\\\CHAPTER THREE

ANTICOAGULANTS

Learning objectives

At the end of this chapter, the student shall be able to:

- Define anticoagulants
- Describe the proportion, mechanism of anticoagulation and advantages of EDTA, Trisodium citrate, double oxalates and heparin anticoagulants.
- Prepare the different anticoagulants in the right concentration

Introduction

Anticoagulants are chemical substances that are added to blood to prevent coagulation. In other words, certain
steps are involved in blood coagulation, but if one of the factors is removed or inactivated, the coagulation
reaction will not take place. The substances responsible for this removal or inactivation are called anticoagulants. While clotted blood is desirable for certain laboratory investigations, most hematology procedures require an anticoagulated whole blood.

For various purposes, a number of different anticoagulants are available. EDTA and sodium citrate remove calcium which is essential for coagulation. Calcium is either precipitated as insoluble oxalate (crystals of which may be seen in oxalated blood) or bound in a non-ionized form. Heparin works in a different way; it neutralizes thrombin by inhibiting the interaction of several clotting factors in the presence of a plasma cofactor, antithrombin III. Sodium citrate or heparin can be used to render blood incoagulable before transfusion. For better long-term preservation of red cells for certain tests and for transfusion purposes, citrate is used in combination with dextrose in the form of acid-citrate-dextrose (ACD), citrate-phosphatedextrose (CPD) or Alserver's solution.

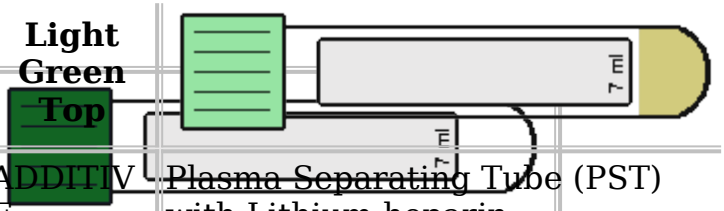
COLLECTION TUBES FOR PHLEBOTOMY

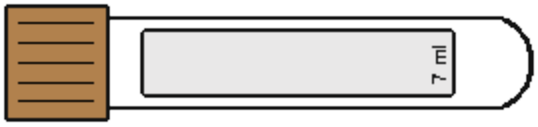
Red Top	
ADDITIVE	None
MODE OF ACTION	Blood clots, and the serum is separated by centrifugation
USES	Chemistries, Immunology and Serology, Blood Bank (Crossmatch)

Gold Top	
ADDITIVE	None
MODE OF ACTION	Serum separator tube (SST) contains a gel at the bottom to separate blood from serum on centrifugation
USES	Chemistries, Immunology and Serology

3.1. Heparin

Heparin is an excellent natural anticoagulant extracted from mammalian liver or pancreas. It is more expensive than the artificial ones and has a temporary effect of only 24 hours. Heparin prevents clotting by inactivating thrombin, thus preventing conversion of fibrinogen to fibrin. It is the best anticoagulant when absolute minimal hemolysis is required (e.g., osmotic fragility test and hematocrit determination). It is unsatisfactory for leucocyte and platelet and leucocyte counts as it causes cell clumping and also for blood film preparation since it causes a troublesome diffuse blue background in Wright-stained smears. It is used in the proportion of 0.1-0.2mg of the dry salt for 1ml of blood.
This is an enzyme based anticoagulant.

Green Top	**Light Green Top**	
ADDITIVE	Sodium heparin or lithium heparin	Plasma Separating Tube (PST) with Lithium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin	Anti coagulates with lithium heparin; Plasma is separated with PST gel at the bottom of the tube
USES	For lithium level, use sodium heparin For ammonia level, use sodium or lithium heparin	Chemistries

Light Brown Top	
ADDITIVE	Sodium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin; contains virtually no lead
USES	Serum lead determination

3.2. Ethylenediamine tetra-acetic acid

Ethylenediamine tetraacetic acid (EDTA) has become the standard hematology anticoagulant because of its very efficient and complete anticoagulation and its lack of effect on the size (morphology) or number of blood cells in the specimen. Its disodium or tripotassium salt are used. The anticoagulant recommended by the ICSH is the dipotassium salt. It is the preferred anticoagulant for cell counts and morphological studies. It is especially the anticoagulant of choice for platelet counts and platelet function tests since it prevents platelet aggregation. It exerts its effect by tightly binding (chelating) ionic calcium thus effectively blocking coagulation. The dilithium salt of EDTA is equally effective as an anticoagulant, and its use has the advantage that the same sample of blood can be used for chemical investigation.

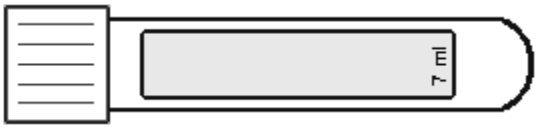
The amount of EDTA necessary for the complete chelation of Calcium is balanced with the desire to
minimize cellular damage so that standardizing bodies have recommended a concentration of 1.5±0.25mg of Na2 or K3 EDTA per 1ml of blood (e.g. 0.02ml of 10% (W/V) solution of K3EDTA is used for 1ml of blood). This concentration does not appear to adversely affect any of the erythrocyte or leucocyte parameters.

Purple Top	
ADDITIVE	EDTA
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Hematology (CBC) and Blood Bank (Crossmatch); requires **full draw** - invert 8 times to prevent

	clotting and platelet clumping

Pink Top	
ADDITIVE	**Potassium EDTA**
MODE OF ACTION	**Forms calcium salts**
USES	**Immunohematology**

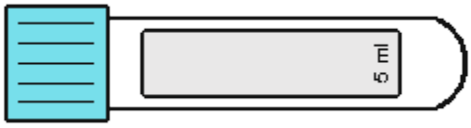
Dark Blue Top	
ADDITIVE	EDTA-
MODE OF ACTION	Tube is designed to contain no contaminating metals
USES	Trace element testing (zinc, copper, lead, mercury) and toxicology

White Top	
ADDITIVE	Potassium EDTA
MODE OF ACTION	Forms calcium salts
USES	Molecular/PCR and bDNA testing

3.3 Trisodium Citrate

Sodium citrate combines with calcium, thereby preventing the conversion of prothrombin to thrombin, and coagulation does not occur. 100-120 mmol/l trisodium citrate (32g/l) is the anticoagulant of choice in coagulation studies. Nine volumes of blood are added to 1 volume of the sodium citrate solution and immediately well

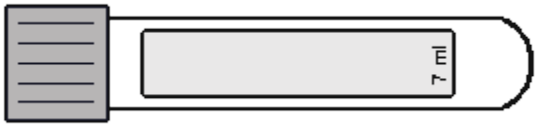
mixed with it. Sodium citrate is also the anticoagulant for the erythrocyte sedimentation rate (ESR); for this, 4 volumes of venous blood are diluted with 1 volume of the sodium citrate solution.

Light Blue Top	
ADDITIVE	Sodium citrate
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Coagulation tests (protime and prothrombin time), **full draw** required

Black Top	
ADDITIVE	Sodium citrate (buffered)
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Westergren Sedimentation Rate; requires **full draw**

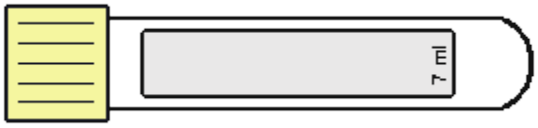
3.4. Balanced or double oxalate

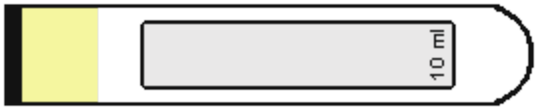
Salts of oxalic acid by virtue of their ability to bind and precipitate calcium as calcium oxalate serve as suitable Anti-coagulants for many hematologic investigations. Three parts of ammonium oxalate is balanced with two parts of potassium oxalate (neither salt is suitable by itself, i.e., ammonium oxalate causes cellular swelling and potassium oxalate causes erythrocyte shrinkage). It is used in the proportion of 1-2mg/ml of blood. Or fluoride oxalate

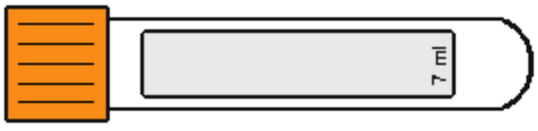
Light Gray Top	
ADDITIV	Sodium fluoride and potassium

E	oxalate
MODE OF ACTION	Antiglycolytic agent preserves glucose up to 5 days
USES	Glucoses, requires **full draw** (may cause hemolysis if short draw)

Additional Vacuutainers

Yellow Top	
ADDITIVE	ACD (acid-citrate-dextrose)
MODE OF ACTION	Complement inactivation
USES	HLA tissue typing, paternity testing, DNA studies

Yellow - Black Top	
ADDITIVE	Broth mixture
MODE OF ACTION	Preserves viability of microorganisms
USES	Microbiology - aerobes, anaerobes, fungi

Orange Top	
ADDITIV	Thrombin

E	
MODE OF ACTION	Quickly clots blood
USES	STAT serum chemistries

Review Questions

1. Define anticoagulant.
2. List the anticoagulants that are commonly used in hematology. How does each of these anticoagulants exert their functions?
3. Write the proportion of the volume of blood to the volume of each if these anticoagulants