

**STABILITY OF ANTICOAGULATED BLOOD  
SEMINAR PRESENTED  
BY  
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TO THE  
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STUDENTS**

**JCS MEDICAL DIAGNOSTIC LABORATORIES AND  
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# SUMMARY

Anticoagulant are substances which when added to blood prevent blood from clotting, or reduced the coagulation of blood . Stability is the capability of a sample material to retain the initial property of a measured constituent for a period of time within specified limit when the sample is stored under defined condition . When EDTA anticoagulated blood cannot be tested within one to two hours it must be refrigerated at 4-8<sup>0</sup>c to prevent cellular changes affecting test result. in EDTA anticoagulated blood, morphological blood cell changes occur soon after blood is collected when it is stored at room temperature (18-25<sup>0</sup>c) and within 3 hours when stored at 4-8<sup>0</sup>c .when citrated blood is stored at 4-8<sup>0</sup>c there is a decrease in the ESR due to changes in erythrocyte shape affecting rouleaux. ESR should be measuring within 4hrs of collecting the blood. Coagulation test should be carried out as soon as possible after blood is collected into citrate anticoagulant . Blood which has been refrigerated must be allowed to warm to room temperature and be well mixed before being tested.

CPD (citrate phosphate dextrose) is used in preservation of blood at 4<sup>0</sup>c for 25 days, ACD (acid citrate dextrose )also preserved blood at 4<sup>0</sup>c for 21days. CPDA [citrate phosphate dextrose plus adenine) is used for the preservation of whole blood and red blood cell for up to 35 days, it extend red cells survival by providing Adenine needed for the maintenance of red cell ATP level . Heparin has a temporary effect of only 24hrs. it does not affect erythrocyte and is less likely to cause haemolysis than EDTA. Gradually broken down on storage and clot appear in the blood, it is only used for urgent blood transfusion at a concentration of 1500 to 300 I.U per 500ml of blood it is not suitable for routine hematology test because it may cause leukocyte and platelet clumping

# STABILITY OF ANTICUAGULATED BLOOD

Anticoagulants [antithrombics fibrinolytic and thromobolytics] are substances which when added prevent blood from clotting or reduced the coagulation of blood.

Common anticoagulants include warfarin and heparin.

## **Laboratory use**

Apart from heparin, most of the chemicals work by binding calcium ions, preventing the coagulation proteins using them,

They include:

Ethylene diamine tetraacetic acid (EDTA): strongly and irreversibly chelates (binds) calcium ions, preventing blood from clotting. It is used in the concentration of 1-2 mg/ml of blood to 2.0 mg/ml.

K<sub>2</sub> = dipotassium

K<sub>3</sub> = tripotassium and Na<sub>2</sub> = disodium are salts used.

Citrate : is in liquid form in the tube and is used for coagulation test, as well as in blood transfusion bags. It binds the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution and it can be reversed with the addition of calcium. It can be in the form of sodium citrate or acid citrate dextrose.

Oxalate: has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride oxalate tubes used to determine glucose and lactate levels.

# STABILITY OF ANTICUAGULATED BLOOD

Stability is the capability of a sample material to retain the initial property of a measured constituent for a period of time within specified limit when the sample is stored under defined condition

When EDTA anticoagulated blood cannot be tested within one to two hours it must be refrigerated at 4-8°C to prevent cellular changes affecting test results.

Manual or automated blood cell counts, reticulocyte counts and PCV change little in EDTA blood at 4-8°C when stored for up to 24hrs. Hemoglobin concentration is stable for 2-3 days at 4-8°C provided there is no hemolysis. In EDTA anticoagulated blood, morphological blood cell changes occur soon after blood is collected when it is stored at room temperature [18-25°C] and within 3hrs when stored at 4-8°C. It is therefore recommended that blood film be made and methanol fixed as soon as possible after blood is collected and never made after overnight storage.

## **Some of the blood cell changes which occur in EDTA blood include:**

- Neutrophil degeneration with neutrophils becoming irregular in shape, nuclear lobes separating and vacuoles appearing in the cytoplasm and there is also loss of granules.
- Segmentation of the nucleus of lymphocytes and monocytes, and vacuoles appearing in the cytoplasm.
- Erythrocyte becoming crenated and spherocytic.
- Platelets disintegrating.

When citrated blood is stored at 4-8°C, there is a decrease in the ESR due to changes in erythrocyte shape affecting rouleaux.

ESR should be measured within 4hrs of collecting the blood. Coagulation test should be carried out as soon as possible after blood is collected into citrate anticoagulant.

**NOTE:** Blood which has been refrigerated must be allowed to warm to room temperature and be well mixed before being tested.

Heparin is used in concentration of 0.1-0.2mg/ml. It has a temporary effect of only 24hours. It does not affect erythrocyte and is less likely to cause haemolysis than EDTA. Heparin is gradually broken down on storage and clot appear in the blood, it is only used for urgent blood transfusion at a concentration of 1500 to 300 I.U per 500ml of blood. it is not suitable for routine hematology test because it may cause leukocyte and platelet clumping.

It is not suitable for blood films staining, since it gives too blue coloration to the background, when films are stained with Romanowsky stains.

CPD [ citrate – phosphate Dextrose] is used in preservation of whole blood at 4<sup>0</sup>c for 25days producing an ATP level of 70%. ACD (Acid citrate Dextrose )

blood preserved in this anticoagulant at 4<sup>0</sup>c for 21days produced an ATP level of 60%

NOTE: the incorporation of purine derivatives to ACD and CPD prolong the storage period of blood beyond 21 days and 28 days respectively.

CPDA citrate phosphate dextrose plus Adenine is incorporated into CPD to give CPDA used for the preservation of whole blood up to 35 days, It extends red cells survival by providing adenine needed for the maintenance of red cell ATP level.

## REFERENCES

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