Interferences From Blood Collection Tube(BCT) Components on Sample Quality.

Presented By

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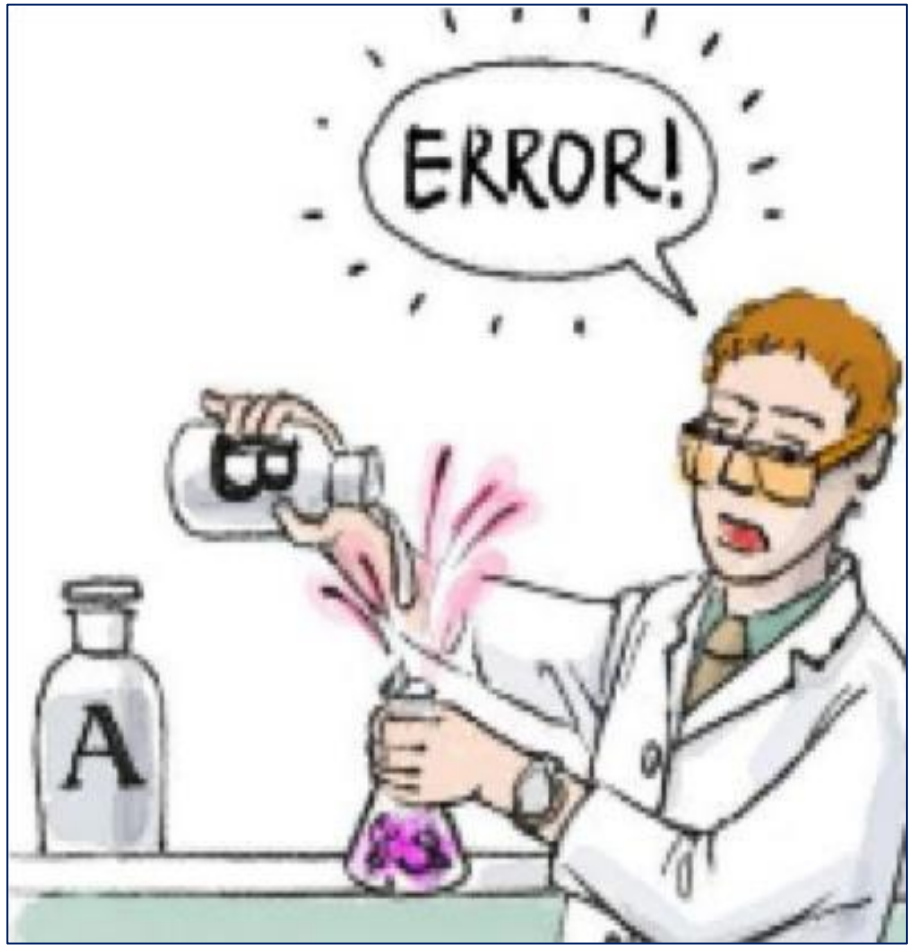
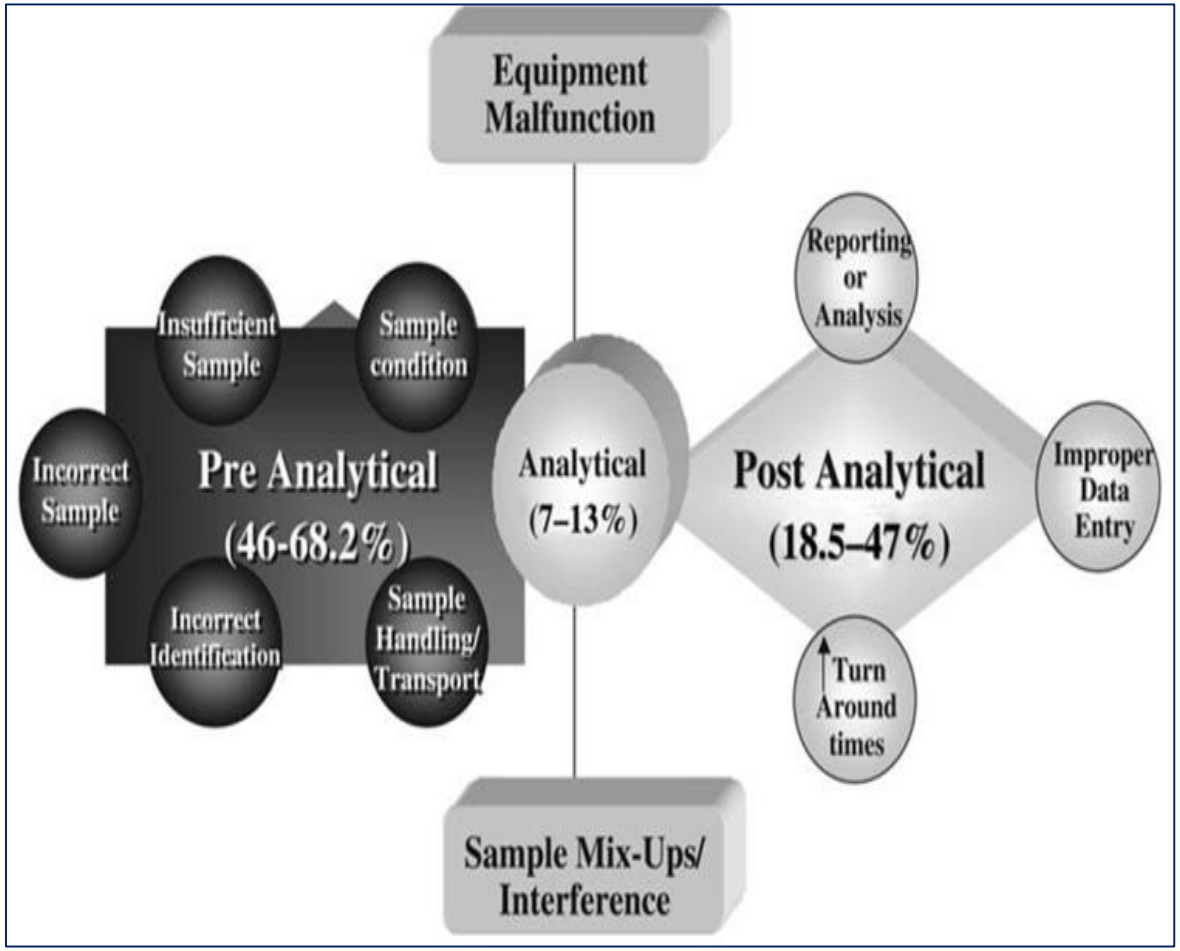
LABORATORY ERRORS





BCTs are often unrecognized/ignored variables in the pre-analytical phase of lab testing.

Interferences from blood collections tube components on clinical chemistry assays.

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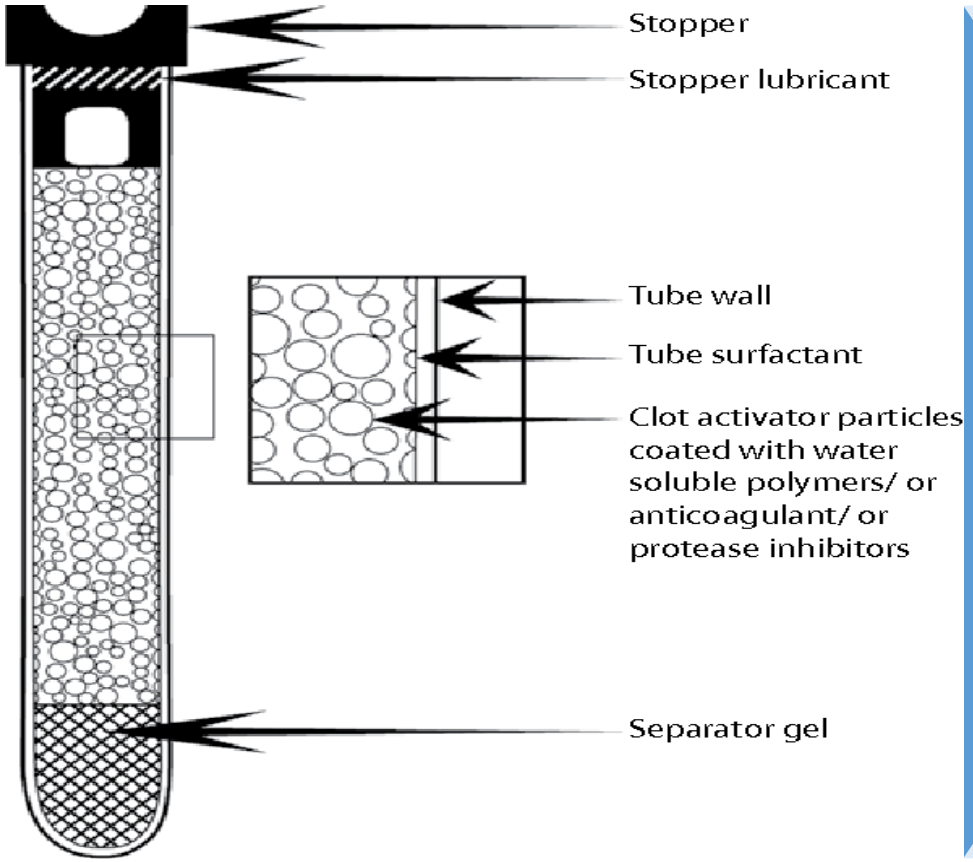
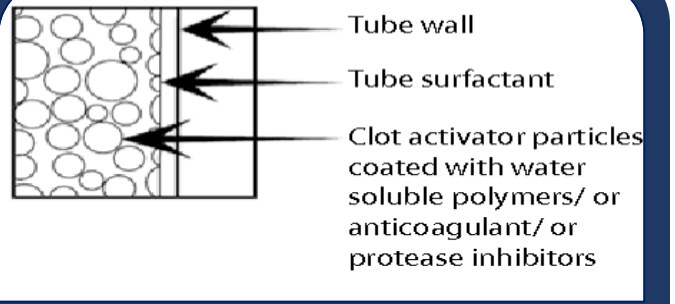


Major interferents would be:

- Tube surfactant
- Clot activator particles (CAP)
- Coatings of the CAP
  - Water soluble polymers
  - Anticoagulants
  - Protease inhibitors
- Separator gel
- Additives



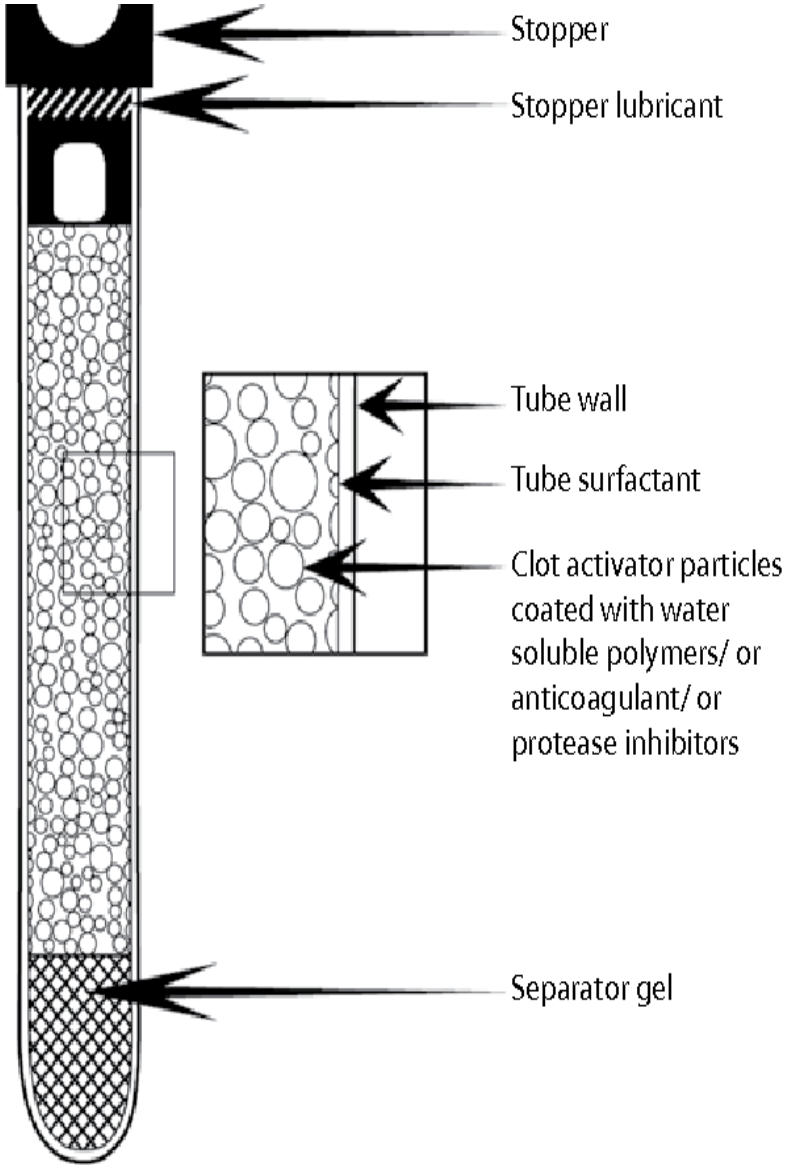
Evacuated tubes were originally made from **soda-lime or borosilicate glass,** but **soda-lime tubes** were found to release **calcium** and **magnesium** into blood specimens

↑↑ **Calcium and Magnesium**

GLASS VERSUS PLASTIC TUBES – OSHA guidelines

Plastic tubes replaced most glass tubes following the establishment of the Occupational Safety and Health Administration (OSHA) guidelines to improve safety and reduce exposure to blood-borne pathogens.

- Has less exposure to biohazardous material following breakage
- Is more easily disposed of by incineration at a lower cost
- Has excellent dimensional precision
- Has a greater shock resistance
- PET tubes have double-walls to minimize evaporation, especially for coagulation-based tests
- Weighs less
- PET(Polyethylene Terephthalate) is unbreakable and maintains a vacuum for a prolonged time
- Tolerates higher centrifugation speeds
- The PP(Polyethylene and polypropylene) plus PET combination improves shelf life and anticoagulant volume retention



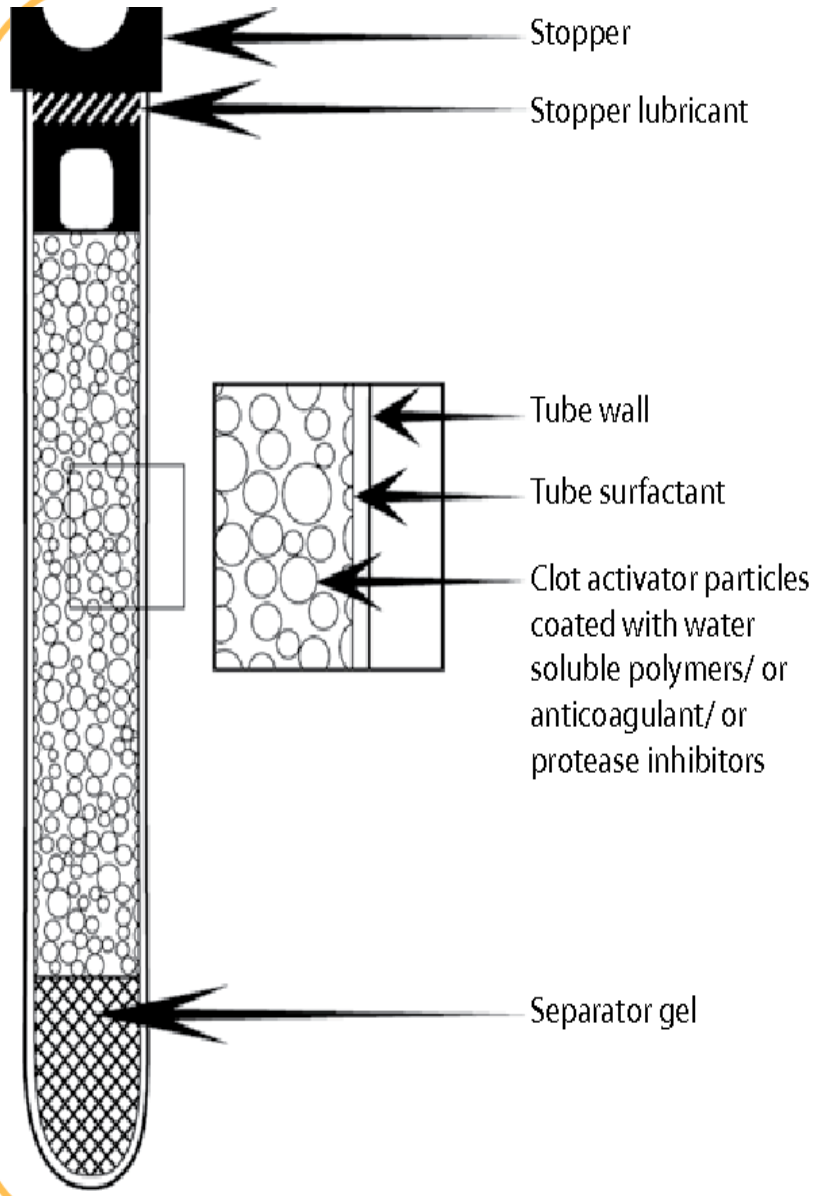
Rubber stoppers are routinely color-coded according to anticoagulant type and the presence of a separator gel.

Stopper Properties

- The stopper should be readily penetrated by a needle and self-seal upon needle removal, maintaining the internal pressure differential.
- Materials : Butyl rubber, a copolymer of isobutylene and isoprene, and halogenated butyl rubber are commonly used materials ;
- Butyl rubber exhibits superior air and moisture impermeability, superior resistance to chemical attack and heat resistance, and good processability.

INTERFERENCES DUE TO STOPPER

- Tris-(2-butoxyethyl)-phosphate (TBEP), which is used to make the stopper soft, displaces certain drugs from plasma-protein binding sites, such as the α1-acid glycoprotein, resulting in increased drug uptake by red blood cells (RBCs), thus artificially lowering serum or plasma levels.
- TBEP has been reported to *alter the drug distribution* of quinidine, propranolol, lidocaine, tricyclic antidepressants, and several phenothiazine drugs, including fluphenazine and chlorpromazine.
- Therefore, most stoppers are manufactured with *low-extractable rubber or have been modified to minimize leaching into the blood specimens* . The complete filling of BCTs dilutes any leached material and helps reduce the effects .
- Further, specimens in tubes with rubber stoppers should be stored at low temperatures (2 °C to 8°C) and in the upright position to minimize leaching.



Stopper Lubricant Properties

- Lubricants, such as silicone oils, fluids, and glycerol, facilitate the insertion and removal of stoppers
- Lubricants minimize red blood cell and clot adherence to stoppers to prevent serum or plasma contamination

Stopper Lubricant Interferences

- Glycerol should not be used to lubricate stoppers used for specimens measuring glycerol or triglyceride when a non-glycerol blank assay is used.
- Siliconized stoppers are generally preferred because they are less likely to interfere with assays
- **BUT**....they may also *falsely elevate ionized magnesium and total triiodothyronine levels* and may *confound peaks during mass spectrometry (MS) analysis and peak interpretation.*

ANTICOAGULANTS

- Ethylenediaminetetraacetic acid (EDTA), heparin, and citrate are the most commonly used anticoagulants.
- **EDTA** – Potassium EDTA , an anticoagulant and chelating agent, interferes with calcium assays and clot generation , but it is preferred for hematology testing.
- Interferences : EDTA binds the *metallic ions europium* (immunoassay reagent), *zinc*, and *magnesium* (enzyme cofactors for immunoassay reagents such as alkaline phosphatase) .

Insufficient sample volumes produce relatively elevated EDTA levels, which can increase the chelation of magnesium and zinc, and can then affect reagent enzymes used for signal generation, such as alkaline phosphatase.

Reagent antibodies recognize divalent cation complex binding sites on proteins; thus, decreased calcium and magnesium levels may induce conformational changes that decrease antibody binding

INTERFERENCE DUE TO K2EDTA IN BCTs

- Patient X – 45/F
- RFT /LFT report
- BUN 15 mg/dL
- Creatinine 0.7 mg/dL
- Calcium 6.0 mg/dL
- Phos 4.1 mg/dL
- Sodium 138 mEq/L
- Potassium 5.9 mEq/L
- Chloride 110 mEq/L
- Magnesium 0.8 mg/dL
- Bil T 0.8 mg/dL
- Bil D 0.1 mg/dL
- T Prot 6.4 g/dL
- Albu 4.0 g/dL
- AST 24 IU/L
- ALT 18 IU/L
- ALP 21 IU/L

History / Examination were normal

Routine check of blood container

- K_2EDTA chelates calcium & magnesium which give ↓ results
- Potassium gives a ↑ result
- ALP is also inhibited leading to a ↓ result

FUNCTIONS AND INTERFERENCE DUE TO Na/Li-HEPARIN IN BCTs

FUNCTION:
Heparin complexes with and induces a conformational change of antithrombin III to accelerate the inhibition of thrombin and Factor Xa , which prevents thrombin activation and the generation of fibrin from fibrinogen.

↓ Albumin
Due to inhibition of the binding of bromocresol green to albumin

Incomplete filling of lithium heparin tubes produced significantly higher creatine kinase and γ-glutamyltransferase activity on a particular Chemistry analyzer.

Exogenously administered heparin alters serum thyroid hormone levels

To be avoided in cryoprotein investigations since it precipitates cryofibrinogen

FUNCTIONS /INTERFERENCE DUE TO Na/Li-HEPARIN IN BCTs (contd)

May produce negative anion gaps due to heparin interference with chloride electrode membranes (unpublished observation).

Slows some antibody-antigen reaction rates , particularly during the precipitation step in second-antibody systems

Solution:

- Dry preparations of electrolyte-balanced heparin
- Low heparin dispersed in a soluble inert web
- Blended lithium–zinc heparin

FUNCTIONS AND INTERFERENCE DUE TO SODIUM CITRATE IN BCTs

FUNCTIONS:
Sodium citrate in acid citrate dextrose and citrate theophylline adenosine dipyrridamole, inhibits platelet activation and is used to measure plasma levels of platelet-derived components.
Trisodium citrate in a 3.2% (109 mmol/L) or 3.8% (129 mmol/L) solution is preferred

INTERFERENCE:
Inhibit both aspartate aminotransferase and alkaline phosphatase by the chelation of cations

INTERFERENCE DUE TO NaF & K OXALATE IN BCTs

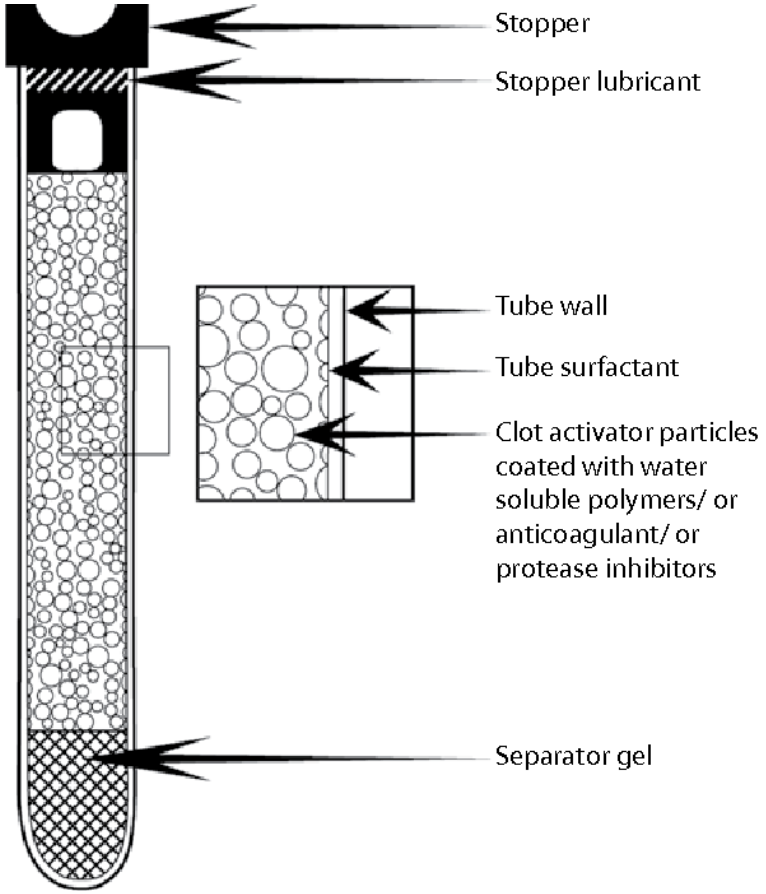
K-Oxalate can decrease hematocrits by as much as 10% by drawing water from cells into plasma and can also inhibit several enzymes, such an amylase, lactate dehydrogenase, and acid and alkaline phosphatase

Na fluoride may be unsuitable for enzymatic immunoassays because of its enzyme inhibitory activity

Fluoride may also interfere with electrolyte measurements by altering cell membrane permeability and promoting hemolysis by red blood cell ATP with subsequent potassium efflux ..↑ K^+

Na-F inhibits glycolysis, but not completely.
It inhibits enolase but not the other upstream enzymes. So in fluoridated, non-separated blood samples, glucose is still metabolized at approximately 5% to 7% per hour at room temperature.
ADA proposal: BCT with EDTA and fluoride in a citrate buffer (pH < 5.9) - immediate inhibition of glycolysis

Separator Gels Properties and Interferences



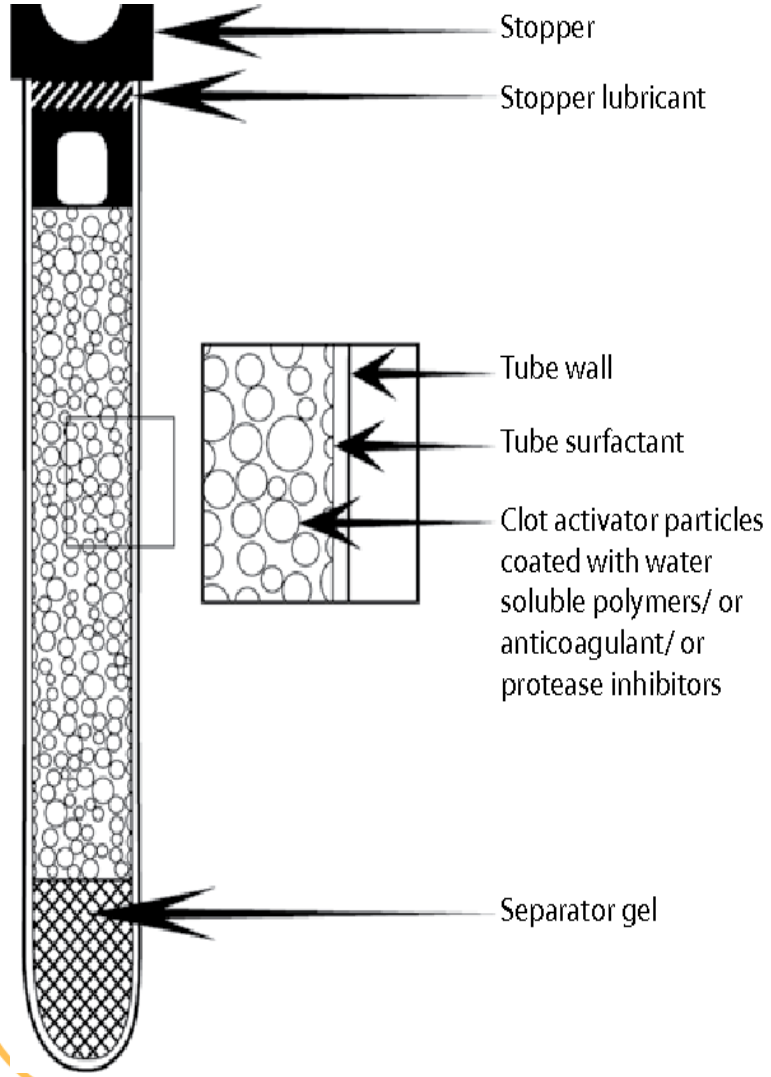
Properties

- Separator gels are used to separate serum from clotted whole blood plasma from cells.

Advantages

- easy to use,
- require short processing times,
- yield higher serum levels,
- require only one centrifugation step,
- allow primary tube sampling, and
- require a single label

Separator Gels Properties and Interferences



Properties

The position of the gel after centrifugation is influenced by many tube characteristics, such as *specific gravity*, *yield stress*, *viscosity*, *density*, and *tube material*. It can also be affected by **temperature, centrifugation speed, acceleration and deceleration, storage, and patient factors, such as heparin therapy, low hematocrit, elevated plasma protein, and serum/plasma specific gravity**

Interferences :

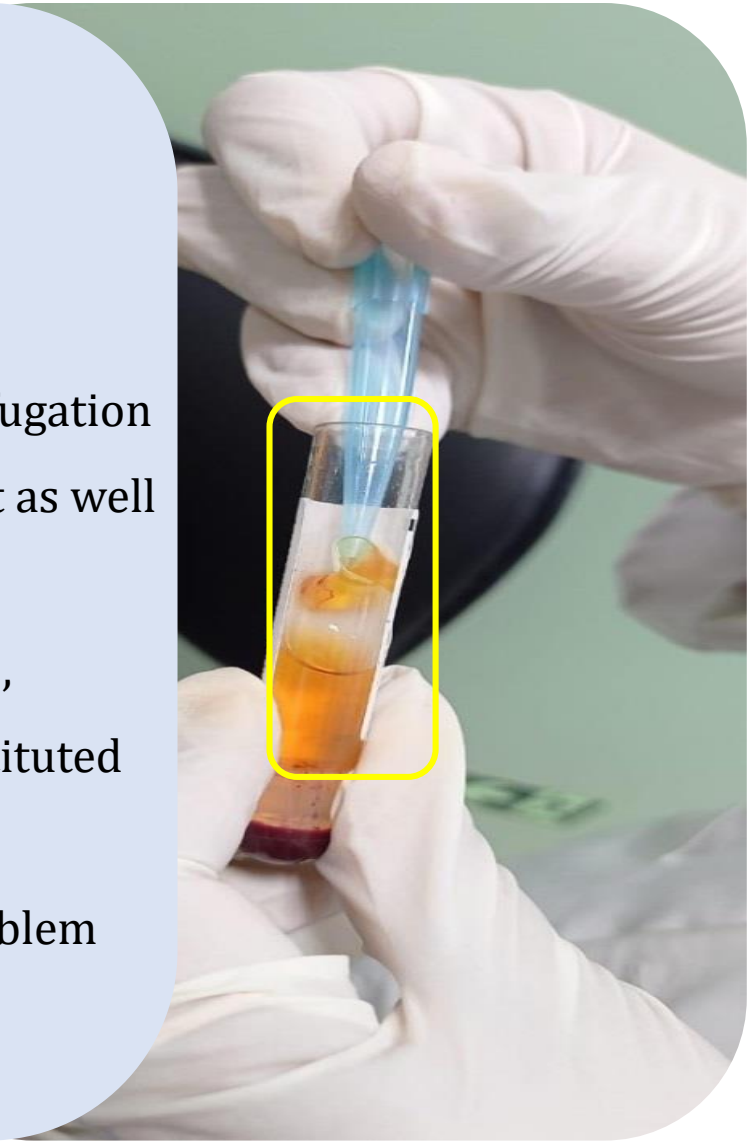
- Several reports of gels affecting analyte concentrations have been published. Hydrophobic drugs, such as phenytoin, phenobarbitol, carbamazepine, quinidine, and lidocaine, can adsorb onto hydrophobic separator gels and lead to a decrease in serum drug concentrations by as much as 20% to 50% after 24 hours at 4 °C.
- Organochlorine, polychlorinated biphenyl, and progesterone levels may also be significantly reduced.

Newer separator gels (e.g. polydimethylsiloxanepolyethylene oxide copolymers) that minimize drug and analyte adsorption have been developed

Separator gels may also release materials (e.g., gel pieces and silicone oil) into the specimens and spuriously interfere with assays, sample probes, systems, and tubes and cuvettes, solid-phase immunoassay electrode surfaces; *the rate of degradation and release may be increased by improper storage or extreme temperatures.*

A FEW YEARS AGO

- During peak of summer
- Gel would fragment on centrifugation
- CAPA revealed cold chain fault as well as inappropriate gel
- To cater to the observed faults, corrective measures were instituted in the manufacture of BCTs
- However, we still face this problem sometimes



SOME EXAMPLES

Patient 1: 57/M

T Prot 18.7 mg/dL

Patient 2: 62/M

T Prot 16.8 mg/dL

Patient 3 : 65/M

T Prot 9.69 mg/dL

Ideally, separator gels should maintain uniform chemical and physical properties for the intended period of use and be inert to the specimens collected in BCTs.

When silica and silicated clot activators are sprayed onto plastic tubes

INTERFERENCE

RELEASE OF

- Pro MMPs
- Active MMPs
- Complexed MMPs

Optimal amounts and composition of clot activators and water-soluble agents must be added to different types and sizes of BCTs in order for these substances to function properly without adversely affecting the quality of the blood specimens and test results.

ANALYTES AFFECTED

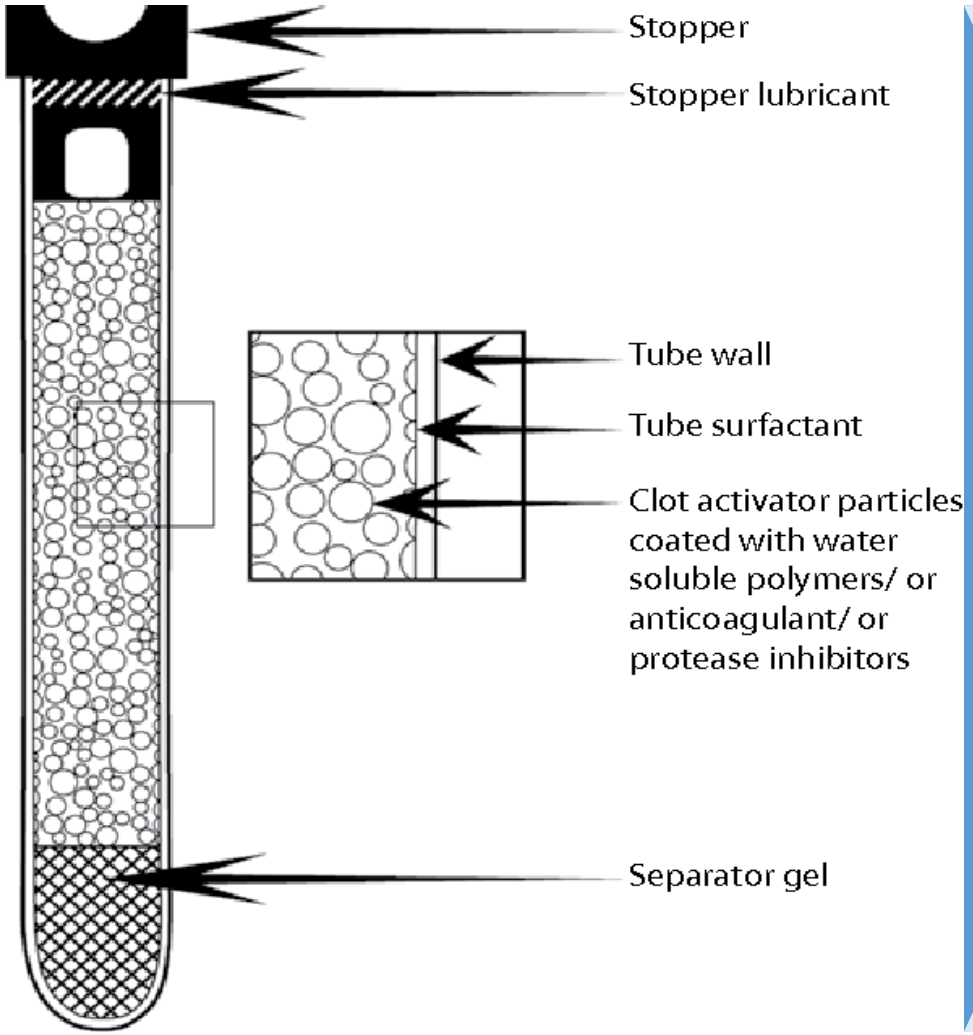
- Ficolin-1/Ficolin-2 levels and their binding capacities
- ↑ Testosterone

When mixing is improper – there is inappropriate pelleting of the clotting activators with the clot

INTERFERENCE

- pipetting device accuracy
- solid-phase binding in immunoassays

FUNCTION OF THE SURFACTANT



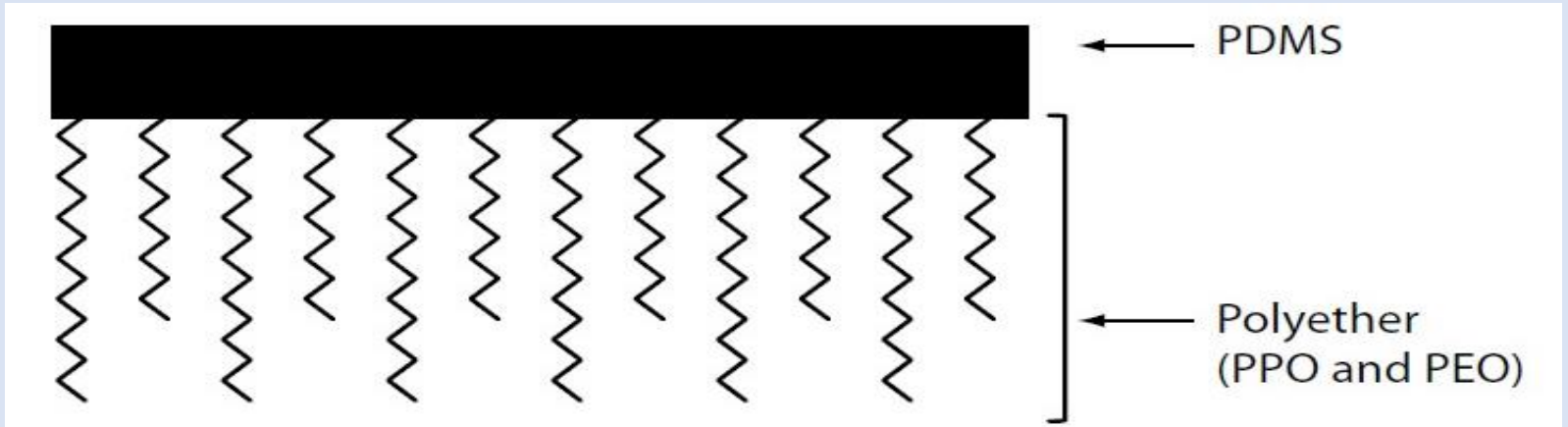
Major role of surfactants(SFs) are:

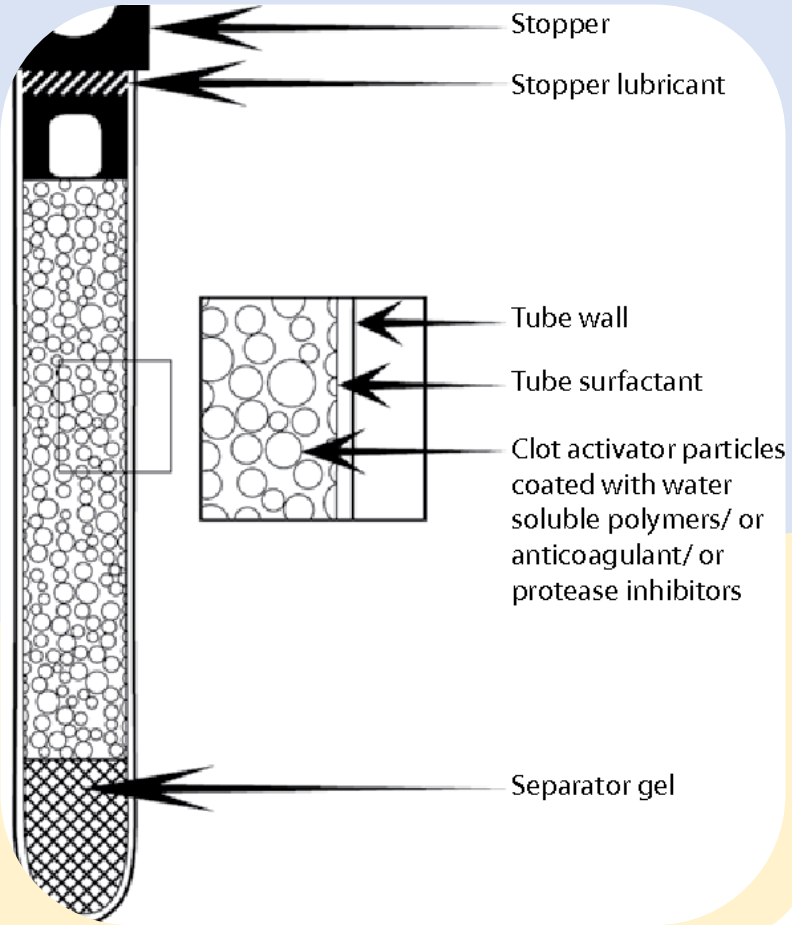
improve blood flow,

distribute clot activator, and

prevent proteins, RBCs, and platelets from adsorbing to tube walls .

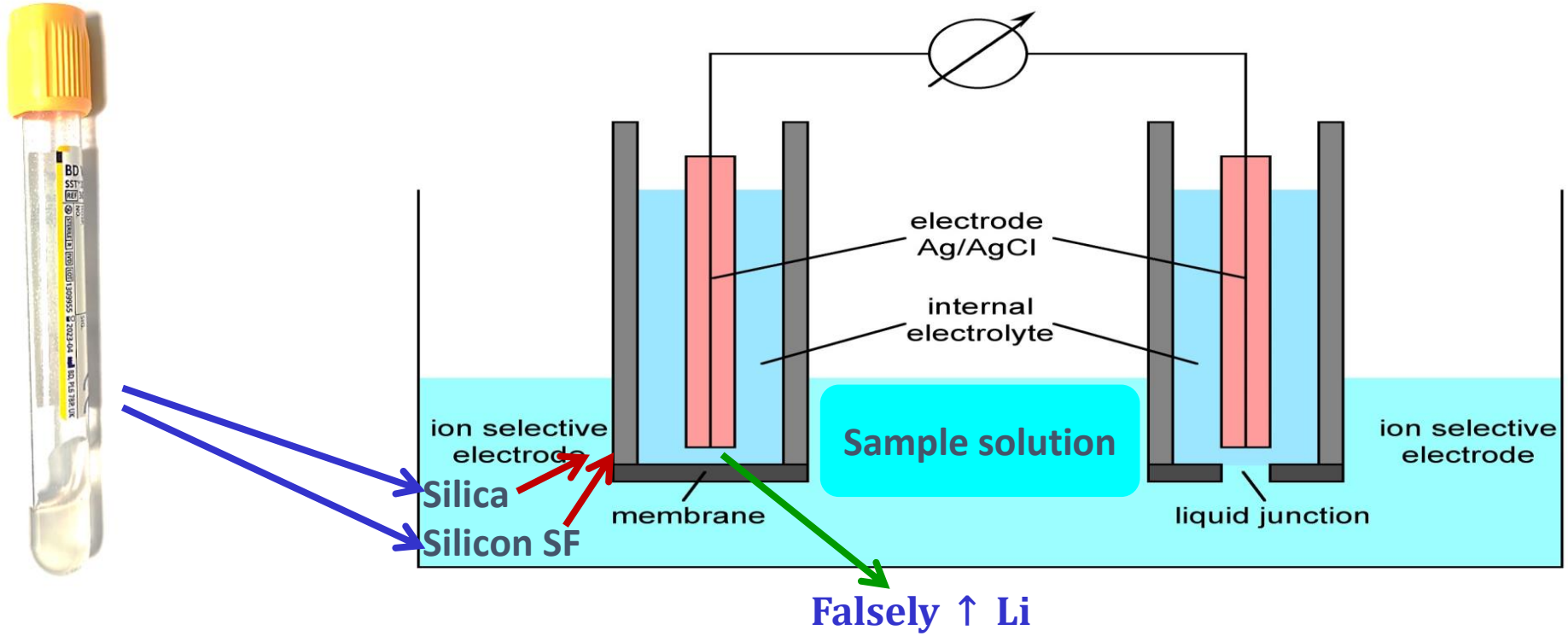
INTERFERENCES DUE TO SURFACTANT IN BCTs





- Alterations in **T_3, Vitamin B_{12}, Ca 15-3** , etc
- Additional peaks in **Mass Spectrometry**, interference in interpretation in low mol wt spectrum (1000-3000 m/z range), e.g. MALDI, SELDI

INTERFERENCES DUE TO SURFACTANT IN BCTs



Silicone SF-coated tubes have been shown to interfere with ion-specific electrode measurement of ionized magnesium and lithium. They seem to interact with ion-specific electrode membranes to increase the measured voltage during magnesium and lithium determinations. In aaddition, water-soluble silicone polymer coatings in separator tubes can physically mask antibodies and alter avidin-biotin binding reactions in immunoradiometric assays.

Large reference lab (RL), uses a brand X BCT

Gets complaints about serum creatinine not matching with clinical parameters

Root Cause Analysis reveals two things:

- Issue reported only in samples received from referring labs
- And ***only those referring labs using different BCT brands*** which were not evaluated by the RL

CAPA: Corrective Action and Preventive Action:
Referring labs should also consider quality of tubes used by referral labs.

Large RL, uses a brand X BCT

Same BCTs supplied to all referring labs

After 2 years- from one location, starts receiving complaints about serum electrolytes

RCA: IQC/EQAS/Laboratory processes were found adequate

Vendor of BCT in the location contacted- vendor refused evaluation of BCT, stating no problems received from any other lab

Same BCTs supplied to all referring labs

Due to loss of business, vendor evaluated BCT and found a problem with recent lot of BCT supplied to that location

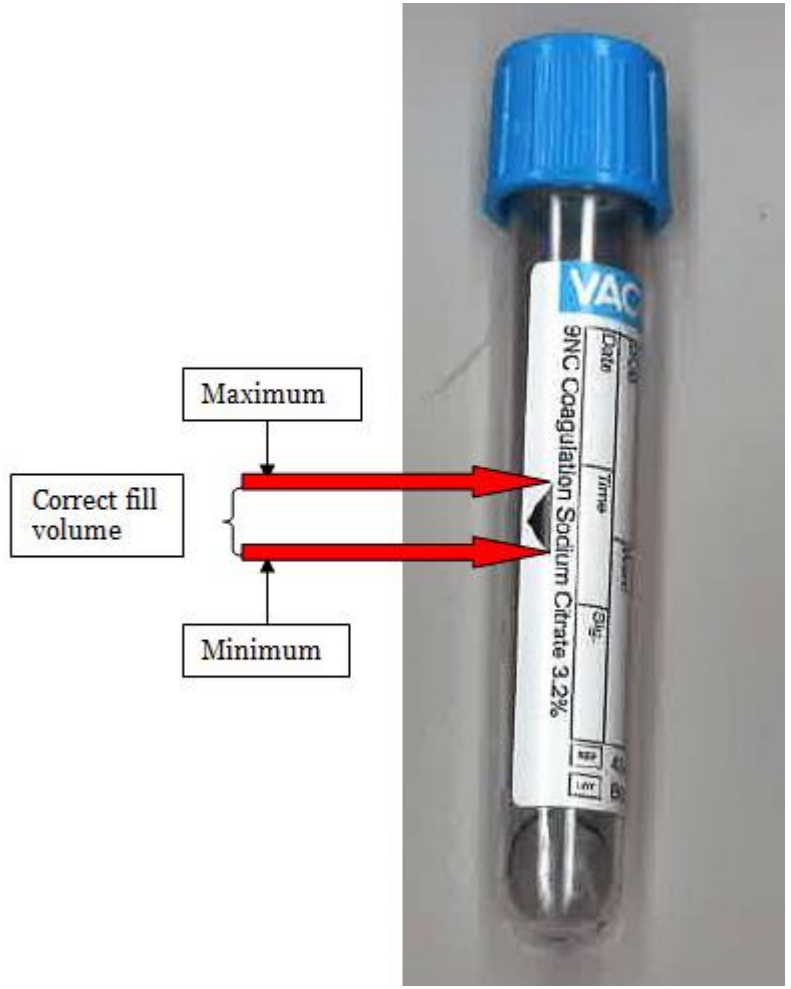
CAPA: Encourage vendors that support science and quality and have people to offer application support

Lab shifted to a new brand of BCTs for coagulation assays

Gets complaints about unusually **prolonged PT APTT assays** from cardiologists and hematologists.

RCA: New BCTs had recommended citrate levels in them, but samples **did not fill up to the mark- 1:9 ratio of citrate: blood not maintained**

CAPA: Vacuum defect evaluation and correction in the BCTs



A large RL decides to go for TLA

BCTs not changed as they were in use for last 7 years without any significant issues

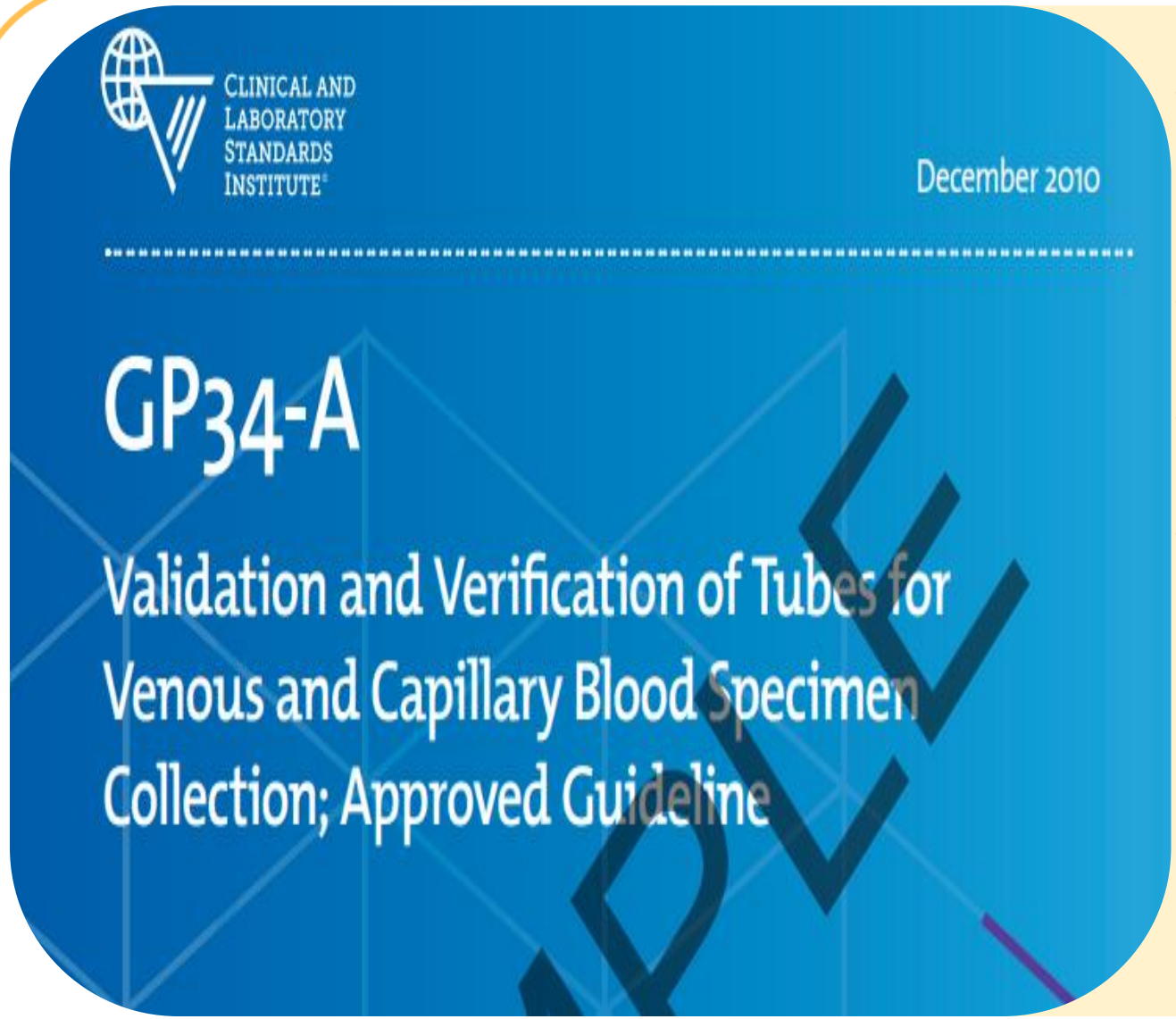
After a week- clinician complaints regarding CBC and some chemistry reports

RCA: Extensive work-up- no obvious flaw at any stage of the process

One lab personnel ***suggests calling representative from BCT manufacturer***

BCT manufacturer revealed that the ***piercing mechanism of the TLA*** platform was the cause of the problem, with **latex particles from the BCT cap accumulating in the samples during cap piercing**

Conclusion: Labs should also consider the potential interference from the hardware components of total automation.



- Blood collection device problems may go unnoticed by laboratorians since routine quality control (QC) practice typically does not assess all aspects of laboratory testing from blood collection, including specimen processing, and test reporting .
- Proficiency testing programs, which do not require blood collection, also fail to detect blood collection device problems.
- QC and proficiency testing specimens in clinical laboratories are analyzed but not processed as patient specimens are.
- When laboratorians change the tubes they use, they should also perform a comparative tube evaluation ; this tube comparison study should be similar to the one described in **CLSI GP34-A guideline.**

TAKE HOME MESSAGE

- ✓ Every lab should identify which BCTs they use and tally it with the analytes they estimate with reference to potential problems.
- ✓ Any possible interferences must be noted
- ✓ Lab personnel/phlebotomist/nursing staff should be trained and specifically instructed about possible BCT-related interferences which can adversely
  - influence patient outcomes,
  - decrease laboratory efficiency,
  - delay test results, and
  - increase the cost per test due to recollection and retesting.
- ✓ All samples should be drawn in the recommended BCTs with the recommended volume of blood/sample to minimise potential errors.
- ✓ Wherever possible, validation of interferences must be done in the lab and documented.


THANK YOU!