

ANTICOAGULANTS USED IN HAEMATOLOGY

DEFINITION

- Anticoagulant is an agent that is used to prevent the formation of blood clots.

Anticoagulants have various uses.



1. Some of them occur naturally in blood-eating animals such as leeches and mosquitoes,
2. Some are used for the prevention or treatment of disorders characterized by abnormal blood clots and emboli.

CHARACTERISTICS OF ANTICOAGULANTS



An anticoagulant selected for use in hematological examination must have the following qualities:

1. It must not alter the size of the cell
2. It must not cause hemolysis
3. It must minimize platelet aggregation
4. It must minimize disruption of staining and morphology of leukocytes
5. It must be readily soluble in water
6. It should be soluble in blood
7. It must be keep the blood in fluid condition


COLOR CODE TUBE SELECTION OF ANTICOAGULANTS COMMONLY USED

Stopper color	Additive	Notes
<p style="color: red; text-align: center;">Red</p> 	No additive	<ul style="list-style-type: none"> •Used for blood bank, some biochemistry Invst. •Collection of serum •10-15 min is required to allow blood to clot before centrifugation
<p style="color: purple; text-align: center;">Lavender (purple)</p> 	EDTA	<ul style="list-style-type: none"> •Collection of whole blood (binds calcium)

COLOR CODE TUBE SELECTION OF ANTICOAGULANTS COMMONLY USED

Stopper color	Additive	Notes
<p>Green</p> 	Sodium or lithium heparin	<ul style="list-style-type: none">•Inhibits thrombin activation.• chemistry studies
<p>Light blue</p> 	Sodium citrate	<ul style="list-style-type: none">•Coagulation studies (bind calcium) (PT &PTT) (ESR).

COLOR CODE TUBE SELECTION OF ANTICOAGULANTS COMMONLY USED

Stopper color	Additive	Notes
	<ul style="list-style-type: none">• Sodium fluoride & potassium oxalate: inhibits enolase (phosphopyruvate dehydrogenase)• Sodium iodoacetate: inhibits glucose-3-phosphate dehydrogenase	<ul style="list-style-type: none">• For glucose determination in chemistry (stabilize glucose in plasma)
	<ul style="list-style-type: none">• Acid citrate dextrose (ACD)	<ul style="list-style-type: none">• For use in blood bank studies, HLA phenotyping, DNA and paternity testing (preserves red cells)

CLASSIFICATION OF ANTICOAGULANTS

ANTICOAGULANTS

1- CALCIUM CHELATORS

A- OXALATES

- AMMONIUM OXALATE
- POTASSIUM OXALATE
- DOUBLE OXALATE

B-EDTA

C- CITRATES

- SODIUM CITRATE
- ACD (ACID CITRATE DEXTROSE)

2-NON CALCIUM CHELATORS

A - SODIUM HEPARIN

B - WARFARIN

COMMONLY USED ANTICOAGULANTS

- EDTA
- OXALATE
- SODIUM HEPARIN
- SODIUM CITRATE
- SODIUM FLUORIDE & POTASSIUM OXALATE

EDTA

(ETHYLENE DIAMINE TETRA ACETIC ACID)

- EDTA is the most frequently used anticoagulant , also known as Sequestrene or Versenate.
- It is an amino carboxylic acid and a colorless, water-soluble solid.

Types/ Forms of EDTA :

Routinely used are

- I. Tri potassium salts...EDTA (K₃ EDTA)
- II. Di sodium EDTA (Na₂ EDTA)

Mode of Action :

- It forms insoluble calcium salts by chelation.

Concentration :

- 0.5 – 2.0 mg EDTA per/ ml of blood will preserve blood excellently for at least 6 hrs.

EDTA

(ETHYLENE DIAMINE TETRA ACETIC ACID)

Advantages :

- Making a blood smear for cell morphology studies.
- used for Tests for CBC, microfilaria, coombs test.
- EDTA preserves the staining and morphology of Leukocytes

EDTA

(ETHYLENE DIAMINE TETRA ACETIC ACID)

- **Disadvantages :**
- Excessive conc% of EDTA will cause shrinkage of RBC's and erroneous PCV, MCV, and MCHC results.
- EDTA interferes with blood chemistry tests as follows
Falsely decreases alkaline phosphates by binding Mg^{++}
- Decreases CO_2 combining power of blood.
- Interferes with jaffes reaction for creatinine test
- Decreases or alters Na^+ , K^+ , and Ca^{2++} con % in plasma

OXALATES

Mode of Action :

These acts by chelating calcium. Calcium oxalate is formed as insoluble precipitate.

These are used for blood chemistry and hematocrit.

Types :

- A. Potassium oxalate
- B. Ammonium oxalate
- C. Double oxalate

POTASSIUM OXALATE

Concentration :

This is used at conc.% of 2 mg/ml of blood .

This anticoagulants is most often used for chemical analysis.

Disadvantages :

Potassium oxalate shrinks the RBC, about an 8% shrinkage in the PCV and therefore it is not recommended for use with blood for PCV and ESR.

DOUBLE OXALATES

POTASSIUM OXALATE & AMMONIUM OXALATE

- Double oxalates is used for ESR and HCT

Concentration :

- Potassium oxalate and ammonium oxalate are used together in a ratio 2:3, this is done to counter the swelling effect of ammonium oxalate and shrinkage effect of potassium oxalate on the RBC.

Advantages :

- Double oxalates can be used for ...
- A. HB
- B. TLC
- C. RBC count
- D. ESR by Wintrob's method

DOUBLE OXALATES

DISADVANTAGES:

- LEUKOCYTE MORPHOLOGY IS NOT WELL PRESERVED
- HENCE NOT SUITABLE FOR P/S STUDIES.
- THE CALCIUM CHELATED IS PRECIPITATED IN CALCIUM OXALATE WHICH IS A TOXIC SUBSTANCE.
- IT IS NEVER TO BE USED FOR BLOOD BANKING APPLICATION.

PREPARATION OF DOUBLE OXALATES

- Potassium oxalate 1.6 grms
- Ammonium oxalate 2.4 grms
- H₂O 100 ml, mix well, 0.2 ml of solution will contain 8 mg of oxalates which prevent clotting of about 3-4 ml of blood

HEPARIN

- It is a natural anticoagulant in the body, found in the liver, and may also be with in basophils and mast cells. Heparin is also called anti thromboplastin or antithrombin.
- It is available in a liquid or dry form as sodium, calcium, ammonium and lithium salt. Each of these will interfere with determination of their respective ions in the plasma.

Mode of Action :

- It interferes with the formation and/or activity of thrombin and the activity of clotting factors IX, X, XI, XII.

Concentration:

- The optimum con% is 0.1-.2 mg/ml of blood.

HEPARIN

Advantages :

- Heparin is the choice of Anticoagulant for blood pH, and blood gas Analysis and Acid base balance.
- It may be used for special trace elements studies and some cytology .
- Excessive heparin does not alter the RBC volume.

HEPARIN

Disadvantages :

- It causes clumping of leukocytes.
- It interferes with staining of leukocytes.
- It is the most expensive anticoagulant
- Blood clot in 8-12 hrs because clotting is only delayed and not prevented.
- It is not suitable for agglutination tests , and coagulation studies.
- It may interfere with some automated biochemical analysis of plasma.

SODIUM CITRATE

- The formal citrate solution (Dacies solution) is used as diluent in the counting of RBCs and PLT's.

Formation

- 3.13 grms of Trisodium citrate is dissolved in 100 ml of water, 1 ml of formaldehyde is added to every 99 ml of the solution.

Mode of action :

- It combines with calcium to form insoluble salt of calcium citrate.

SODIUM CITRATE

Concentration used

- The standard concentration 1 part (3.8%) for 9 parts of blood

Advantages :

- Sodium Citrate is the anticoagulant for choice for studies of Platelets function and morphology.

Disadvantages :

- It interferes with many chemical tests
- Used alone it preserves blood for only few min.
- It has a tendency to shrink cells. Sodium citrate is generally not used for CBC.

ACID CITRATE DEXTROSE (ACD)

- It is prepared from disodium hydrogen citrate and is the anticoagulant of choice for blood transfusion.

Formation:

- 2 grms of Na_2 hydrogen citrate and 3 grms dextrose are added to 120 ml of water autoclaved for 30 min at 20 PSI and used the ratio 1 part acid to 4 parts of blood.

SODIUM FLUORIDE & POTASSIUM OXALATE MIXTURE

Mode of Action :

- Sodium fluoride inhibits the glycolytic enzymes responsible for the break down of glucose in the blood. (At RT. About 10% glucose is lost per hour from an untreated sample)
- The potassium oxalate is the primary anticoagulant as Sodium fluoride has a poor anticoagulant effect. **Concentration:**
- The optimum concentration : 1 mg of mixture per 1 ml of blood

Use

- Glucose determination

SODIUM FLUORIDE & POTASSIUM OXALATE MIXTURE

Disadvantages :

- It is poisonous
- It inhibition of urease, and glycolytic enzymes may interfere with urea and glucose determinations that employ enzyme activity.
- Alkaline phosphatase, amylase and uric acid cannot be determine in blood containing sodium fluoride.

Thank You