is necessary for blood culture collections to ensure sterility of the sample.

- Selecting collection method most appropriate for patient: Use of evacuated tube system, winged infusion sets, syringe, or skin puncture should be decided based on the location, depth, and accessibility of the patient's veins.

- Correct angle of needle insertion/anchoring of vein: This assures the needle enters the vein successfully.

- Order of draw: Inaccurate test results can occur if an additive from a previous tube contaminates the tube being collected.

- Hemolysis: Traumatic venipuncture, blood collected from area with a hematoma, vigorous shaking of tubes after collection, use of small gauge needle with regular size evacuated tubes, pulling too hard on a syringe barrel can all cause the blood specimen to hemolyze, which can affect test results.

- Timing of specimen collection: If specimens are not collected at the appropriate time for timed draws, peak/trough levels for therapeutic drug monitoring, fasting, etc., the test results will not correctly represent the patient's condition which can lead to improper treatment.

- Collection tubes: Incorrect tube drawn, incorrect fill volume, tubes with additives and anticoagulants not thoroughly mixed will all affect laboratory test results.

LABORATORY TEST DIRECTORIES

All laboratories must have some form of a test directory. The test directory contains information about the analyte, the type of specimen needed (whole blood, serum, plasma), specimen requirements (protect from light, maintain specimen at body temperature, etc.), analytical methodology, and reference range. The test directory provides laboratory personnel with information to follow to assure accurate and precise lab test results, and to assist the physician in interpretation of patient test results.

Following are examples of test directories from three large reference laboratories – Mayo Medical Laboratories, ARUP, and LabCorp. These examples are provided only for the purposes of showing the detailed process of laboratory testing. As can be told from these examples, some laboratories provide more information than other laboratories.

Mayo Medical Laboratories

Amylase, Total, Serum

- Specimen
  Specimen Type Serum
  Specimen Container/Tube: Preferred: Serum gel **Acceptable:** Red top
  Specimen Volume: 0.5 mL
  Additional Information: Patient's age and sex are required.
  Specimen Minimum Volume 0.25 mL
  Reject Due To

Hemolysis	Mild OK; Gross reject
Lipemia	Mild OK; Gross OK
Icterus	NA
Other	NA

Specimen Stability Information

Specimen Type	Temperature	Time
Serum	Frozen (preferred)	30 days
	Ambient	7 days
	Refrigerated	30 days

- **Cautions** Amylase results may be elevated in patients with macroamylase. Macroamylase refers to a high molecular weight form of amylase that is present in a patient's serum. Different causes of macroamylase have been suggested, the most common being amylase complexed with an immunoglobulin. The large size of the macroamylase complex prevents its excretion in the urine. As a result, the serum amylase is usually elevated. This elevated amylase is not diagnostic for pancreatitis. By utilizing serum lipase and urinary amylase, the presence or absence of macroamylase may be determined.

- **Clinical Reference**
  1. Soldin SJ, et al: Pediatric Reference Ranges, AACC Press, Washington DC 1997
  2. Tietz Textbook of Clinical Chemistry. Editors Burtis and Ashwood.WB Saunders Company, Philadelphia, 1999
  3. Swaroop VS, Chari ST, Clain JE: Acute pancreatitis, JAMA 2004;291:2865-2868

- **Method Description** The liquid Roche amylase (AMYL) method is an enzymaticcolorimetric test using 4,6-ethyliden (G7)-p-nitrophenol (G1)-a, D-maltoheptaoside (ethylidene-G7PNP) as a substrate. Human salivary and pancreatic amylase convert the substrateat approximately the same rate. The a-amylase cleaves the substrate into G2, G3, G4 PNP fragments. The G2 and G3 andG4 PNP fragments are further hydrolyzed by an a-glucosidase to yield p-nitrophenol and glucose. The rate of increase in absorbance at 415 nm (measuring the increase in p-nitrophenol) is proportional to amylase activity (Roche AMYL reagent package insert, Indianapolis, IN, February 2000)

- **Day(s) and Time(s) Test Performed** Monday through Sunday; Continuously
- **Analytic Time** Same day/1 day
- **Maximum Laboratory Time** 2 days
- **Specimen Retention Time** 1 week
- **Reporting Name** Amylase, Total, S

ARUP

Glucose, Plasma or Serum : 0020024
Mnemonic: GLU

Methodology:	Quantitative Enzymatic
Performed:	Sun-Sat
Reported:	Within 24 hours
Specimen Required:	Collect: Gray (sodium fluoride/potassium oxalate), plasma separator tube, or serum separator tube. Specimen Preparation: Separate serum or plasma from cells ASAP or within 2 hours of collection. Transfer 1 mL serum or plasma to an ARUP Standard Transport Tube. (Min: 0.2 mL) Storage/Transport Temperature: Refrigerated. Stability (collection to initiation of testing): After separation from cells: Ambient: 24 hours; Refrigerated: 1 week; Frozen: 1 year
Reference Interval:	By report (reports may vary based on instrumentation)
Interpretive Data:	Refer to report.
CPT Code(s):	82947

LabCorp

Cocaine Metabolite Screen and Confirmation, Blood
Synonyms:

- Coke
- Crack
- Snow

Test Number: 766555 CPT Code: 80101

Test Includes:	Cocaine metabolite
Specimen:	Whole blood
Volume:	12 mL
Minimum Volume:	4 mL
Container:	Two gray-top 6 mL **or** three gray-top 4 mL (sodium fluoride) tubes (preservative in gray-top tubes is essential for cocaine analysis)
Special Instructions:	Chain-of-custody documentation is required for samples submitted for preëmployment, random employee testing, and forensic purposes. For other applications, use the standard request form. Please mark chain-of-custody test number on the request form.
Collection:	Routine venipuncture. Mix blood by inversion after collection. Label tube with patient information. Seal stopper with tamper-evident tape after collection.
Storage Instructions:	Maintain specimen at room temperature. If arrival extends beyond seven days, then refrigerate.
Causes for Rejection:	Quantity not sufficient for analysis; improper specimen (serum, plasma, clotted blood); incomplete chain-of-custody documentation; incomplete specimen identification
Reference Interval:	Detectability: 20 ng/mL
Use:	Drugs of abuse testing
Methodology:	Initial testing by immunoassay; confirmation of positives by gas chromatography/mass spectrometry (GC/MS). See drug profiles for multidrug testing.

At the end of this course, a very simple test directory is provided. This directory will be used to answer the CE course test questions.

ORDER OF DRAW – WHO, WHAT, WHEN, WHERE, & WHY

Who determines the order of draw?

The approved order of draw for venipuncture is found in the most current edition of the Clinical and Laboratory Standards Institute (CLSI) GP41 *Collection of Diagnostic Venous Blood Specimen, 7th edition*. This standard replaced standard GP41-A6, formerly H03-A6. The document was first published in 1977, and has been updated as needed. The latest edition was published in April 2017.

> The Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Commission on Clinical Laboratory Standards (NCCLS), is an international, nonprofit organization that develops standards and guidelines for patient testing and related healthcare issues. Healthcare professionals voluntarily participate in CLSI projects. Individuals involved in the phlebotomy standards include experts in the profession of phlebotomy and experts employed by the makers of venipuncture equipment.

The order of draw is changed when new research indicates a probable interference or inaccuracy in test results. Healthcare facilities should be using the most current recommended order of draw unless they have documentation supporting an alternate order of draw. For example, the Mayo Clinic requests tubes for lead and/or trace metal testing be collected first. Their studies show that the back-end of a needle (the end that punctures the tube stopper) picks up trace metal contamination from stoppers other than tan or navy and this contaminates the blood collected for trace metals.

What is the CLSI recommended order of draw for venipuncture?

The recommended CLSI order of draw is used for both glass and plastic venous blood collection tubes. The same order of draw is also used for collections using a syringe or an evacuated (collection tube and tube holder) system. Many laboratory accreditation agencies such as the College of American Pathologists (CAP) and The Joint Commission require CLSI standards to be followed.

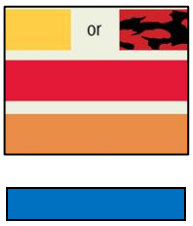
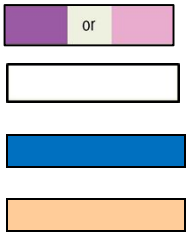
CLSI lists the order of draw as follows.

1. Blood culture tube
2. Coagulation tube (eg, blue closure)
3. Serum tube with or without clot activator, with or without gel (eg, red closure)
4. Heparin tube with or without gel plasma separator (eg, green closure)
5. EDTA tube with or without gel separator (eg, lavender closure, pink closure, pearl closure)
6. Glycolytic inhibitor (eg, gray closure)

However, a review of the tube types on page 8 reveals more than the above listed tubes. To identify where the other tubes are placed in the order of draw, the tube

additive must be known. This information is on the tube label. Once this information is known, additives are grouped together in the order of draw. For example, the tan shield tube contains K_2EDTA so its place in the order of draw is with the other tubes that contain EDTA – lavender, pink, and white.

CLSI RECOMMENDED ORDER OF DRAW

Order	Tube	Stopper/Shield Color	
1	Blood culture, i.e., sterile specimens	• Blood culture bottles • Yellow stopper SPS tubes • Any other test that must be sterile	
2	Coagulation tube (tubes with citrate additives)	• Light blue stopper/shield • Clear shield over blue stopper	
3	Serum tube (with or without clot activator, with or without gel)	• Red & black stopper • Gold shield • Red stopper/shield • *Royal blue shield with clot activator****	
4	Heparin tube (with or without gel plasma separator)	• Green & gray stopper • Light green shield • Green stopper/green shield	
5	EDTA tube	• Lavender stopper/shield • Pink stopper/shield • White (pearl) shield • *Royal blue shield with EDTA** • *Tan tube with EDTA****	
6	Glycolytic inhibitor tube	• Gray stopper/shield	
7	All other tubes in no particular order unless otherwise directed		

***Important note:** If a royal blue shield tube or tan tube is being collected for **trace metal analysis**, the tube should be collected first or via a separate second venipuncture to assure there is no trace metal contamination on the needle from puncturing the other tube stoppers. A separate venipuncture for trace metal analysis must be performed if blood cultures are ordered at the same time.

When is the order of draw followed?

This order of draw is followed for every venipuncture blood collection. It is used for outpatients, inpatients, home care, and all ages. The same order of draw is followed regardless of what equipment is used – syringe/needle, winged infusion set/evacuated tubes, and tube holder with needle/evacuated tubes. The order of draw is the same for

glass and plastic tubes. However, the order of draw differs for skin puncture (capillary) collections; see page 17.

Where is information found about the order of draw for venipuncture?

The CLSI website is www.clsi.org. The order of draw for venipuncture is discussed in the approved standard CLSI GP41 *Collection of Diagnostic Venous Blood Specimens, 7th edition,* published in 2017.

If a laboratory has researched an alternate order of draw, this information will be provided in their laboratory test directory.

Why is the order of draw so important?

Laboratory testing methods are based on scientific principles involving biology, chemistry, and physics. Very small quantities of substances (analytes) are measured by sophisticated techniques. Because the quantities are small, other substances can readily interfere with the accuracy of the test result. If a patient's test result is not accurate, he/she may not receive the appropriate care and treatment.

The purpose of the order of draw is to avoid possible test result error due to cross contamination from tube additives. While it might seem impossible for the very small amounts of additives in tubes to cause inaccurate test results, extensive research has been performed that indicates this is possible.

1. EDTA is rich in potassium and can falsely elevate a patient's potassium test results. Because of this, tubes used to test for potassium must be collected before tubes containing EDTA. Therefore,

   - red & black stopper, gold shield, green & gray stopper, light green shield, red stopper, red shield tubes

     are collected before

   - lavender stopper, lavender shield, pink shield, and white shield tubes.

2. The additives in gray stopper/shield tubes distort the microscopic appearance of blood cells on a white blood cell (WBC) differential test. Therefore,

   - lavender stopper/shield tubes

     are collected before

   - gray stopper/shield tubes.

3. Clot activators can interfere with coagulation tests such as prothrombin (PT) and activated partial thromboplastin time (aPTT), resulting in shortened test results. Therefore,

   - light blue stopper/shield tubes

     are collected before

- red/gray stopper, gold shield, red shield, gray/yellow stopper, and orange shield tubes.

4. Bacteria from non-sterile tube stoppers/shields can contaminate blood collected into bottles/tubes used for blood cultures, resulting in the growth of bacteria erroneously leading a physician to think his/her patient has a blood infection. Therefore,

   - Blood culture bottles and yellow SPS tubes (or other tests requiring sterile blood)

     are collected first before

   - any other tubes.

REMEMBERING THE ORDER OF DRAW

Many people have developed mnemonics to remember the order of draw. The course author has used the following statement as a memory aid for the most commonly used evacuated tubes.

Sally brings really good grease and leaves the gravy.

Sally = **s**terile
Brings = **b**lue (light blue)
Really = **r**ed
Good = **go**ld
Grease = **gre**en
and
Leaves = **l**avender
the
Gravy = **gra**y

OTHER EVACUATED TUBES

Some laboratories use a tube with a black stopper/shield containing sodium citrate for erythrocyte sedimentation rate testing. Its place in the order of draw is with other tubes that contain sodium citrate, eg, light blue stopper/shield tubes.

Other tubes manufactured by BD *(*Becton*,* Dickinson and Company) that are available for diagnostic laboratory testing include the following.

- BD Vacutainer® CPT™: Cell Preparation Tube
  - There are two tube types – one with sodium citrate as an additive and one with sodium heparin as an additive. Both tubes are glass.
  - After centrifugation, the mononuclear cells present in the blood are used for PCR (sodium citrate tube) or cell cultures for immunotyping and cell function studies (sodium heparin tube)
  - The sodium citrate tube has a blue and black stopper; the sodium heparin tube has a red and green stopper.
  - BD's product information states that the CPT tube is collected after other EDTA tubes and before any tubes containing liquid additives.

- BD Vacutainer® PAXgene® Blood RNA System
    - This system contains a tube and a winged infusion set. The use of a winged infusion set is required to assure there is no backflow of the tube additive into the patient blood.
    - This plastic tube contains 6.9 mL of a propriety additive that preserves intracellular RNA in the whole blood for RNA analysis. The tube is filled with 2.5 mL of blood.
    - This tube is always drawn last. If it is the only tube being collected, a discard tube must be used to fill the volume of the blood collection set tubing so the PAXgene® tube fills to the correct volume.
    - The tube has a red stopper with a white shield

Other types of tubes are manufactured for research purposes only. Manufacturer's instructions should always be followed when collecting blood for research purposes.

FREQUENTLY ASKED QUESTIONS ABOUT TUBES/ORDER OF DRAW

QUESTION: ***My previous employer told us to draw electrolytes in a green top tube. At my current job, they tell me to draw electrolytes in a red top tube. Who is right?***

Answer: Both of your employers are right! It is the laboratory's responsibility to determine the type of specimen to collect for testing, thus the requirements vary from lab to lab. The determination is based on analytical method, type of analyzer used for the testing, and the manufacturer's recommendations for specimen type. In the case of electrolytes, different methods call for use of plasma, use of serum, or even the use of whole blood.

Sometimes the situation dictates the specimen type. For example, some laboratories may request a green top tube for STAT testing as the testing can be done on plasma. The tube can be centrifuged immediately upon receipt and there is no wait time required for specimen clotting. An orange shield tube is used by some laboratories when a STAT test requires serum. This tube contains thrombin, which makes blood clot quicker in the tube.

QUESTION: ***Where I used to work, we were required to collect a little bit of blood in a discard tube before drawing a protime (PT). At my new job, I am told that this is not necessary. Who is right?***

Answer: Your new employer is correct. In the past, there was concern that the first blood collected into a tube or syringe was contaminated with tissue thromboplastin resulting from the entry of the needle into the skin. The presence of tissue thromboplastin in a blue top tube could alter the results of the routine coagulation tests, protime (PT) and activated partial thromboplastin (aPTT). Therefore, it was necessary to collect a small quantity of blood in another tube to assure that no tissue thromboplastin would contaminate the blue top tube.

Research now indicates that trauma to the skin during a venipuncture is minimized with today's single use and finely sharpened needles. The amount of tissue thromboplastin

generated from a venipuncture today is minimal and will not affect the results of PT and aPTT testing. However, studies have **not** been performed for other coagulation tests so a discard tube should be filled with a small volume of blood prior to collecting blood into a blue top tube.

NOTE: The "no discard tube needed" policy applies only when using a routine venipuncture with needle/tube holder or needle/syringe. If using a winged infusion set, refer to the following question.

QUESTION: ***Why is a discard tube used when drawing a blue top using a winged infusion set?***

Answer: When using a winged infusion set to collect blood for coagulation testing (PT, aPTT, and other coagulation tests), a small volume of blood must first be collected into a discard tube. The discard tube is only needed to allow the air within the tubing to be moved out. If the air is not first removed, the air will become trapped in the blue top collection tube. This air will cause the tube to be underfilled with blood.

Do not use a plastic red top tube as a discard tube, as this type contains a clot activator.

SKIN PUNCTURE ORDER OF COLLECTION (CAPILLARY DRAWS)

A different order of draw is recommend for skin puncture (capillary) specimens and is discussed in Clinical and Laboratory Standards Institute (CLSI) *GP42 Collection of Capillary Blood Specimens*, 7th edition, September 2020.

This different order of draw is to assure that specimens requiring anticoagulation are collected first, as this lessens the likelihood that clots will appear in these specimens. The presence of clots in a lavender micro-collection tube results in specimen rejection. The lavender top micro-collection tubes are filled first, as the skin puncture itself will attract platelets to the site. Clumping together of platelets will interfere with CBC results, so it's important to obtain the CBC specimen before this has a chance to occur.

1. Lavender (EDTA) micro-collection tube
2. Plasma additive micro-collection tubes such as green (lithium heparin), light green (lithium heparin with gel), gray (sodium fluoride and potassium oxalate)
3. Serum micro-collection tubes such as red (no additive), gold (clot activator and gel)

CONCLUSION

To assure the accuracy of patient test results, evacuated tubes must be filled in a particular order. Contamination of the blood by the needle entering a stopper or by contact with additives in the tubes can cause erroneous patient test results, leading to improper diagnosis and treatment of the patient.

TEST DIRECTORY EXAMPLE

Use this test directory to answer the questions for this CE course.

Important note: This is only an <u>example</u> of a test directory. The laboratory you collect blood specimens for may have different specimen collection requirements.

Analyte Name	Specimen Collection Requirements
ABO & Rh Type	• Red cells & plasma • Pink shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Acid Phosphatase	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Alanine Transaminase (ALT)	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Albumin	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Alcohol	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Alkaline Phosphatase	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Alpha-Fetoprotein (AFP)	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Aluminum	• Plasma • Royal blue stopper • Minimum 2.0 mL
Ammonia	• Plasma • Green & gray stopper/Light green shield • Minimum 2.0 mL • Immediately after collection and labeling, place tube in ice slurry
Amphetamines (methamphetamine, amphetamine)	• Plasma • Green stopper/shield • Minimum 2.0 mL • Tubes with gel are **not** acceptable
Amylase	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Antibody Identification	• Red cells & plasma • Pink shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Antibody Screen	• Red cells & plasma • Pink shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Antinuclear antibodies (ANA)	• Serum • Red stopper or gold shield • Minimum 2.0 mL
Antistreptolysin O (ASO) Titer	• Serum • Red stopper or gold shield • Minimum 2.0 mL
Arthritis Panel	• Serum • Red stopper or gold shield • Minimum 2.0 mL
Aspartate Aminotransferase (AST)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL

Analyte Name	Specimen Collection Requirements
Barbiturates (phenobarbital, secobarbital, pentobarbital)	• Plasma • Green stopper/shield

	• Minimum 2.0 mL • Tubes with gel are **not** acceptable
Basic Metabolic Panel (BMP)	• Plasma • Green and gray stopper or light green shield • Minimum 2.0 mL • Serum may also be used; red, red & black stopper, or gold shield acceptable
Benzodiazepines [diazepam (Xanax®, Valium®)]	• Plasma • Green stopper/shield • Minimum 2.0 mL • Tubes with gel are **not** acceptable
Bilirubin	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Bleeding Disorders Screen	• Plasma • Light blue stopper/ light blue shield • Critical: Tube must be filled to correct volume • Critical: If testing cannot be immediately performed, freeze plasma
Blood Culture, Acid Fast Bacteria	• Whole blood • Yellow SPS tube • Minimum 5.0 mL
Blood Culture, Aerobic and Anaerobic Bacteria	• Whole blood • Aerobic & anaerobic blood culture bottles • Minimum 7.0 mL in each bottle
Blood Urea Nitrogen (BUN)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
B-type Natriuretic Peptide (BNP)	• Plasma • Lavender stopper/shield • 2.0 mL • Pink shield also acceptable
CA 15-3	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
CA 19-9	• Serum or plasma • Red & black stopper/gold shield or green & gray stopper/light green shield • Minimum 2.0 mL
CA-125	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Calcium	• Serum or plasma • Red & black stopper/gold shield or green & gray stopper/light green shield • Minimum 2.0 mL
Cannibinoids (marijuana)	• Plasma • Gray stopper or gray shield • Minimum 2.0 mL • Red stopper/red shield or lavender stopper/shield, or pink shield also acceptable
Carbon Dioxide (CO_2)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Carcinoembryonic Antigen (CEA)	• Serum • Red stopper/gold stopper • Minimum 2.0 mL
Cardiac Troponin I (cTnI)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL • Pink shield, light blue stopper/shield or green stopper/shield (**lithium heparin only**) also acceptable
Chloride (Cl)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Cholesterol	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Cocaine and Benzoylecognine	• Plasma • Green stopper or green shield • Minimum 2.0 mL • Tubes with gel are **not** acceptable

Analyte Name	**Specimen Collection Requirements**
Cold Agglutinins	• Serum

	• Red & black stopper or gold shield • Minimum 2.0 mL • Blood specimen must be kept at 37°C immediately after collection and until serum is separated after centrifugation
Complement (C3, C4, CH 50)	• Serum • Red stopper/shield • Minimum 2.0 mL • If testing cannot be performed within 2 hours, freeze serum after centrifugation
Complete Blood Cell Count (CBC)	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Comprehensive Metabolic Panel (CMP)	• Serum or plasma • Red & black stopper or green stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
C-Reactive Protein (CRP)	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Creatine Kinase Isoenzymes	• Serum • Red & black stopper or gold shield • Minimum 2.0 mL
Creatine Kinase MB (CK-MB)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Creatinine	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Cryoglobulin	• Serum • **Whole blood drawn in a pre-warmed (37°C) syringe and immediately transfer to a pre-warmed plain red stopper.** • Specimen may be drawn directly into a pre-warmed plain red stopper tube • **Maintain whole blood at 37°C until clotting is complete; do not refrigerate or freeze at any time.** • Separate serum from cells using a 37°C centrifuge, if possible. • Minimum 2.0 mL
Cyclic Citrullinated Peptide Antibody (anti-CCP)	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
D-Dimer	• Plasma • Light blue stopper/shield • Critical: tubes must be filled to correct volume
Digoxin	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
Direct Antihumanglobulin Test (DAT)	• Red cells • Pink shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Direct Bilirubin	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Drugs of Abuse/Toxicology Screening	• Plasma • Green stopper/shield • Minimum 2.0 mL • Tubes with gel are **not** acceptable
Electrolytes	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL • Even slight hemolysis will falsely elevate potassium level
Epstein-Barr Virus (EBV) by PCR	• Serum or plasma • Red & black stopper or gold shield or lavender stopper/shield or pink shield • Minimum 2.0 mL
Erythrocyte Sedimentation Rate (ESR)	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL

Analyte Name	**Specimen Collection Requirements**
Fibrinogen	• Plasma • Light blue stopper/shield

	• Critical: tubes must be filled to correct volume
Fluorescent Treponemal Antibody (FTA)	• Serum • Red stopper/shield • Minimum 2.0 mL
Gamma Glutamyl Transferase	• Serum or plasma • Red & black stopper or gold shield or pink shield • Minimum 2.0 mL
Globulin	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Glucose	• Serum or plasma • Gray stopper/shield or red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Hematocrit	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Hemoglobin	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Hemoglobin Electrophoresis	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Hepatic (Liver) Function Panel	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Hepatitis B DNA by PCR	• Serum or plasma • Red stopper or lavender stopper/shield • Minimum 2.0 mL
Hepatitis B Surface Antibody (HBsAB)	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL
Hepatitis B Surface Antigen (HbsAG)	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL
Hepatitis C Virus RNA Quantification by bDNA	• Plasma • White shield • Minimum 5.0 mL
Hepatitis Panel	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL
Herpes Simplex Virus (HSV) Antibody	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL
High Density Lipoprotein (HDL)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
HIV Antibody Test	• Serum • Red & black stopper or gold shield or red stopper/shield • Minimum 2.0 mL
HIV RNA by PCR	• Plasma • Lavender stopper/shield or pink shield or green stopper/shield • Minimum 2.0 mL
HLA B27 Antigen	• White blood cells from plasma • Lavender stopper/shield or green stopper/shield • Minimum 4.0 mL
HLA Class II Molecular Typing Disease Association	• White blood cells from plasma • Yellow ACD solution B • Minimum 6.0 mL
Human Chorionic Gonadotropin (HCG)	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL
Human Immunodeficiency Virus (HIV) Antibody Confirmation	• Serum • Red & black stopper or gold stopper • Minimum 2.0 mL

Analyte Name	**Specimen Collection Requirements**
Indirect Antihumanglobulin Test	• Red cells & plasma • Pink shield

	• Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Lactic Acid	• Plasma • Gray shield • Minimum 0.5 mL • Keep on ice
Lactic Dehydrogenase	• Serum or plasma • Red & black stopper/gold shield or green & gray stopper/light green shield • Minimum 2.0 mL
Lead	• Plasma • Tan shield • Royal blue shield also acceptable • Minimum 7.0 mL (royal blue) or 4.0 mL (tan shield)
Lipase	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Lipid Profile	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Lithium	• Serum or plasma • Red stopper/shield or green stopper/shield – **sodium heparin only** • Minimum 2.0 mL • Specimens collected in tubes with lithium heparin are **not** acceptable
Low Density Lipoprotein	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Myoglobin	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Nutritional Elements Analysis	• Plasma • Royal blue shield • 5.0 mL
Obstetric Quadruple Screen (Maternal Serum Screen)	• Serum • Red stopper/shield • Minimum 6.0 mL
Opiates (codeine, morphine, Darvon®, Percocet®, Vicodin®, Oxycontin®)	• Plasma • Gray stopper/shield • Minimum 2.0 mL • Red stopper/shield or lavender stopper/shield or pink shield also acceptable • Tubes with gel are **not** acceptable
Partial Thromboplastin Time (aPTT or PTT)	• Plasma • Light blue stopper/shield • Critical: Tube must be filled to correct volume
Phencyclidine (PCP)	• Plasma • Gray stopper/shield • Minimum 2.0 mL • Red stopper/shield or lavender stopper/shield or pink shield also acceptable • Tubes with gel are **not** acceptable
Phosphorus	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Platelet Count	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Platelet Function Assays	• Plasma • Clear shield over blue stopper – CTAD • Minimum 2.7 mL
Potassium (K)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Prostatic Specific Antigen (PSA)	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Protime (PT)	• Plasma • Light blue stopper/shield • Critical: Tube must be filled to correct volume •
Analyte Name	**Specimen Collection Requirements**
Rapid Plasma Reagin (RPR)	• Serum • Red stopper or gold stopper • Minimum 2.0 mL

RBC Indices • Mean Corpuscular Volume (MCV) • Mean Corpuscular Hemoglobin (MCH) • Mean Corpuscular Hemoglobin Concentration (MCHC) • Red Cell Distribution Width (RDW)	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Red blood Cell Count	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Renal (Kidney) Function Panel	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Reticulocyte Count	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Rheumatoid Factor	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Ristocetin Cofactor	• Plasma • Light blue stopper/shield • Critical: Tube must be filled to correct volume
Rubella Titer	• Serum • Red stopper or gold shield • Minimum 2.0 mL
Serum Protein Electrophoresis	• Serum • Red stopper or gold shield • Minimum 2.0 mL
Sickle Cell Screen	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
Sodium	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Tacrolimus	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
Theophylline	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
Thrombin time	• Plasma • Light blue stopper/shield • Critical: Tube must be filled to correct volume
Thyroid Function Panel	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Thyroid Stimulating Hormone	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Thyroxine	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Tobramycin	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
Total protein	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Trace Metal Analysis	• Serum or whole blood • Royal blue shield • Minimum 5.0 mL • **Critical**: royal blue shield tube must be collected first if other blood tests are ordered. Once the phlebotomy needle has punctured another rubber stopper, it is contaminated and should not be used for trace metal specimen collection •
Analyte Name	**Specimen Collection Requirements**
Triglycerides	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL

Triiodothyronine	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Type & Crossmatch	• Red cells & plasma • Pink stopper/shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Type & Screen	• Red cells & plasma • Pink stopper/shield • Minimum 3.0 mL • Red stopper/shield or lavender stopper/shield also acceptable
Uric Acid	• Serum or plasma • Red & black stopper or gold shield or green & gray stopper or light green shield • Minimum 2.0 mL
Valproic Acid	• Serum • Red stopper/shield • Minimum 2.0 mL • Gel tubes unacceptable
Venereal Disease Research Lab (VDRL)	• Serum • Red stopper/shield • Minimum 2.0 mL
Vitamin B12	• Serum • Red stopper/shield • Minimum 2.0 mL • Protect from light immediately after collection and labeling
von Willebrand Factor Antigen (vWF)	• Plasma • Light blue stopper/shield • Critical: Tube must be filled to correct volume
WBC Differential	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL
White Blood Cell Count	• Whole blood • Lavender stopper/shield • Minimum 3.5 mL

* = Federal workplace cutoff values

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TEST QUESTIONS

Phlebotomy Order of Draw #1221524

Directions:

- Answer sheets: Read the instructions to assure you correctly complete the answer sheets.
- Online: Log in to your User Account on the NCCT website www.ncctinc.com.
  - **NOTE:** If the online test questions differ from the course test that follows the reading material, the CE course you are using is outdated or the question has been revised since you downloaded it. The online question is the most current and it should be answered accordingly.
- Select the response that best completes each sentence or answers each question from *the information presented in the course*.
- If you are having difficulty answering a question, go to www.ncctinc.com and select *Forms/Documents*. Then select *CE Updates and Revisions* to see if course content and/or a test questions have been revised. If you do not have access to the internet, call Customer Service at 800-875-4404.

1. Why is plasma preferred over serum for many STAT lab tests?

    a. Fibrinogen in serum interferes with lab testing.
    b. Lab tests performed on plasma are more accurate.
    c. Serum cannot be used for STAT testing.
    d. Patient test results can be available quicker.

2. Which one of the following evacuated tube types requires no mixing at the time of collection?

    a. Light blue stopper
    b. Red stopper, silicon coated
    c. Green shield
    d. Gold shield with gel

3. Fill volume is critical in all of the following tube types EXCEPT _____.

    a. Clear shield over light blue stopper
    b. Light blue shield
    c. Yellow ACD Solution A
    d. Orange shield

4. Which organization is responsible for recommending an order of draw?

    a. CLSI
    b. CAP
    c. The Joint Commission
    d. Mayo Clinic

5. The phlebotomy order of draw for venipuncture is reversed when blood is collected with a syringe.

    a. True
    b. False

6. Which of the following determines the order of draw?

    a. The color of the tube closure
    b. The type of specimen, eg plasma
    c. The type of additive(s) in the tube
    d. The quantity of blood required

7. If you have an order to collect blood cultures and a trace metal analysis at the same time, which one of the following is most appropriate?

    a. Perform the venipuncture and draw the royal blue tube followed by the blood cultures.
    b. Perform two venipunctures – one for the trace metal analysis and one for the blood cultures.
    c. Perform the venipuncture and draw the blood cultures followed by the royal blue tube.
    d. Perform a venipuncture using a syringe and transfer blood into the trace mineral tube and then into the blood culture bottles.

8. If a lavender shield tube is collected before a light green shield tube, which of the following could occur?

    a. Clotting of the light green shield tube
    b. Distortion of blood cells on WBC differentials
    c. Shortened PT time test results
    d. Falsely elevated potassium test results

9. In which of the following situations would you collect a discard tube before collecting a light blue tube for a protime/INR?

    a. Blood collected using a winged infusion set
    b. Blood collected using a syringe
    c. Blood collected using a tube holder
    d. A discard tube is always collected before a light blue tube

10. You have orders to collect a CBC, type and screen, RPR, and basic metabolic panel. Using the test directory provided what tube types do you collect and in what order do you collect them?

    a. Light green shield, gold shield, pink shield, lavender shield
    b. Lavender shield, gold shield, pink shield, light green shield
    c. Gold shield, light green shield, lavender shield, pink shield
    d. Pink shield, lavender shield, light green shield, gold shield

11. You have orders to collect a hepatitis panel, activated partial thromboplastin time (aPTT), CBC, and comprehensive metabolic panel. Using the test directory provided what tube types do you collect and in what order do you collect them?

    a. Gold shield, lavender shield, light blue shield
    b. Lavender shield, gold shield, light blue shield
    c. Light blue shield, lavender shield, gold shield
    d. Light blue shield, gold shield, lavender shield

12. You have orders to collect a type and crossmatch, erythrocyte sedimentation rate, basic metabolic panel, and acid fast bacteria blood culture. Using the test directory provided what tube types do you collect and in what order do you collect them?

    a. Yellow SPS stopper, light green shield, lavender shield, pink shield
    b. Blood culture bottles, light green shield, lavender shield, pink shield
    c. Yellow ACD solution A stopper, light green shield, lavender shield, pink shield
    d. Light green shield, lavender shield, pink shield, yellow SPS

13. You have the following tube types on your tray: green shield, light green shield, pink shield, red shield, red/black stopper, light blue shield, and gray shield. You are collecting a hemoglobin and hematocrit with the last lavender shield tube. Blood ceases to flow into this tube almost immediately after the venipuncture. What tube on your tray could you use as a substitute for the lavender shield tube?

    a. Green shield
    b. Light blue shield
    c. Light green shield
    d. Pink shield

14. Which of the following is the correct order of collection for blood obtained by skin puncture (capillary draw)?

    a. Serum tubes, EDTA, plasma additive tubes
    b. Plasma additive tubes, serum tubes, EDTA
    c. EDTA, plasma additive tubes, serum tubes
    d. EDTA, serum tubes, plasma additive tubes

End of test

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3. Rate the author in terms of knowledge, organization, and effectiveness (circle one):

1 2 3 4 5 (1 = low/poor; 5 = high/excellent)

4. Rate your overall satisfaction with the course content (circle one):

1 2 3 4 5 (1= not satisfied; 5 = extremely satisfied)

What can NCCT do to make the CE courses better for you?

What would you like to learn about in the future? Please list *specific* topics!

Please include this evaluation with your answer sheet