

## **The Effect of Storage on Full Blood Count in Different Anticoagulant**

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**Abstract:** *The effect of storage on full blood count in different anticoagulant was determined in view of its importance on the reliability and validity of test results. The study was designed to know the effect of storage on full blood count in different anticoagulant which is Ethylenediamine tetra acetic acid and citrate phosphate dextrose Adenine. A total of 50 samples of apparently healthy individuals were analyzed for their packed cell volume, Haemoglobin concentration, Red blood cell, Platelet, white blood cell and differential Neutrophils, Lymphocytes and monocytes, by storing 2mls each of their blood sample in Ethylenediamine tetra acetic acid and Citrate phosphate dextrose adenine (CPDA) anticoagulant for a period of 24hrs and 168hours at 4<sup>0</sup>C. Changes were observed in some but not all haematological parameters measured on LDW-3600 Auto haematological analyzer, specifically the HB, PCV, Lymphocytes and Monocytes showed a drastic increase on day seven compared to day one and more decreased on platelet and WBC on seven day compared to day one. For this study no statistical change was observed for Rbc. Based on these findings, samples stored for 24hours at 4<sup>0</sup>C would not result in significant changes in blood parameters, therefore haematological laboratories are advised not to keep samples beyond 24hours at 4<sup>0</sup>C for reliability of test result.*

**Keywords:** *Anticoagulant, Full blood count, HB, PCV and Leucocytes.*

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### **I. Introduction**

The determination of the effects of storage on full blood count in different anticoagulant is an aspect of quality assurance. Quality assurance involves the application of all possible means to guarantee that the results reported by the laboratory are both reliable and valid. Reliability is concern with the consistency of work. Validity is concern with the degree of the test measuring what is supposed to measure with accuracy (Tatsumi et al., 2002).

Excessive delay in processing blood samples for haematological testing could compromise the reliability of the result. One of the element that contribute to quality assurance is the nature of the specimen, blood deteriorate rapidly if not under ideal condition, (Baker et al., 2001).

Blood is a connective tissue made up of cells suspended in a fluid medium known as plasma and the plasma is made up of minerals, salt, vitamins, coagulation factors cell and organic elements. Full blood count (FBC) consist of investigation of cellular content of blood mainly white blood cells (leucocytes) and its different types platelets (thrombocytes) and the red blood cell (erythrocyte) with related parameters (packed cell volume, haemoglobin concentration) (Wood et al., 2004).

In vivo red cells are carried and protected by the plasma which helps to provide carefully regulated temperature, controlled PH, adequate glucose supply necessary for proper metabolic waste. In donated blood the cells are removed from their in vivo environment and subjected to numerous conditions that stress their ability to survive, artificial means are employed to maintain the environment. The formulation of anticoagulant for storage and maintenance of appropriate metabolism in vivo is employed; the sum total of these measures is what we refer to as blood storage. The components necessary for blood storage are the anticoagulant/preservative and refrigerator. An anticoagulant is a substance that prevents coagulation. In order to maintain the blood in its fluid state anticoagulant must be added, such solution also provides capability and nutrients for cellular metabolism during storage (Hess et al., 2000).

Apart from heparin most of the chemicals work by binding calcium ions preventing the coagulation proteins from using them (Beutler et al., 2005).

The anticoagulant to be used in this work includes: Ethylenediamine tetra-acetic acid (EDTA) and Citrate phosphate dextrose adenine (CPDA). The change in blood on storage are directly related to the storage time and the type of anticoagulant used (Ochei and Kolhatka; 2007).

Haematology sample should be refrigerated at a temperature of 4<sup>0</sup>C. This is found to favor optimal preservation of the blood and also prevents multiplication of any bacterial which might be present, however specific information concerning the suitability or unsuitability of specimen older than one day for various laboratory test including full blood count and differential is scarce particularly in the recent literature (Gene Gulatie et al., 2002).

This study intends to know the changes that occur in various parameters of full blood count during storage of blood at 4<sup>0</sup>C for several days in different anticoagulant.

#### **AIM**

To compare the effect in Blood storage on full blood count in different anticoagulants.

#### **OBJECTIVES**

To compare the effect of ethylenediamine tetra acetic acid (EDTA) and citrate phosphate dextrose adenine (CPDA) anticoagulant on these haematological parameters packed cell volume (PCV), white blood cell (WBC) differential count and platelet count when used with anticoagulated blood.

To ascertain the extent of deteriorous change that could occur when blood is stored in this anticoagulant.

To recommend and to confirm the anticoagulant of choice for haematological investigation.

#### **STUDY AREA**

This study was conducted in Rivers State Hospital Management Board Located in the city of Port Harcourt Rivers State Nigeria. Port Harcourt is urban area were some commercial and industrial centre and seaport occurs. It is the state capital of Rivers State and has a population of 1,382,592 people according to the 2006 census. The geographical location of Rivers State is latitude 4' .31 – 5' .31 and longitude 6' .30 – 7' .21.

#### **STUDY POPULATION**

In this research work a total of 50 samples was analyzed in Rivers State Hospital Management Board. 35 samples were collected and store in Ethylenediamene Tetra acetic acid (EDTA) and 15 samples were collected also and store in CPDA Citrate Phosphate Dextrose Adenine. The donors tested negative for HCV, HbsAg, Syphilis HIV I and 2.

#### **SAMPLE COLLECTION**

Blood collection was performed as described by Monica (2000). Blood was collected from each of the donors with care and adequate safety precautions to avoid contamination and infection from blood transmissible pathogens, protective gloves were worn during collection, syringes were sterile and blood collected materials were discarded safely to avoid injury from needles. In this study 10mls of blood sample was collected through venepuncture from the vein, which was well cleaned with 70% alcohol and dry. After collection, it was dispensed into a 2.5mg/ml of dipotassium EDTA anticoagulant immediately and 2ml of the same blood into 0.5ml of plastic specimen plane container CPDA. The collected blood sample was mixed properly in order to avoid clotting of the samples, the samples container was labeled properly with the date of collection and patient name. The test was carried out 2hrs after collection for haematological parameters; full blood count (packed cell volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell (RBC) White Blood Cell (WBC) and differential (Neutrophils, lymphocytes and monocytes).

## **II. Laboratory Diagnosis**

#### **MATERIALS/REAGENTS**

Lyse 500ml, Detergent 1000ml and LDW – 3600 Auto hematology Analyzer.

#### **PROCEDURE**

The test was carried out automatedly. The LDW-3600 Auto hematology Analyzer was switch on and allow to boot. The anticoagulated samples were mixed with a roller mixer. After mixing, the samples were placed under the probe, the button on the machine was click okay using the mouse. After 60 seconds the result was ready in a printed form. the following parameters were analyzed packed cell volume PCV, Haemoglobin concentration (Hb), Red Blood Cell (RBC), platelet, White Blood Cell (WBC) and differential neutrophils, lymphocytes and monocytes, After which it was stored in the refrigerator at 4<sup>0</sup>C for day 1, on the seventh day the same test was repeated using the same machine LDW-3600 Auto hematology Analyzer and the same samples. The LDW – 3600 Auto analyzer works on the principle of Electronic impedance, it enumerates. 23 parameters with 3-part differential of white blood cell.

**DATA REPORT**

Data was analyzed and presented using statistical evaluation, mean standard deviation and coefficient of variance, standard error was also considered.

**SAMPLE DESIGN**

Blood was collected randomly from male and female using EDTA and CPDA and was analyzed in Rivers State hospital Management Board.

**III. Results**

**Table 1: Statistical evaluation showing the Haematological parameters Analyze in EDTA bottle for day one and after Seven days (N=35)**

DAYS	PARAMETERS	MEAN ± STANDARD DEVIATION	COEFFICIENT OF VARIANCE	STANDARD ERROR
DAY 1	Hb	11.6143 ± 1.5797	0.138	0.2703
DAY 7		12.0917 ± 2.7688	0.16175	0.3241
DAY 1	PCV	36.2571 ± 5.0785	0.21342	0.8709
DAY 7		38.9143 ± 6.2398	0.16223	1.0686
DAY 1	RBC	4.6686 ± 0.8148	0.17966	0.1320
DAY 7		4.9 ± 0.66.7	0.14877	0.3184
DAY 1	WBC	6.7743 ± 2.4150	0.3617	0.4101
DAY 7		2.3971 ± 1.1812	0.49993	0.2024
DAY 1	PLATELETS	267.9143 ± 91.8746	0.34793	15.7564
DAY 7		118.6857 ± 73.6992	0.40638	13.1779
DAY 1	NEUTROPHILL	60.457 ± 12.7056	0.19602	1.9888
DAY 7		36.0857 ± 9.5713	0.26535	1.6219
DAY 1	LYMPHOCYTE	25.7143 ± 8.1406	0.31658	1.4080
DAY 7		37.6286 ± 9.0913	0.24161	1.5367
DAY 1	MONOCYTE	12.9429 ± 4.0143	0.31015	0.6785
DAY 7		25.7143 ± 8602	0.19854	0.8726

**Table 2: Statistical evaluation showing the Haematological parameters analyze in CPDA bottle for day one and after seven days (N=15)**

DAYS	PARAMETERS	MEAN ± STANDARD DEVIATION	COEFFICIENT OF VARIANCE	STANDARD ERROR
DAY 1	Hb	12.8 ± 1:6024	0.13312	0.4305
DAY 7		19.9533 ± 29.1859	0.13346	0.4414
DAY 1	PCV	35.4 ± 4.3939	0.12848	1.1743
DAY 7		36.5333 ± 5.0181	0.14218	1.3411
DAY 1	RBC	4.7067 ± 0.6557	0.1442	0.1919
DAY 7		4.7867 ± 0.6879	0.14875	0.2108
DAY 1	WBC	5.6 ± 1.3981	0.25842	0.4079
DAY 7		2.36 ± 1.4741	0.64656	0.3966
DAY 1	PLATELETS	161.3333 ± 47.4477	0.30442	12.6809
DAY 7		86.8667 ± 47.2424	0.55747	12.62600
DAY 1	NEUTROHILS	51.7333 ± 13.3938	0.26799	3.5797
DAY 7		29.4667 ± 14.8498	0.52164	3.9687
DAY 1	LYMPHOCYTE	3.2 ± 8.2704	0.26752	2.2104
DAY 7		52.2667 ± 13.0357	0.25816	3.4839
DAY 1	MONOCYTE	17.4667 ± 4.12903	0.24469	1.1035
DAY 7		18.2667 ± 3.9744	0.22521	1.0621

**IV. Discussion**

There is substantial evidence from invitro studies documenting the change that haematological parameters undergo during storage. It was observed that heamatological parameters analyze at 4<sup>0</sup>C increase and decreased.

Haemoglobin and packed cell volume showed a drastic increase on day seven compared to day one this is in an agreement with the scientist who used turkey on their experiment their result showed a rapid increase in haemoglobin and packed cell volume.

On day one there was more increase on platelet compared to day seven and decrease on day seven compared to day one. This agrees with the work done by Cohle et al., (1981) who uses coulter Gen.s. on 40k3(Tripotassium ethylenediamine tetra acetate), EDTA anticoagulated blood specimen found that the mean platelet volume (mpv) initially increased steadily reaching a maximum mean percent change on day five ,on day six, and seven mean percentage change had drastically increased.

When white blood cells (WBC) values on day one was compared to day seven, it was observed that there was a rapid decrease on day seven and more increase on day one. These changes in white blood cells are mostly likely due to the changes in sum effects of the loss of individual cell characteristics specifically degeneration that is known to occur as the cell ages. Cohle et al 1981 and Trombridge et al., 1985. There is more increased in neutrophils on day one compared to day seven and more decreased on day seven compared to day one. This agreed with (Dacie et al., 2001) when blood is store at longer period the neutrophils are affected. A progressing increase in lymphocytes and monocytes on day seven was noticed in this study, there is more increased on sample analyse on day seven in EDTA and CPDA compared to the day the sample was collected and store in EDTA and CPDA being day one and decreased on Day one this agree with (Davey et al., 1986). This research deals with the effect of blood storage on full blood count in different anticoagulant. The anticoagulant used in this research work is ethenyldiamine tetra acetate and citrate phosphate dextrose Adenine. The research is summarize under five chapters, chapter one consist of background of the study, statement of the problem, objective, justification of the study and scope of the study, followed by chapter two that deal with the literature review and explanation of the different parameters used which comprises of packed cell volume, haemoglobin, platelets, white blood cell and differential neutrophils, lymphocytes and monocytes. Chapter three is based on the research methodology used, which includes study area, study population, sample collection data analysis and study design. Chapter four shows the result which is calculated using standard deviation, mean, coefficient of variance and standard error and discussion of the result which reveals that there is increase and decreased that occurs when blood is store for a long time

### **Conclusion**

There are degenerative changes observe in full blood count in sample store in ethylenediamine tetra-acetic acid (EDTA) and citrate phosphate dextrose adenine (CPDA).

### **V. Recommendation**

Blood analysis (Full blood count and differential) should be done immediately after collection to get accurate and more reliable results. My study suggest that clinically reliable results may not be obtained for most full blood count and differential parameters from specimen older than one day.

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