

# Preanalytical Variables Best Practices in Blood Collection and Handling

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**Date:** 08/30/2021



# Learning Objectives

At the end of this session, you will be able to:

- 1 Verbalize the three phases of testing

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- 2 Identify errors that occur in the preanalytical phase

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- 3 Understand and identify contributing factors in specimen collection, processing, handling and storage that affect specimen quality and test results

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- 4 Use this knowledge to minimize preanalytical errors in your institution, enhance sample quality, improve patient satisfaction and enhance patient care



# Today's Clinical Lab Testing

## Impacts up to 70% of medical decisions

- 1 Assists with diagnosis of disease in conjunction with other medical signs and symptoms
- 2 Identifies pre-disposition for developing diseases and/or conditions
- 3 Reduces potential for less invasive tests and treatments
- 4 Guides management strategies for specific diseases
- 5 Estimates disease progression and treatment response



Risk Assessment



Diagnosis



Prognosis



Treatment Selection



Disease Monitoring and Management

# Three Phases of Laboratory Testing



## Preanalytical

- Physiological/biological factors (healthcare practitioners can't control)
  - Technical factors (healthcare practitioners have the ability to control)
- 



## Analytical

- Instrument and reagent dependent
- 



## Postanalytical

- Result reporting and clinical interpretation



“

The results I give the doctor depend entirely on **the quality of the sample I receive.**

”

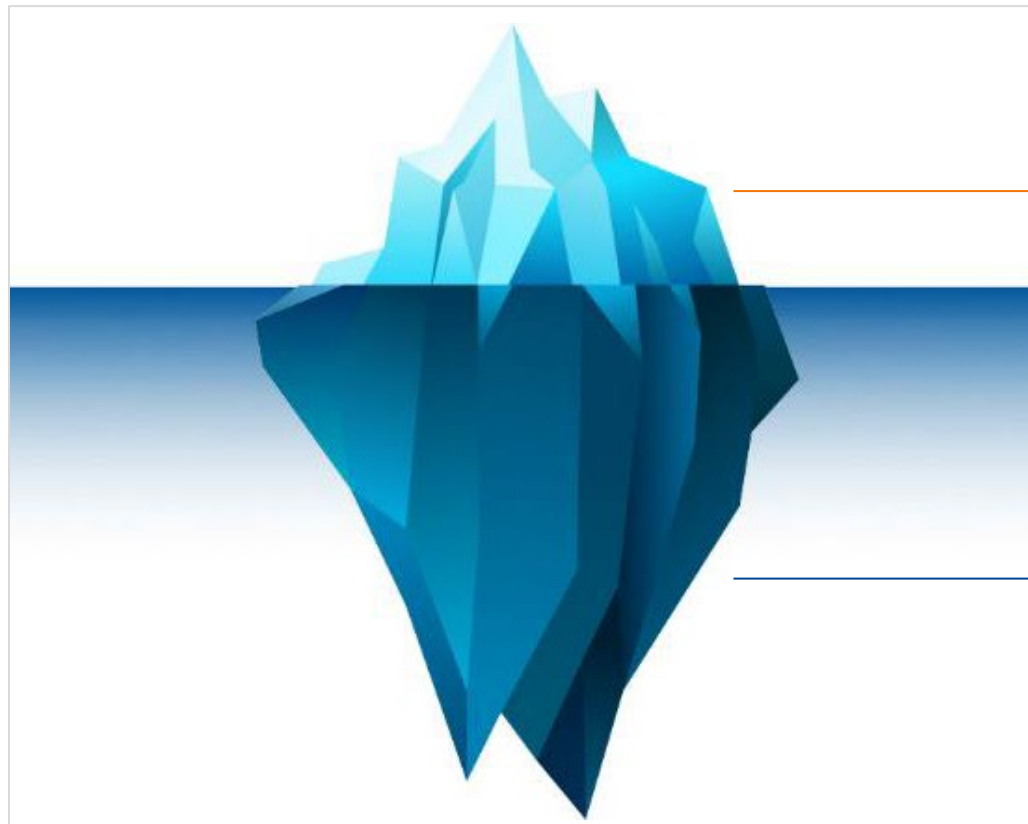
# Preanalytical Variables and Specimen Collection Manual



**Establish a specimen collection manual that includes instructions for the following:**

- 1 Patient preparation
- 2 Type of collection container, amount of specimen required
- 3 Need for special timing for collection
- 4 Types, amounts of preservatives, anticoagulants
- 5 Need for special handling between collection and receipt times
- 6 Proper specimen labeling
- 7 Need for appropriate clinical data when indicated

# The Erroneous Results in the Laboratory Medicine Iceberg



**30%**

**Analytical and  
Postanalytical Errors**

**70%**

**Preanalytical Errors**



# Preanalytical Variables and Factors that Impact Specimen Quality



## Before sample collection:



Patient identification errors



Sample identification errors



# Preanalytical Variables and Factors that Impact Specimen Quality

## During sample collection:

- ✓ Wrong container or sample matrix
- ✓ Wrong additive
- ✓ Inappropriate Blood-to-Additive Ratio
- ✓ Insufficient volume
- ✓ Clotted samples/platelet clumping
- ✓ Spurious hemolysis
- ✓ Sample contamination
- ✓ Site selection/preparation
- ✓ Tube/needle selection
- ✓ Tourniquet placement/time
- ✓ Difficult venous access (DVA)
- ✓ Draws from intravenous (IV) catheters


# Preanalytical Variables and Factors that Impact Specimen Quality




## After sample collection:

- 1 Labeling error
- 2 Inappropriate sample management (e.g. mixing)
- 3 Insufficient centrifugation
- 4 Inappropriate transportation
- 5 Inappropriate storage (time, temperature)


# Preanalytical Variables and Patient Identification

 Use two unique identifiers (full name, date of birth, etc. according to institutional protocol)

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 Ask patient to provide information

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 Have measures to accommodate hearing limitations / language barriers

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 Minimize interruptions, distractions

 1 Follow institution's protocol for unconscious patients

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 2 Verify information on labels, patient ID band, report discrepancies


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 3 Positive patient ID must be made and discrepancies resolved before specimen collection


# Preanalytical Variables and Causes for Patient ID Errors

 Failure to check ID wristband every time


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 Missing ID wristband


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 Placing wrong wristband on patient

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 Relying on verbal identification by patients("are you Mr. Smith?")

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 Relying on other caregiver to provide identification

 1 Identifying patient only as John / Jane Doe

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 2 Failure to distinguish between patients with the same/similar names

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 3 Malfunctioning barcode scanner

# Preanalytical Variables and Specimen Labeling / Identification

## Label specimens immediately

- Critical for patient care

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## Tubes must be labeled in front of the patient

- At bedside
- At phlebotomist chair

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## Label must be affixed with the following information:

- Full name
- ID number
- Date, time as required, i.e.,  
Therapeutic Drug Monitoring
- Collector's initials

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## Impact of improper specimen labeling:

- Recollection
- Reanalysis
- Misdiagnosis
- Malpractice cases
- Labor costs



# Preanalytical Variables and Causes of Mislabeled Specimens



Carrying multiple specimen labels into patient room



Labeling away from bedside



Labeling specimens by someone other than collector



Failing to use blood bank labeling protocol



Failing to document collection date, time



Re-labeling of specimens in the lab



Using multiple label types applied to specimen



Labeling tubes prior to collection

# Preanalytical Variables and Site Selection

## Best Sites for Venipuncture

Superficial veins of the upper limb



1

### Median Cubital Vein

A superficial vein, most commonly used for venipuncture, it lies over the cubital fossa and serves as an anastomosis between the cephalic and basilic veins.

2

### Cephalic Vein

Shown in both forearm and arm, it can be followed proximally where it empties into axillary vein.

3

### Basilic Vein

Shown in the forearm and arm, it divides to join the brachial vein.



# Preanalytical Variables and Site Selection - Hand



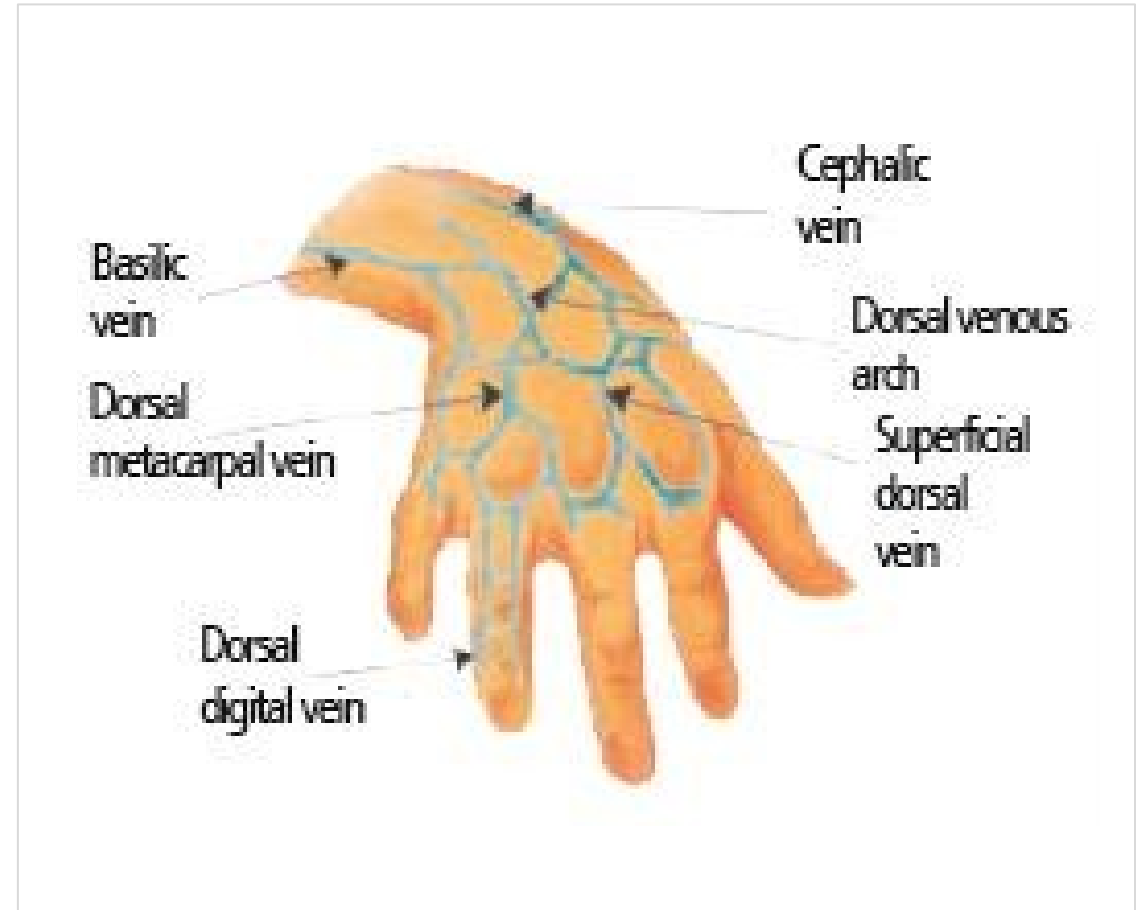
Hand or wrist veins may only be used when ante - cubital fossa veins are unsuitable or unavailable.



Extra care needed to anchor these veins.



A small gauge needle and small volume evacuated tubes may be required because these veins have a narrow circumference



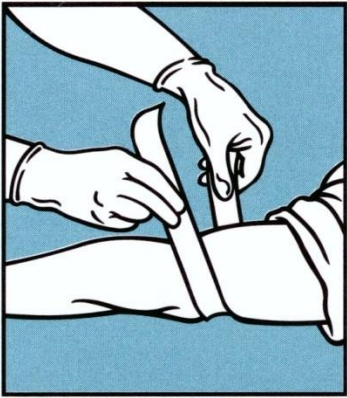
# Preanalytical Variables and Inappropriate Sites for Venipuncture

Sites That Must Not Be Used	
Site	Rationale
Fistula, arm with a fistula, or vascular graft	<ul style="list-style-type: none"> <li>Threatens the integrity of fistulas and vascular grafts, which can lead to serious patient complications</li> </ul>
Arteries <sup>12,13</sup>	<ul style="list-style-type: none"> <li>Risk of misinterpretation of results and patient mismanagement if arterial blood is used rather than venous blood; <b>NOTE:</b> Arterial and venous blood specimens are not equivalent for many analytes.</li> <li>Poses a significantly higher risk of injury and complications than venous access</li> </ul>
Veins on lateral and palmar surface (underside) of the wrist <sup>14-21,25</sup>	<ul style="list-style-type: none"> <li>Increased risk of nerve, tendon, and arterial involvement</li> </ul>
Infected sites	<ul style="list-style-type: none"> <li>Potential for altered test results, exacerbation of infection, and patient discomfort</li> </ul>

# Preanalytical Variables and Inappropriate Sites for Venipuncture

Sites That Require Physician's Permission	
Site	Rationale
Limbs on the side of a mastectomy	• Risk of lymphedema and the potential for altered test results <sup>22-24</sup>
Any part of the lower extremities	• Risks tissue necrosis in diabetic patients and thrombophlebitis in patients with coagulopathies
Sites That Should Be Avoided	
Site	Rationale
Extensive scarring, healed burns	• Palpation and needle insertion complications • Inability to detect adverse reactions
Hematoma	• May cause discomfort to the patient and potential altered test results
Above and below infusing fluids or from a VAD	• Possible contamination of specimen with IV fluids <sup>26</sup> (see Subchapter 5.3.2)
Inflamed sites (including inflamed tattoos)	• Patient discomfort and possible complications
Edematous sites	• Potential for altered test results
Extremity affected by stroke and injury	• Inability to detect adverse reaction, eg, nerve injury, pain, infection

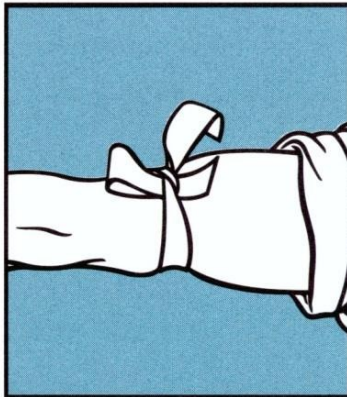
# Preanalytical Variables and Tourniquet Application



Position the tourniquet  
3-4 inches above the  
venipuncture site



Tuck a portion of one  
end under the opposite  
end to form a loop



Cross the Tourniquet  
over the patient's arm



A properly applied  
tourniquet for  
easy release

## Preanalytical Variables and Tourniquet Use

- ✓ Use no more than one tourniquet
- ✓ Use non-latex tourniquets to prevent latex sensitivity and allergic reactions
- ✓ Use single-use tourniquets to prevent spreading of MRSA
- ✓ Apply pressure for no more than 1 minute to prevent hemo- concentration
- ✓ Release tourniquet as soon as blood flows into first tube
- ✓ If pressure exceeds one minute before accessing the vein, release and reapply after two minutes
- ✓ Constriction must not be excessive or provide discomfort to the patient; may be used over clothing to prevent pinching
- ✓ Blood pressure cuff inflated below patient's diastolic pressure may be used by those trained to use such devices



# Preanalytical Variables and Improper Tourniquet Use



**Poses a risk to patients**



**May affect test results**



**Hemo-concentration causes erroneously high values for:**

- Protein-based analytes
- Packed Cell Volume
- Cellular elements

## Affected analytes include:

- ✓ Albumin
- ✓ Alkaline phosphatase
- ✓ Calcium
- ✓ CBC parameters – RBC, WBC, Differential, Hemoglobin, Hematocrit
- ✓ Potassium
- ✓ Glucose
- ✓ Triglyceride
- ✓ Total protein



# Preanalytical Variables and Fist Clenching



## Clenching the fist is not mandatory

Veins do become more prominent,  
easier to enter

Fist should be clenched and held until blood flows  
into collection device, then released

Fist pumping or vigorous motion to open and close  
the hand is to be avoided

## Pumping can cause significant elevations

- ✓ Potassium
- ✓ Ionized Calcium



# Preanalytical Variables and Site Preparation, Disinfection

- 1 Disinfect site to minimize microbiological contamination of specimen, patient
- 2 70 percent isopropyl alcohol solution with clean gauze pad or prepared pad; chlorhexidine also acceptable
- 3 Scrub with back-and-forth motion with friction
- 4 Use non-alcohol cleanser if blood alcohol analysis ordered
- 5 Use facility-specific cleanser if blood cultures are ordered
- 6 Allow to air dry completely before venipuncture; at least 30 seconds for blood cultures
- 7 Avoid blowing or waving at site to encourage drying
- 8 Avoid using non-sterile gauze to dry the area
- 9 Re-palpating vein requires that the site be cleansed again

# Preanalytical Variables and Site Preparation, Disinfection



Residual disinfectant can be introduced into the sample if drying is not complete



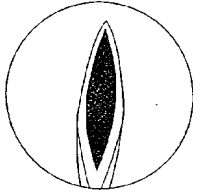
Alcohol can cause hemolysis, increased ethanol level



Benzalkonium compounds may affect electrolyte results

## Betadine contamination:

- ✓ False elevation of phosphorus, uric acid, potassium
- ✓ Oxidative effect causes false-positive stool hemoglobin, urine glucose



# Preanalytical Variables and Needle Selection



**Determine appropriate gauge based on amount of blood to be drawn, age of patient, and vein size**

- 19-23 gauge most commonly used
- 22-23 gauge in children
- 25 gauge can result in more hemolysis, slower fill – however, the new BD RightGauge™ Ultra-Thin Wall Cannula Technology modifies these issues

## Goal:

To minimize hemolysis and thrombogenicity due to pre-analytical variables



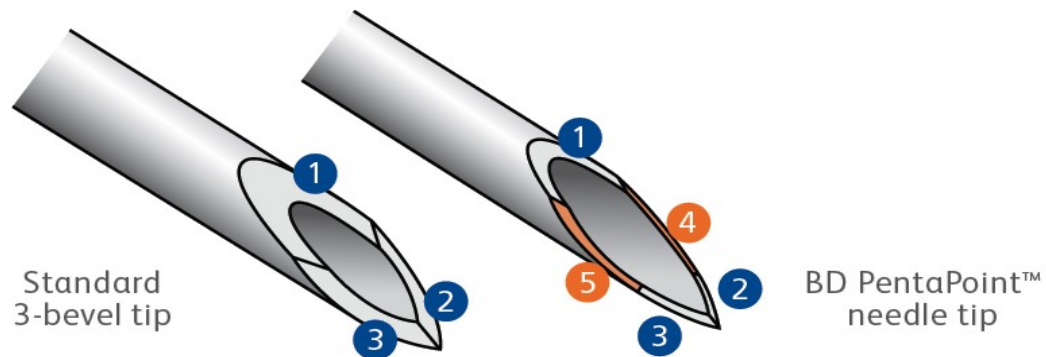
Clinical and Laboratory Standards Institute, document GP41

# 25g Needle Technology

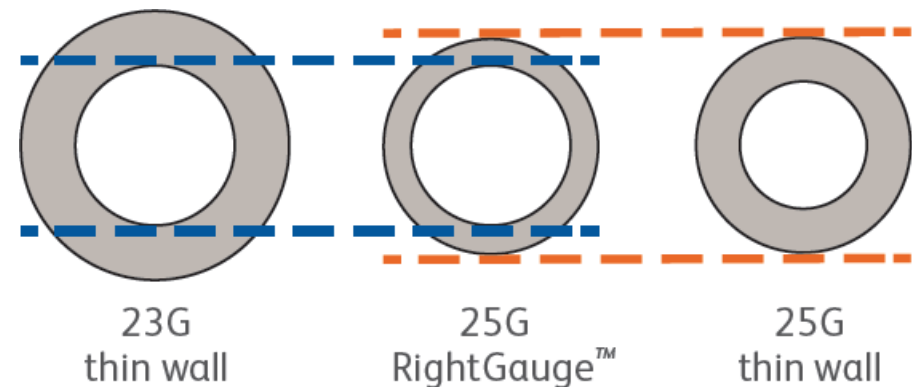
## Uses 5 Bevels and BD RightGauge™ Ultra-Thin Wall Cannula Technology with an Inner Diameter Similar to 23g



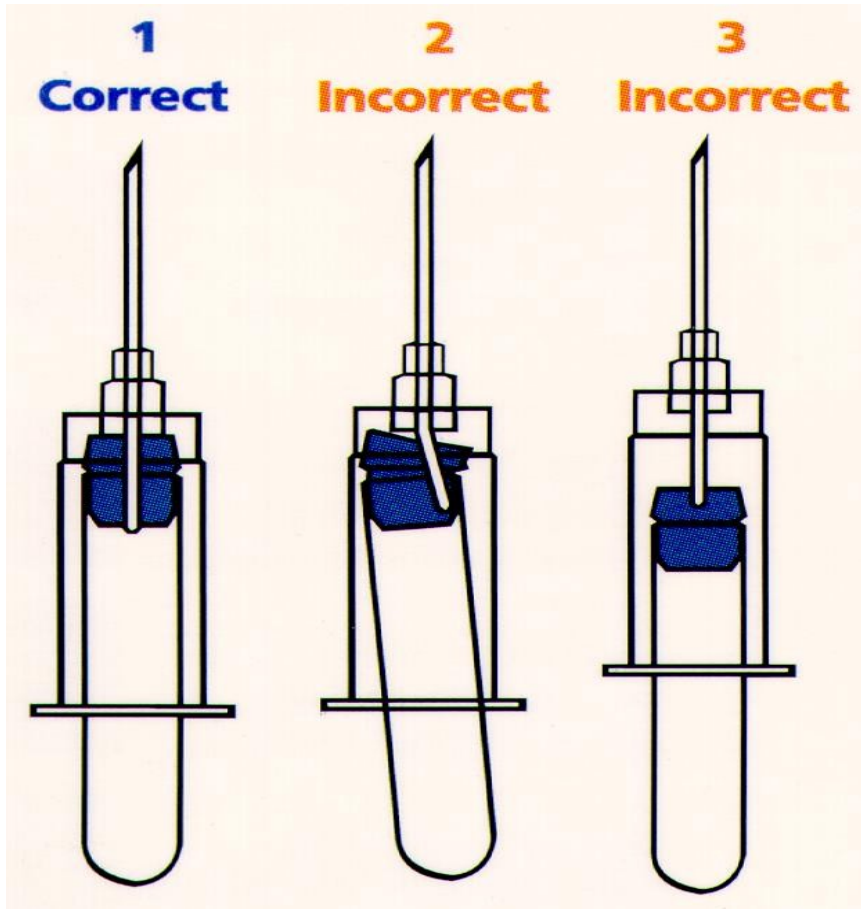
- 1** 5 bevels instead of 3 **minimizes patient discomfort.** The new technology requires 32% less penetration force when compared with equivalent gauges of the current BD PBBCS with a thin wall 3-bevel cannula.



- 2** Ultra-Thin Wall Cannula enables **easier access to small, difficult veins** without compromising sample quality or tube fill time.

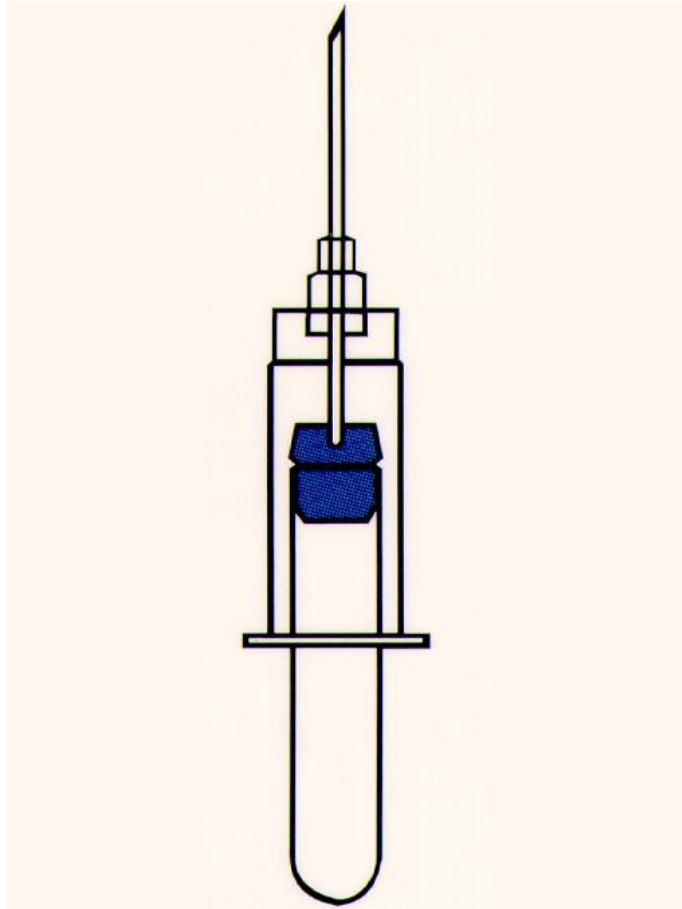


# Preanalytical Variables and Proper Tube Alignment - Insertion



- 1 Correct**  
Proper insertion of tube into holder.
- 2 Incorrect**  
Improper insertion resulting in an incompletely punctured stopper.
- 3 Incorrect**  
Partially punctured stopper.

# Preanalytical Variables and Proper Tube Removal



**Remove tube from contact with the back end of the blood collection needle before removing from patient's arm**



No blood leakage from needle tip when removing the needle



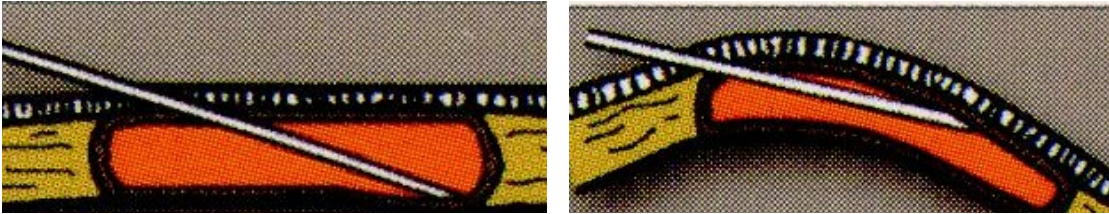
Minimizes blood exposure to patient or self



Safety

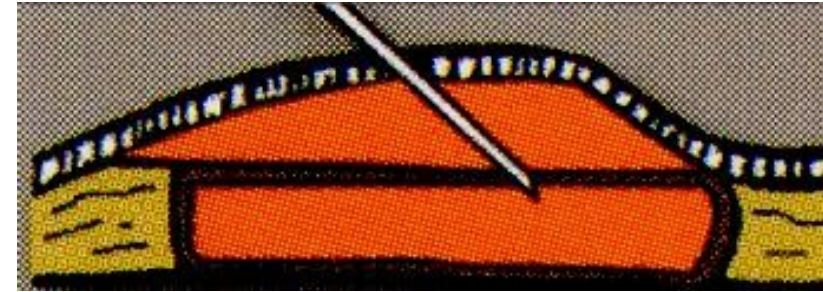
# Preanalytical Variables and Needle Positioning in Vein

## Bevel on lower or upper vein wall



- 1 Pull back slightly
- 2 Avoid rotating or changing needle angle

## Partial needle insertion

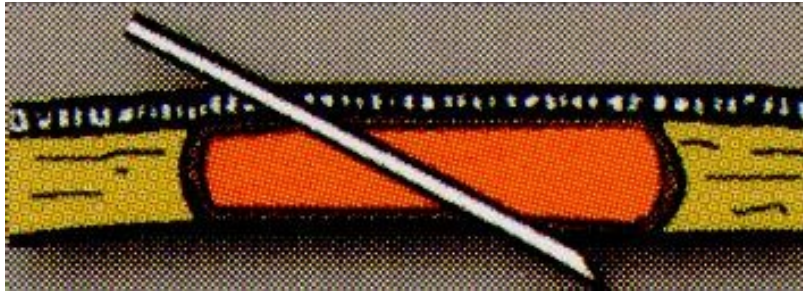


- 1 Should a hematoma form, immediately remove tourniquet and needle
- 2 Elevate arm above the patient's heart and apply pressure. Do not bend patient's arm



# Preanalytical Variables and Needle Positioning in Vein

## Puncture through vein



- 1** Withdraw the needle slightly to establish blood flow

## Collapsed vein



- 1** Tighten tourniquet by grasping the ends with one hand and twisting together.  
Should blood flow fail to resume, remove tube, wait a few seconds for blood flow to reestablish, and insert a smaller volume tube.
- 2** Remove Needle

# Preanalytical Variables and Difficult Venous Access



Needles should run in the same direction as the vein

Insert needle quickly and smoothly at a 15 degree angle to the skin



# Preanalytical Variables and Tube Selection



- ✓ Tubes must be within acceptable use date; not past expiration date
- ✓ Tubes may or may not contain additives
- ✓ Tube tops color-coded by additive or use



## Improper choice of additive/ anticoagulant for intended testing may affect test results

- $K_2$ EDTA (spray-coated) vs.  $K_3$ EDTA (liquid)
- Plasma vs. serum
- Sodium vs. Lithium heparin
- Presence of gel for therapeutic drugs
- Grey top tube (Glucose, C&S)
- Royal blue tube ( $K_2$ EDTA, clot activator)



# Preanalytical Variables and Order of Draw

## Blood culture tube or bottle

**Coagulation tube**  
(e.g., citrate, blue closure)

**Serum tube with or without clot activator, with or without gel**  
(e.g., red closure)

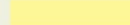

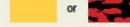




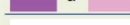

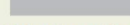
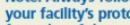
**Heparin tube with or without gel plasma separator**  
(e.g., green closure)

**EDTA**  
(e.g., lavender closure)

**Glycolytic inhibitor**  
(e.g., gray closure)

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

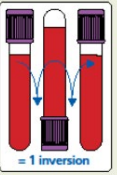
*Designed for Your Safety* Reflects change in CLSI recommended Order of Draw (H3-A5, Vol 23, No 32, 8.10.2)

Closure Color	Collection Tube	Mix by Inverting
<b>BD Vacutainer® Blood Collection Tubes (glass or plastic)</b>		
	• Blood Cultures - SPS	8 to 10 times
	• Citrate Tube*	3 to 4 times
 or 	• BD Vacutainer® SST™ Gel Separator Tube • Serum Tube (glass or plastic)	5 times 5 times (plastic) none (glass)
	• BD Vacutainer® Rapid Serum Tube (RST)	5 to 6 times
 or 	• BD Vacutainer® PST™ Gel Separator Tube With Heparin • Heparin Tube	8 to 10 times 8 to 10 times
 or 	• EDTA Tube	8 to 10 times
	• BD Vacutainer® PPT™ Separator Tube K <sub>2</sub> EDTA with Gel	8 to 10 times
	• Fluoride (glucose) Tube	8 to 10 times

\* When using a winged blood collection set for venipuncture and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection set tubing's "dead space" with blood but the discard tube does not need to be completely filled. This important step will ensure proper blood-to-additive ratio. The discard tube should be a nonadditive or coagulation tube.

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

Handle all biologic samples and blood collection tubes in a safe, controlled, low-velocity and low-turbulence environment according to the policies and procedures of your facility. Exercise appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury) since they may transmit viral hepatitis, HIV, AIDS, or other infectious diseases. Utilize any built-in used needle protector if the blood collection device provides one. Do not use and do not reuse needles used needles, but the practice and procedure of your facility may differ and must always be followed. Discard any blood collection "waste" in biohazard containers approved for their disposal.

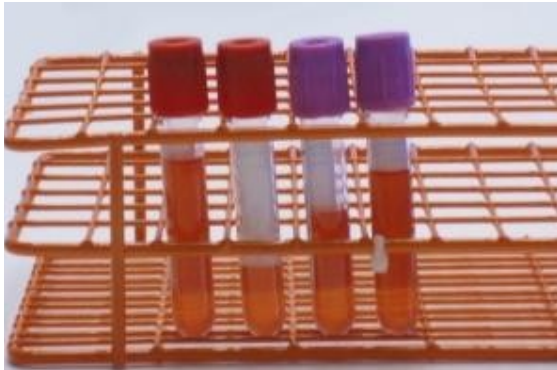


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# Preanalytical Variables and Blood-to-Additive Ratio



**Tubes contain additives, clot activator or anti-coagulant**

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**Additive is engineered with designated Blood-to-Additive Ratio**

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**Ratio not achieved when tubes either under or over filled**

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**Winged blood collection sets contain dead-air space in tubing depending upon the tubing length**

- 0.3 mL for 7-inch tubing
  - 0.5 mL for 12-inch tubing
- 

**When drawing coagulation (Sodium Citrate light-blue top) tubes, use a discard tube first**

- Filling of discard tubes unnecessary, just enough to clear dead space
- Can be Coag tube or non-additive tube

# Preanalytical Variables and Syringe Draws



- ! Avoid venipuncture with traditional needle and syringe for safety reasons
- ! Syringe stopper materials can contaminate specimen, interferes with drug assays
- ! Needle should enter at 30 degree angle or less
- ! Needle should remain as stable as possible while maintaining a slow, steady withdrawal of blood
- ! Excessive pulling pressure must be avoided
- ! Transfer into tubes should be done with a designated blood transfer device, not an injection needle

# Preanalytical Variables and Coag Tube Minimum Fill Level

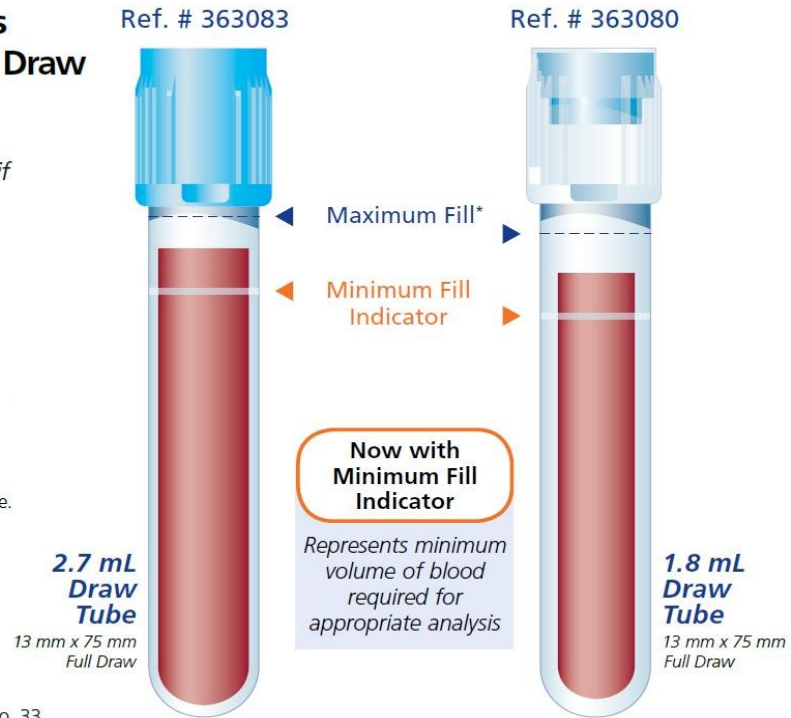
## BD Vacutainer® Plus Plastic Citrate Tube

### BD Vacutainer® Plus Plastic Citrate Tube Draw Volume Guide

Sufficient volume achieved if blood drawn falls above minimum fill indicator. For blood transfer, **do not** fill above illustrated dashed maximum line.

**Note:** The quantity of blood drawn into evacuated tubes varies with altitude, ambient temperature, barometric pressure, tube age, venous pressure and filling technique.

\*According to CLSI guideline, Dec. 2003, Doc. H1-A5, Vol. 23, No. 33.



# Preanalytical Variables and Impact of Under-Filled Tubes

## EDTA

- Cell shrinkage or swelling – time dependent
- Abnormal cell morphology, cell counts

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## Sodium Citrate

- Prolonged PT, aPTT

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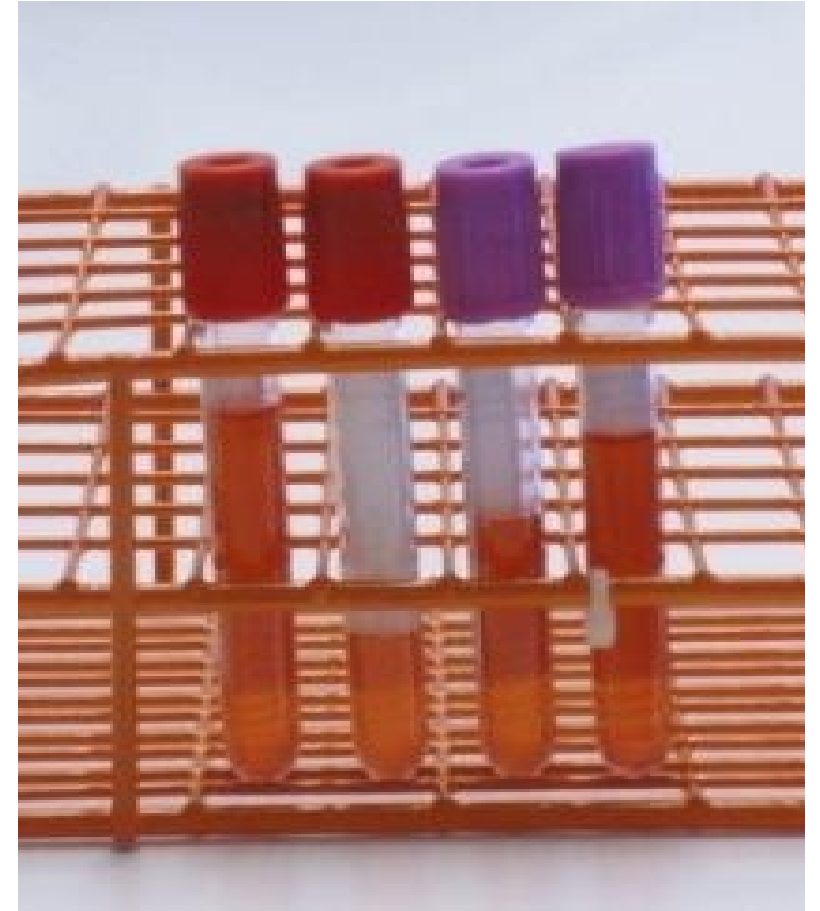
## Heparin

- Troponin
- CK
- Creatinine
- Aminoglycosides (Gentamicin, Tobramycin)

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## Oxalate Fluoride

- Hemolysis





# Preanalytical Variables and Causes of Short Filled Tubes



Using winged blood collection sets



Using expired evacuated tubes (vacuum)



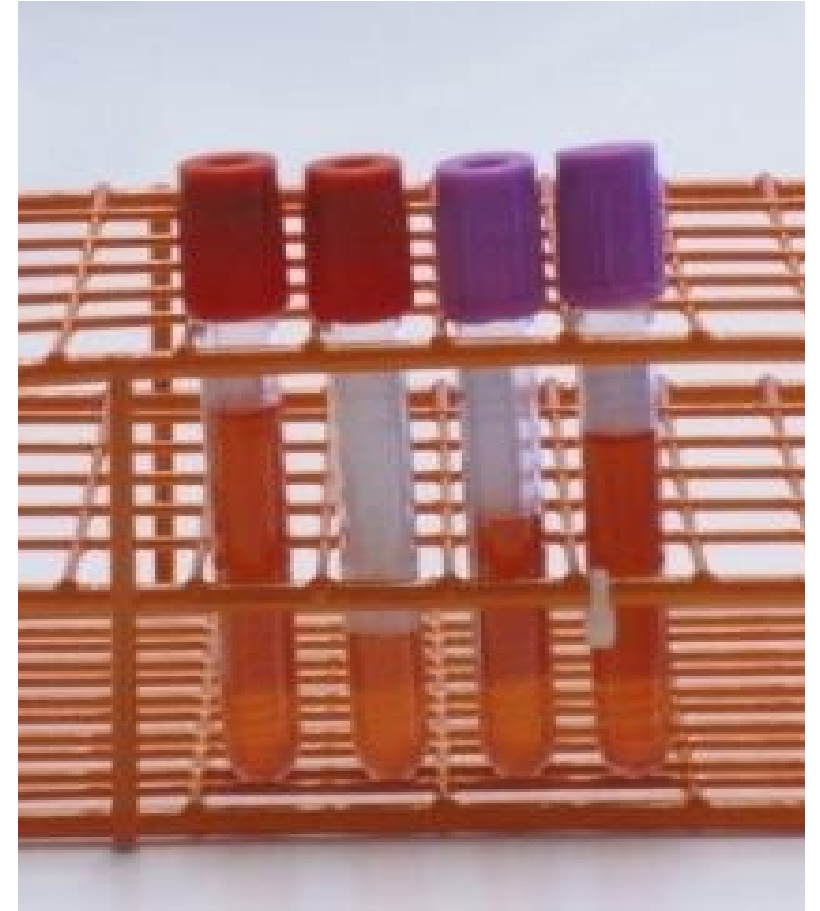
Transferring blood from a syringe to evacuated tube



Collapsing vein



Removing tube prematurely during venipuncture



# Preanalytical Variables and Ways to Avoid Short Fills



Using a discard tube with a winged blood collection set



Using blood collection tubes within acceptable use date, prior to expiration



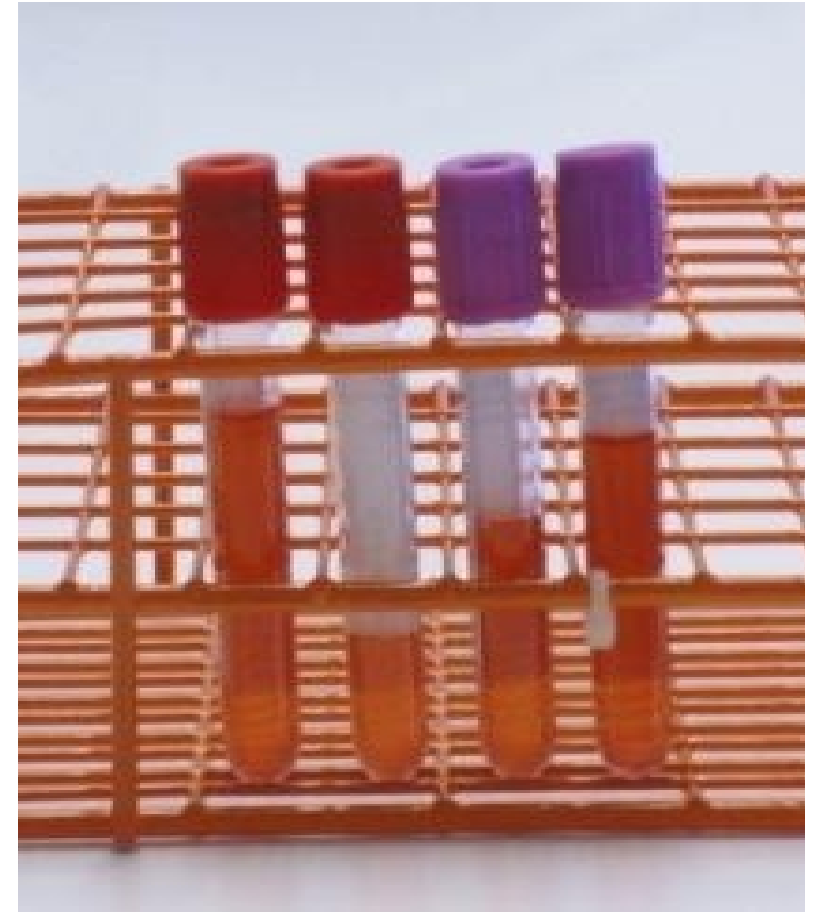
Allowing vacuum to aspirate blood from syringe



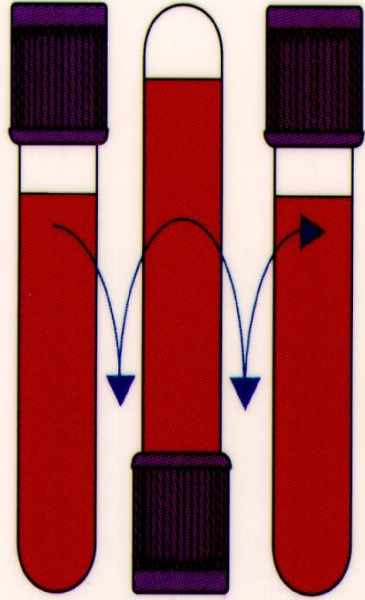
Allowing vein to recover if collapsed



Allowing tube to remain in place until blood flow ceases



# Preanalytical Variables and Proper Tube Mixing



One Inversion

## Specimen mixing – gently invert, not shake – tubes ASAP after collection

- SST (gold top) tubes: Invert 5 times
- Sodium (Na) citrate (blue top) tubes: Invert 3 to 4 times
- Other additive tubes: Invert 8-10 times

---

## Insufficient or delayed mixing can cause:

- Fibrin strands, micro-clots, platelet clumping, clotted specimens
- Hemolysis

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**Excessive mixing of coag tubes (>4 inversions) can cause activated platelets**

# Preanalytical Variables and Serum Separator Tubes (SST)



- Gently invert 5 times to mix clot activator with blood.

Clot  
**30**  
Minutes



- Allow blood to clot for a minimum of 30 minutes in a vertical position.
- Observe a dense clot.

Spin  
**10**  
Minutes

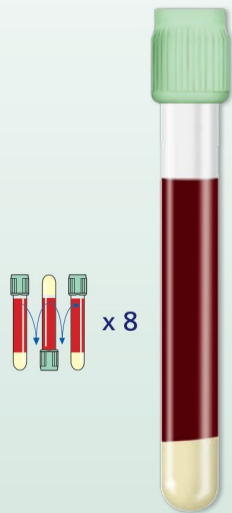


- Centrifuge at FULL SPEED (between 1100 and 1300g) for 10 minutes for swing-head units or 15 minutes for fixed angle units (balance tube in centrifuge).
- Barrier will form, separating serum specimen from clot.
- Transport spun tube to laboratory.

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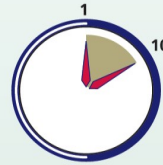
# Preanalytical Variables and Plasma Separator Tubes (PST)

**Invert  
8-10  
Times**



- Gently invert 8-10 times immediately after collection to mix lithium heparin anticoagulant with blood.
- Insufficient mixing may lead to microclot and fibrin strand formation.

**Spin  
10  
Minutes**



**Centrifuge at  
1100 - 1300g**



- Centrifuge at full speed  
1100 – 1300g for 13 mm Plus Plastic tubes  
1000 – 1300g for 16 mm Plus Plastic tubes  
for 10 minutes in a swing-bucket unit or  
15 minutes for a fixed-angle unit  
(balance tubes in centrifuge).
- Gel barrier will form to separate plasma from red blood cells.

**Ready  
for  
Analysis**



- Use in laboratory for plasma determinations in chemistry.

## Preanalytical Variables and Fibrin



**Can cause significant  
disruption to  
instrument operation  
and process workflow**

# Preanalytical Variables and Clotted Samples

Account for 65% of CBC rejections\*



## Causes

- Overfilling additive tubes
- Mixing inadequate or delayed
- Drawing from DVA patient
- Filling evacuated tubes slowly
- Drawing blood into syringe, then transferring
- Improper choice of needle or other equipment



## Impact

- Specimen rejection leads to specimen recollection
- Instrument downtime due to probe or cell clogging
- Micro-clots and clotted blood influence key parameters



# Preanalytical Variables and Ways to Avoid Clotted Samples



Mix anti-coagulated specimens adequately to distribute additive

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Avoid drawing in syringes

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Conduct period in-servicing of all personnel involved in blood collection

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Enforce collection policies and specimen rejection criteria

# Preanalytical Variables and Platelet Clumps



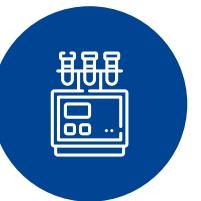
## Impact

- ↓Platelet Count
- ↑WBC Count



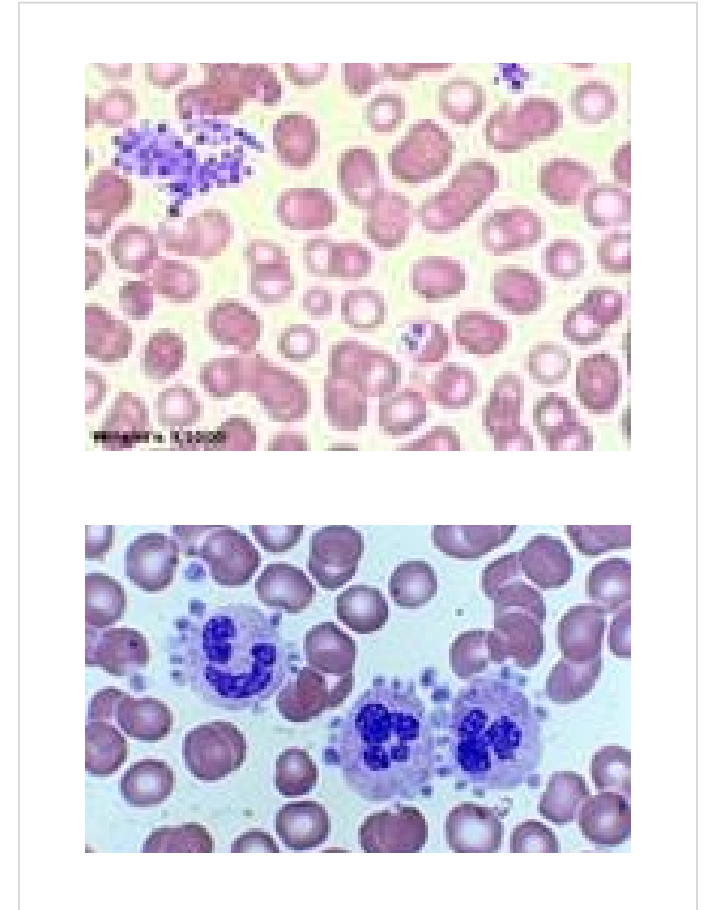
## Causes

- Poor mixing after collection
- Overfilling additive tubes
- EDTA induced



## Remedies

- Verify Platelet Count with blood drawn in NaCitrates
- Collect & analyze at 37°C
- Vortex sample



# Preanalytical Variables and Special Situations

# Preanalytical Variables and Timing of Specimen Collection

## Medications & Timed Testing

1 Therapeutic Drug Monitoring (TDM)

---

2 Glucose

---

3 Blood Cultures  
• Antibiotic therapy

---

4 Anticoagulation Monitoring

---

5 Cardiac Markers

## Diurnal variations

1 Cortisol

---

2 Adrenocorticotropin

---

3 Other hormones

## Preanalytical Variables and Collection near IV site

**If a site distal to the IV site needs to be used:**

Have the responsible caregiver turn off IV for at least two minutes

---

Apply tourniquet below IV site

---

Select vein other than the one with the IV

---

Perform venipuncture, discarding the first 5mL of blood

---

Indicate the IV solution, arm used, and drawn below IV



# Preanalytical Variables and Collection from patients receiving IV fluids



**Blood should be obtained from the arm opposite the one receiving IV solution**

## **When IVs are in both arms:**

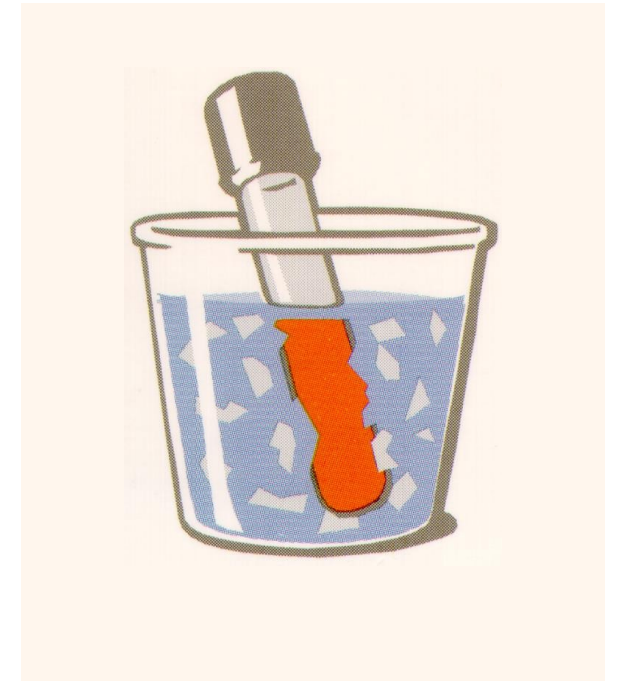
- ✓ Draw blood specimen from a vein distal (peripheral) to the IV site
- ✓ Place tourniquet between the IV and venipuncture sites

# Preanalytical Variables and Transport Temperature

**Analytes that require the metabolic processes to be slowed down must be transported in an ice slurry to avoid erroneous lab results.**

## Examples:

- |                                       |                       |                  |
|---------------------------------------|-----------------------|------------------|
| ✓ ACTH                                | ✓ Gastrin             | ✓ Lactic Acid    |
| ✓ Acetone                             | ✓ Parathyroid Hormone | ✓ Pyruvate       |
| ✓ Angiotensin Converting Enzyme (ACE) | ✓ Catecholamines      | ✓ Renin Activity |
| ✓ Blood Ammonia                       | ✓ Free Fatty Acids    | ✓ Vitamin D      |





# Preanalytical Variables and Light-sensitive samples



**Wrap light-sensitive specimens in aluminum foil for transport**

## Light-sensitive analytes include:

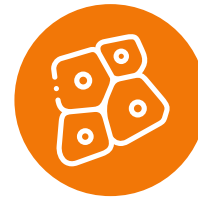
- ✓ Bilirubin
- ✓ Erythrocyte Protoporphyrin
- ✓ Carotene
- ✓ Vitamin A
- ✓ Vitamin B6



# Preanalytical Variables and Clot Formation



## Clot Formation



Normal blood clots in 45 min.  
+/- 15 min



Specimens from patients on  
anticoagulant therapy or with  
coagulopathies take longer to clot

# Preanalytical Variables and Centrifugation

## Centrifugation: Time and Speed

- ✓ **Complete** clotting of sample or maintenance of anticoagulation

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- ✓ Centrifuge to separate cells from serum/plasma

---

- ✓ Centrifuge at speeds, time recommended by tube manufacturer
  - Horizontal swing bucket recommended

---

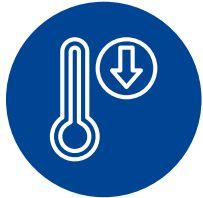
- ✓ Do not centrifuge original tube a second time

---

- ✓ Remove serum or plasma and place in a secondary tube if a second round of centrifugation is indicated
  - ↑ K
  - ↓ Glucose

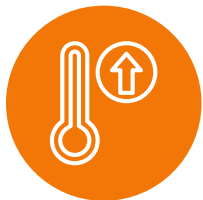


# Preanalytical Variables and Storage Temperature



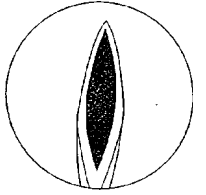
## Risks related to low storage temperatures

- Increased potassium from RBC rupture
- Increased RBC swelling, increased HCT, MCV
- Decreased Platelets, MCHC, Sed Rate
- Increased WBC from platelet clumping
- Cold-induced activation of intrinsic coagulation system



## Risks related to high storage temperatures

- Accelerate degradation of labile coagulation factors
- Serum/plasma proteins



# Preanalytical Variables and Causes of Hemolysis



- ✓ Needle gauge size either too small or large for vein
- ✓ Use of syringes:
  - Withdraw too quickly
  - Syringe volume too large
  - Expel blood into tube with force
- ✓ Drawing from intravenous or central lines
- ✓ Improper blood to additive ratios
- ✓ Filling tubes by hand
- ✓ Mixing too vigorously or rough transport
- ✓ Failing to allow alcohol to dry completely
- ✓ Using tube with volume too large, too much vacuum
- ✓ Incompatible collection devices
- ✓ Blood frothing due to poor connections
- ✓ Drawing blood from hematoma

# Preanalytical Variables and Impact of Hemolysis

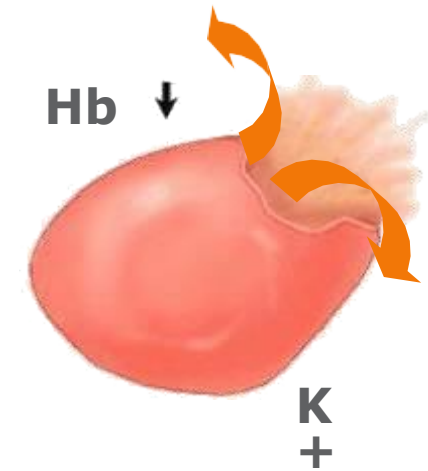
Can affect results in all laboratory disciplines:  
Chemistry, hematology, coagulation, microbiology



Rupture of red blood cells leads to contamination of the serum or plasma with intracellular components



Hemolysis – (L-R) Good Specimen, Trace Hemolysis, Moderate Hemolysis, and Gross Hemolysis



# Preanalytical Variables and Impact of Hemolysis

✓ Often results in recollection

---

✓ Delays test results and patient treatment

---

✓ Decreases patient satisfaction

✓ Causes increased values:

- Potassium, LD, AST, ALT, iron, total protein, phosphorus, magnesium
- 

✓ Causes falsely decreased values:

- RBC, HCT, WBC
- Albumin, Alkaline phosphatase, sodium (from specimen dilution)

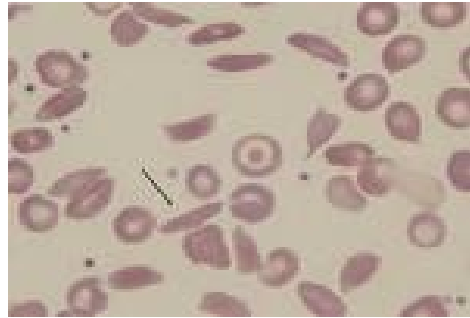
**Hemolysis may not be apparent in whole blood assays, such as point-of-care tests**



# Preanalytical Variables and *In-vivo* Hemolysis



**Metabolic Disorders**



**Physical Agents**



**Chemical Agents**



**Infectious Agents**





“

The results I give the doctor depend entirely on **the quality of the sample I receive.**

”

# Conclusions

- 1 The 3 phases of laboratory testing are: \_\_\_\_\_.
- 2 Preanalytical factors are responsible for \_\_\_\_\_% of laboratory errors.
- 3 Preanalytical errors can lead to erroneous test results and misinterpretation.
- 4 Preanalytical errors often result in the need to re-collect samples, longer turnaround time, increased costs, delayed patient treatment, and reduced patient satisfaction.
- 5 Good phlebotomy technique and awareness of the factors that can affect specimen quality are essential for patient care.
- 6 Preanalytical factors can affect specimen quality.

## References

Clinical and Laboratory Standards Institute (CLSI) – C56: *Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators or Interferences in Clinical Laboratory Analysis*, 1st Edition, July 2012.

---

CLSI – H02: *Procedures for the Erythrocyte Sedimentation Rate Test*, 5th Edition, May 2011.

---

CLSI – H21: *Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays*, 5th Edition, January 2008.

---

CLSI – GP33: *Accuracy in Patient and Sample Identification*, 1st Edition, March 2010.

---

CLSI – GP39: *Tubes and Additives for Venous and Capillary Blood Specimen Collection*, 6th Edition, December 2010.

---

CLSI – GP41: *Collection of Diagnostic Venous Blood Specimens*, 7th Edition, April 2017.

---

CLSI – GP44: *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests*, 4th Edition, May 2010.

---

CLSI – M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*, 4th Edition, May 2014.

---

BD Evacuated Blood Collection System Package Insert 7/2010.



## References

Becan-McBride, K. (ed) Preanalytical Phase and Important Requisite of Laboratory Testing. *Advance for Med. Lab. Prof.* Sept 28, 1998: 12-17.

---

Bush, V. Green, Sol. Managing Preanalytical Variables AACC Workshop #2211. July 2002.

---

CAP Q-Probe 92-05, Hematology specimen acceptability 1993.

---

Gerhardt W, Nordin G, Herbert AK, Burzell BL, Isaksson A, Gustavsson E, Haglund S, Muller-Bardorff, and Katus HA. Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: Lower recoveries in early than in late phases of myocardial injury. *Clin Chem* 2000;46:817- 821.

---

Green, SF. The cost of poor blood specimen quality and errors in pre-analytical processes. *Clinical Biochemistry.* 46: 1175-1179, 2013. <http://dx.doi.org/10.1016/j.clinbiochem.2013.06.001>

---

Guder, Narayanna, Wisser, Zawta. *Samples From the Patient to the Laboratory* Git Verlag July 1996:pp.6-50. Darmstadt, Germany.

---

Gulati GL, Asselta A and Chen C. Using a vortex to disaggregate platelet clumps. *Lab Med* 1997;28(10):665-667.

---

McPherson, RA and Pincus, MR (Eds.), *Henry's Clinical Diagnosis and Management by Laboratory Methods.* 23rd Edition. St, Louis: Elsevier, 2017.



## References

Ross JW, Boone DJ. Assessing the effect of mistakes in the total testing process on the quality of patient care [Abstract 102]. Martin L. Wagner W Essie JDK eds. 1989 *Institute of Critical Issues in Health Laboratory Practice* 1991 Dupont Press Minneapolis, MN.

---

Young D.S., Bermes, E.W. Specimen Collection and Processing: Sources of Biological Variation. Ashwood ER. ed. *Tietz Textbook of Clinical Chemistry*. Philadelphia: W.B. Saunders Company, 1999, pp.42-72.

