How Can the Presence of Inappropriate Anticoagulant in Specimen Collection Compromise Sample Quality?

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About the Speaker

- Bench tech experience in hematology, hemostasis, urinalysis, toxicology, and chemistry in a large multi-system medical center
- Program Director of a hospital based MT Program as well as Safety and Education Coordinator
- Lab Director of Immediate Response Lab and Critical Access Lab
- MLS instructor at University of Cincinnati for online and on campus students
Objectives

1. Review venous specimen collection and tube order of draw
2. Review the function of common anticoagulants
3. Identify errant test results based on inappropriate anticoagulant use
Pre-Analytical Errors

1999 IOM *To Err is Human: Building A Safer Health System*

46-68.2% of errors in the lab are pre-analytical*
- 25% result in inappropriate patient care*
- Hospital costs are increased
  - Patient management
  - Recollection and repeat testing
  - Lab investigation
  - Consumables and supplies

Kaushik, Nitin, and Green, Sol. Pre-analytical errors: Their impact and how to minimize them. MLO, 5/2014
Pre-Analytical Errors

- Patient non-compliance
- Misidentification
- Labeling and clerical errors
- Sample transport and storage
- Inadequate specimen collection
  - Inadequate specimen volume/ratio of blood to anticoagulant
  - Incorrect order of draw
  - Contamination by other anticoagulants
Standard Procedures

WHO – World Health Organization
- *WHO guideline on drawing blood: best practices in phlebotomy*

CLSI – Clinical Laboratory Standards Institute
- GP41-A6 *Procedures for the collection of diagnostic blood specimens by venipuncture - Approved guideline, 2007*
  - Addresses the need to prevent errors in the lab induced by additive contamination
Order of Draw

- Sterile tubes
- Sodium citrate
- Clot activator/SST
- Lithium heparin/PST
- EDTA
- Sodium fluoride
Common Anticoagulants

To provide specimens appropriate for laboratory testing
- Sodium Citrate
- Lithium Heparin
- EDTA
Sodium citrate

- 3.2% buffered Sodium citrate
  - Chelates calcium
  - When filled, provides a 9:1 ratio of blood to anticoagulant

Anticoagulant of choice for coagulation assays
Coagulation Cascade

Intrinsic

surface contact

XII → XII_a

XI → XI_a

IX → IX_a

(VIII, PL, Ca^{++})

X → X_a

(prothrombin)

thrombin (serine protease)

fibrinogen

fibrin

XIII → XIII_a

stable fibrin clot

Extrinsic

tissue damage

TF:VII_a

Common

XII – Hageman factor, a serine protease
XI – Plasma thromboplastin, antecedent serine protease
IX – Christmas factor, serine protease
VII – Stable factor, serine protease
XIII – Fibrin stabilising factor, a transglutaminase
PL – Platelet membrane phospholipid
Ca^{++} – Calcium ions
TF – Tissue Factor

(\_a = active form)
Heparin

- Sodium, Lithium or Ammonium salts
- Binds to antithrombin to cause a conformational change
  - Interferes with binding to coagulation factors XIIa, Xia, X, and Thrombin
- Anticoagulant of choice for electrolytes, pH, blood gases, ionized calcium
Coagulation Cascade

**Intrinsic**
- XII → XIIa
- XI → XIa
- IX → IXa
- IX (VIII, PL, Ca++) → X (Xa)

**Extrinsic**
- TF: VIIa
- Tissue damage

**Common**
- thrombin (serine protease)
- fibrinogen → fibrin
- XIIa → stable fibrin clot

XII – Hageman factor, a serine protease
XI – Plasma thromboplastin, antecedent serine protease
IX – Christmas factor, serine protease
VII – Stable factor, serine protease
XIII – Fibrin stabilising factor, a transglutaminase
PL – Platelet membrane phospholipid
Ca++ – Calcium ions
TF – Tissue Factor (a = active form)
EDTA

Potassium ethylene diamine tetra-acetic acid
– K$_2$ EDTA or K$_3$ EDTA
– Chelates calcium and other ions
– Commonly used in hematology for morphological and quantitative properties of cellular components
– Use in Immunohematology for plasma and cellular testing in antigen and antibody detection
Coagulation Cascade

**Intrinsic**
- Surface contact
- XII → XIIa
- XI → XIa
- IX → IXa
- (VIII, PL, Ca++)

**Extrinsic**
- TF:VIIa
- tissue damage

**Common**
- prothrombin → thrombin
  - (serine protease)
- fibrinogen → fibrin
  - stable fibrin clot

**Factors**
- XII – Hageman factor, a serine protease
- XI – Plasma thromboplastin, antecedent serine protease
- IX – Christmas factor, serine protease
- VII – Stable factor, serine protease
- XIII – Fibrin stabilising factor, a transglutaminase
- PL – Platelet membrane phospholipid
- Ca++ – Calcium ions
- TF – Tissue Factor
  - (a = active form)
Errors in Collection Processes

- Transfer of blood from one collection tube to another
  - Mixing of anticoagulants
  - Changing anticoagulant to blood ratio
- Out of sequence order of draw
  - Sample carryover from rubber septum of needle
- Droplet transfer from syringe/transfer needle tip into fresh tube
  - Anticoagulant carryover
Impact of Sodium Citrate Collection Error

- Not suitable for Ca\(^{2+}\) testing due to chelating action of anticoagulant
- Not suitable for AST and ALP testing
- Incomplete fill volume
- Mixing two tubes for full volume
Impact of Heparin Collection Error

- May interfere with antibody – antigen reactions, decreasing the rate of reaction
  - Inhibits DNA polymerase in PCR reactions
- Insufficient quantity of blood in tube
  - Fill volume 90% of recommended volume
- Prolongation of PT, aPTT
- False increase in Na\(^+\), BUN, or Li\(^+\) testing
Impact of EDTA Collection Error

- Not suitable for Ca\(^{2+}\) testing due to action of anticoagulant
- Under filling of tubes increases EDTA concentration
- If the recommended order of draw during collection is not followed, K\(_2\)EDTA salts can be carried over
  - Adverse effect on chemistry and coagulation assays
Impact of EDTA - Coagulation

- Elongated activated partial thromboplastin time (APTT)
- Elongated prothrombin time (PT)
  - Lowers activity for Factors V, VIII
- Reduced fibrinogen level
Impact of EDTA - Chemistry

- Not suitable for Ca^{2+}, Zn^{2+}
- Not suitable for Alkaline Phosphatase and Creatine kinase enzymes
  - Chelation of enzymes within the reaction reagent
  - Not suitable for K^{+}, Zn^{2+}
Impact of EDTA – Chemistry

Due to presence of $K_2$EDTA/ $K_3$EDTA, see

- **Hyperkalemia**
  - Potassium >5.5 mmol/L
    - True increase would indicate impending cardiac arrest

- **Falsely elevated hypokalemic value**

- **Most likely due to incorrect order of draw and carry over of EDTA**

- **Identify through Delta checks, unbelievable values**
Impact of EDTA - Chemistry

- **Hypocalcemia**
  - Values <2 mmol/L

- **Hypomagnesium**
  - Values <0.7 mmol/L

- **Hypozincemia**
  - Values <11 µmol/L
Impact of EDTA Contamination

~13,000 Electrolyte Profiles
- 3.1% elevated K⁺ suggested EDTA contamination

~7300 Ca²⁺ assays
- 14.3% of hypocalcemic showed contamination

~570 Mg²⁺ assays
- 4.5% of hypomagnesemic showed contamination

~300 Zn²⁺ assays
- 1.4% hypozincemic showed contamination

92% normalized upon recollection
Impact of EDTA Contamination

UK study of 28,000 BUN/Lytes values
- 117 had K+ values >6.0 mmol/L
- 28 had EDTA concentrations >0.1 mmol/L
  • 10 reported before identifying contamination!
- 27 that were retested were normal

Concluded that 25% of hyperkalemic lab values were due to EDTA contamination
Identifying EDTA Contamination

- Delta check

- Hyperkalemia with hypocalcemia, hypernatremia
  - $>6\text{mmol/L } K^+$
  - $<0.1 \text{ mmol/L } Ca^{2+}$
  - $>155 \text{ mmol/L } Na^+$

- Assay for EDTA directly
  - Analyzer
  - Test strips
Conclusion

- Quality of testing is impaired
- Patient safety is jeopardized
- Incorrect presence of anticoagulant results in laboratory errors and repeat testing
  - Resources are wasted
Conclusion

- Tube manufacturer recommendations regarding fill volume and mixing should be followed
- Staff should be trained in proper specimen collection, tube selection and order of draw
References


CLSI Procedures for the handling and processing of blood specimens for common lab tests –Approved Guideline GP44-A4. Wayne, PA, 2010

CLSI Procedures for the collection of diagnostic blood specimens by venipuncture - Approved guideline. GP41-A6, Wayne, PA 2007


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- Kaushik, Nitin, and Green, Sol. Pre-analytical errors: Their impact and how to minimize them. MLO, 5/2014
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Image:

Coagulation Cascade. Dr Graham Beards - Own work, CC BY-SA 3.0. April 2012
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