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Changes In HB And Blood Glucose Levels Of Blood Stored In CPDA-1 Under Bonaberi Baptist Hospital Storage Conditions

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Abstract

The aim of this study was to evaluate the changes in Haemoglobin and glucose levels of whole blood stored in CPDA-1 for a 28days storage period. The study was a longitudinal cohort study conducted using 45 healthy donors Blood for transfusion was collected from prospective male blood donor found to be in good health, aged between 18 and 52 years, with haemoglobin levels within the range of 13.5 g/dl – 16 g/dl, body weight within 55 kg – 75 kg, into plastic bags containing anticoagulant CPDA-1, and handled under strict sterile condition. The blood was stored in a blood bank refrigerator with a constant temperature of 2 to 8oc under proper inspection at intervals for colour, turbidity, haemolysis and clot formation. 5ml of the sample was collected aseptically into a plain tube from the blood bag and analyzed using the ACCU-answer. Results: Results showed significant changes in whole blood haemoglobin and glucose from day 7 to day 28. Conclusion: It is pertinent therefore to note that the use of CPDA-1 does not completely stop the changes that occur in RBC as there are several changes occurring in stored blood collectively called “storage lesions”. Therefore, it is advisable that blood should be transfused within 14 days of storage to avoid transfusion of blood products that have lost most of their content to recipients, and where possible whole blood should be processed.

INTRODUCTION

A Blood bank is a centre where blood is gathered for the purpose of blood donation and is stored and preserved for later use for blood transfusion. The term “Blood bank” typically refers to a division of a hospital where proper testing is performed to reduce the risk of transfusion-related adverse events. (1).

The first recorded blood transfusion was made between dogs, by an English doctor, Richard lower around 1666.In 1667, French scientist Joan Bautista Danys transfused a human with animal blood. In 1990, Karl Landsteiner identifies some of the blood substances responsible for the agglutination of red blood cells, identifying blood group for the first time and some of their incompatibilities.

Direct Transfusion was still not practised in the 20th century because it was impossible to keep blood unaltered outside the body; after 6 to 12 minutes coagulation begins to manifest, that is the blood gradually becomes more viscose and ends with almost complete solidification. It is of important to note that coagulation is a defence mechanism, as it helps the organism to minimize bleeding by forming clot. Clotting is almost totally formed by platelets, fastened by a network of filaments of fibrin. Fibrin does not normally exist in the blood it is created by the action of thrombin enzyme. Similarly, thrombin is not naturally present in blood, its created by a precursor prothrombin in a process that involve platelets, some calcium, and substances produced by lesion material since clot is not created if there is a lack of some of these elements, This aid in the discovery of anticoagulant that prevents blood from clotting, by removing one or more of these substances needed for blood clot. One of the first anticoagulants for blood transfusion to be realized was sodium citrate, which eliminates calcium ions from blood thereby preventing coagulation. The possibility to store blood invitro without it being altered and the increase need for transfusion of safe blood let to the collection and preservation of blood also known as blood banking.

Another important breakthrough came from 1939 to 1940, when Karl Landsteiner, Alex Weiner, Philip Levine and Steton discovered the Rhesus blood group, which was found to be the cause of majority transfusion reaction up to that time. The introduction by Loutit and Patrick Mollison of Acid Citrate Dextrose solution (ACD), which reduces the volumes of blood and allowed longer-term storage.

Today the storage and preservation of whole blood or blood component, also known as Blood banking, is a necessary facility for all laboratories, as it provides a quick, and safe transfusion of blood. Blood transfusion has remained, the number one therapeutic way of managing patients with anemia, severe bleeding and kidney failure. Although it exposes the patient to some risk like, immunomodulation, transmission of undetected viral and bacterial antigens, and transfusion reactions.

Blood Hemoglobin (Hb) and glucose are two important components, which can be measured accurately in whole blood. Hb the most important component of red blood cells is a conjugate protein, whose prosthetic group (non-protein component) is made up of 4 heme group (Fe), and that gives red blood cell its typical intense red colour, that attaches to a protein unit called globin, the exist various globin as discussed in chapter two of this work but the standard structure of the molecules consist of two alpha and two beta polypeptide chains. Haem is a metal complex containing an iron atom in the centre of a porphyrin structure, the Hb formation starts at the intermediate level (polychromatic normoblast) of Erythropoiesis and it is completed by the time of the RBC maturation in the bone marrow. There are about 2 to 3 million Hb in a red cell, each having a molecular weight of 64,458.The life span of Hb is the same as that of RBC that accommodates it, that is about 120days.Healthy adult losses and produces 6.25g of Hb a day .A liter of normal blood is capable of carrying 200ml of oxygen and 1.0gram of Hb combines with 1.34ml of oxygen for delivery of oxygen to the cells of the body for tissue respiration, to produce adenosine triphosphate (ATP) for energy. Oxidization converts haem into haematin. The iron content of hemoglobin molecule is 3.4nanograms. Hb serves as the most significant buffer in blood, and plays a vital role in controlling erythrocytes metabolism. The normal reference range of Hb in whole blood in male is 14 to 18g/dl and for female is 12 to 16g/dl; this decrease in female is because of menstruation where blood is lost every month.

Blood glucose($C_6H_{12}O_6$) a monosaccharide usually produced from apolysaccharide is the principal source of energy for red blood cells and the body, only when all glucose sources of the body are exhausted, in a healthy individual will the body start to produce energy from non glucose substances. Glucose after being released from carbohydrate (polysaccharides), it's absorbed into circulation by the single-layer capillaries, which then send into the hepatic portal vein, which transport it to the liver where it's either store as glycogen or distributed to the other cells of the body, depending on the body need for glucose. The normal reference range for blood glucose, before breakfast is 70 to 105mg/dl (3.9 to 5.8mmol/l).

MATERIALS AND METHODS

Study setting and period

This research was carried out at the Bonaberi Baptist Hospital (Blood Bank). Bonaberi is a port in the Littoral region of Cameroon. It is located on the western side of the harbor across the Wouri River from the larger port of Douala. It is served by a station on the national railway system. More than 75 of Cameroon's industrial strength is located Douala and Bonaberi. Bonaberi with about 8091 citizens is located 206km west of Yaounde, the capital city of the Republic of Cameroon.

Study design and duration

The study was a longitudinal cohort study, which are studies carried out on same sample over a period

of time and makes repeated observations, the blood samples were donated by ten healthy voluntary donors and the changes in Hb and blood glucose were determined for 28days and the study ran from April to May 28 2022.

Study procedure

Procedure for measurement of hemoglobin and glucose

Measuring Principle

The ACCU-ANSWER® ISAW® multi-monitoring system employs electrochemical biosensor technology to measure enzymatic chemical reaction. When blood is applied to an electrochemical test stripe, an electric current is produce. The multi meter measures the current and calculates the level, displays the result, and stores the result in its memory.

Hemoglobin Testing

- Started with the meter off, an Hb test strip from its vial was removed and inserted into the measuring port to turn on the meter, with clean, dry hands, the test strip can be touched anywhere on its surface without bending, cutting or modifying the test strips in any way.
- The meter turned on immediately and showed a code and the test (Hb) to be performed. The code and test displayed on the meter was matched with the one on the vial bottle for each sample before proceeding.
- After 5 seconds, the meter displayed Hb with a symbol indicating blood should be applied on the test pad of the strip, ready to perform an Hb test.
- The sample was applied by touching and holding a drop of blood to the narrow channel in the top edge of the test strip and the blood was drawn into the strip by holding the drop of blood to the top edge of the strip until the confirmation window was full. The Hb test results were read when the meter detected blood in the test strip.
- The Hb levels of the samples appeared on the display, along with the unit of measurement, and the date and time of the test.
- The meter was turned off after running all the blood samples and the used strips discarded into the safety box.

Blood Glucose Testing

- Start with the meter off, a blood glucose test strip from its vial was removed and inserted into the measuring port of the meter to turn the meter on.
- The meter turned on immediately and showed a code and the test (BG) to run, displayed on the screen. The code and test performed were matched on the meter with the one on the vial bottle before proceeding.
- After 5 seconds the meter displayed BG with symbols indicating blood should be

applied on the meter, ready to perform a blood glucose test.

- The blood glucose test results were read when the meter detected blood in the test strip along with the date and time of the test.
- The meter was turned off after all the samples were run and the test strips discarded into the safety box.

28days and the study ran from April to May 28 2022

Study population

Healthy voluntary blood donors, aged 20 to 52 years, who have been screened free from blood transfusion disease at the Bonaberi Baptist Hospital Blood Bank.

Sample size

45 sterile dry tubes of blood samples, of 4ml each from a 450ml CPDA-1 blood bag that was used to collect the donors using standard venipuncture techniques and placed on a quarantine shelf of the blood bank refrigerator, with a constant temperature of 2^0c to 8^0c

Inclusion criteria

- Must be above 18 years old
- Must have been screen free from blood transfusion diseases like hepatitis virus, syphilis, HIV/Aids.
- Most have a normal respiration rate, pulse, heart beat
- Most have a blood glucose of more than 5.8mmol/l or 105mg/dl, and Hemoglobin of 12 to 16g/dl for the male, and 11.5 to 15.5g/dl for the female
- Body weight from 50kg to 100kg and body temperature of 35.5^0c +/-1 to 37^0c +/-1

Exclusion criteria

- Male donors of HB below 12g/dl and female donors with HB below 11.5g/dl
- Underweight donors
- Donors who tested positive for any of the screening test
- Donors with abnormal vital signs
- Paid donors
- Donors with diabetes (type 1 or 2) or hypoglycemia
- Pregnant women and menstruating women
- Donors who didn't give their informed consent to the study.

Sampling technique

A random sampling technique by numbered tag was used, as the first forty-five healthy voluntary donors who came to donate blood at the Bonaberi Baptist Hospital Blood Bank were taken, and their blood samples were collected into 450 CPDA-1 blood bags and 4ml of each sample was transferred into plain sterile tubes to be used for the research and the other part will be used for transfusion in the hospital.

Ethical authorizations

Ethical clearance to carry out this study was obtained from the director of the Regional delegation of Public Health and the authorization was forwarded to the administrator of the Bonaberi Baptist hospital

who signed a permission form for data collection in the establishment.

All information about patients was being kept with strict confidentiality, as codes were used to identify samples and not patient identity. Also, details of the study were given to all participants so they can also realize its benefit.

Data management and analysis

For this work to be achieved, we used scientific calculators, tally sheets, text, Microsoft word, Excel, frequency distribution tables, and graphs. These various method helped in converting the raw data collected in the field as values from the meter into meaningful data which can be easily understood.

RESULTS

Presentation of result

The evaluation of the effect of storage on both Hb and blood glucose concentration was carried out using CPDA-1 anticoagulant, blood drawn from forty five healthy volunteers donors both male and female of ages 20 to 52 years (mean age 28years) and placed on the quarantine shelf of the blood bank refrigerator. This blood was kept for 28days and samples were evaluated on day; 0, 7, 14, 21, and 28 days.

Table 1 below shows the difference in whole blood Hb and blood glucose concentration for 4weeks (28days) for the different donors.

Table 1: Presentation of Results

Donors	DAYS									
	0		7		14		21		28	
	HB(g/dl)	BG(mg/dl)	HB(g/dl)	BG(mg/dl)	HB(g/dl)	BG(mg/dl)	HB(g/dl)	BG(mg/dl)	HB(g/dl)	BG(mg/dl)
B1	15.8	482.82	14.9	383.48	14.4	306.70	13.76	221.91	13.24	198.5
B2	16.5	526.84	13.29	458.72	13.14	380.39	13.13	307.90	12.09	286.72
B3	13.9	455	12.01	360.5	11.7	298.89	11.61	209.9	10.45	186.71
B4	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B5	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9

B6	15.6	514.63	15.44	432.85	14.31	362.48	13.93	299.5	13.09	266.3
B7	15.5	516.33	13.99	448.35	13.2	379.1	13.19	308.02	12.26	265.2
B8	14.6	480.52	14.01	371.21	13.51	300.96	13.49	281.05	12.7	200.9
B9	16.2	396.48	15.1	302.33	14.00	288.40	13.95	208.93	13.38	188.1
B10	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B11	13.9	455	12.01	360.5	11.7	298.89	11.61	209.9	10.45	186.71
B12	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B13	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9
B14	15.6	514.63	15.44	432.85	14.31	362.48	13.93	299.5	13.09	266.3
B15	15.5	516.33	13.99	448.35	13.2	379.1	13.19	308.02	12.26	265.2
B16	15.8	482.82	14.9	383.48	14.4	306.70	13.76	221.91	13.24	198.5
B17	16.5	526.84	13.29	458.72	13.14	380.39	13.13	307.90	12.09	286.72
B18	14.6	480.52	14.01	371.21	13.51	300.96	13.49	281.05	12.7	200.9
B19	16.2	396.48	15.1	302.33	14.00	288.40	13.95	208.93	13.38	188.1
B20	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B21	15.5	516.33	13.99	448.35	13.2	379.1	13.19	308.02	12.26	265.2
B22	15.8	482.82	14.9	383.48	14.4	306.70	13.76	221.91	13.24	198.5
B23	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B24	13.9	455	12.01	360.5	11.7	298.89	11.61	209.9	10.45	186.71
B25	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94

B26	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9
B27	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B28	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9
B 29	15.6	514.63	15.44	432.85	14.31	362.48	13.93	299.5	13.09	266.3
B30	16.5	526.84	13.29	458.72	13.14	380.39	13.13	307.90	12.09	286.72
B31	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9
B32	16.2	396.48	15.1	302.33	14.00	288.40	13.95	208.93	13.38	188.1
B33	15.5	516.33	13.99	448.35	13.2	379.1	13.19	308.02	12.26	265.2
B34	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B35	15.8	482.82	14.9	383.48	14.4	306.70	13.76	221.91	13.24	198.5
B36	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B37	15.6	514.63	15.44	432.85	14.31	362.48	13.93	299.5	13.09	266.3
B38	14.6	480.52	14.01	371.21	13.51	300.96	13.49	281.05	12.7	200.9
B39	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B40	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B41	16.2	396.48	15.1	302.33	14.00	288.40	13.95	208.93	13.38	188.1
B42	15.4	590.44	15.19	471.34	14.4	398.96	14.08	306.2	13.90	293.9
B43	13.3	521.9	12.90	442.40	12.5	371.5	12.44	296.89	11.94	216.5
B44	14.8	450.80	13.21	372.32	12.68	316.8	11.11	210.52	10.95	191.94
B45	14.6	480.50	14.03	371.41	13.49	300.80	13.48	281.04	12.7	200.5

4.2 Statistical Presentation of Variation in Haemoglobin Concentration for a Period of 28days.

Table 2 below presents descriptive statistics for whole blood Hb concentration over a storage time of 28 days. The highest concentration of whole blood Hb was recorded on day 0 while the lowest was recorded on day 28. Measurements from the 45 donors were closest to the mean on day 14 as indicated by a relatively low standard deviation of 0.91431 while measurements from the 10 donors deviated the most from the mean on day 7 as indicated by a relatively high standard deviation of 1.14543.

Table 2: Descriptive statistics for whole blood hemoglobin concentration over a storage period of 28 days.

Storage Time	Mean hemoglobin content (mg/dl)	Std. Deviation	N
Day 0	15.1600	1.00797	45
Day 7	14.0040	1.14543	45
Day 14	13.3840	0 .91431	45
Day 21	13.0690	1.03143	45
Day 28	12.4000	1.09110	45

Figure 1 below is a graphical presentation of the variation of whole blood Hb content over a storage period of 28 days. A trend was observed in which mean whole blood hemoglobin concentration decreased as storage time increased. However, the decrease in whole blood hemoglobin concentration was found to vary weekly as indicated by variations in the slope of the line weekly. In this regard, the highest drop in whole blood hemoglobin concentration was recorded in the first week (day 0 to day 7) while the smallest drop was recorded in the third week (day 14 to day 21).

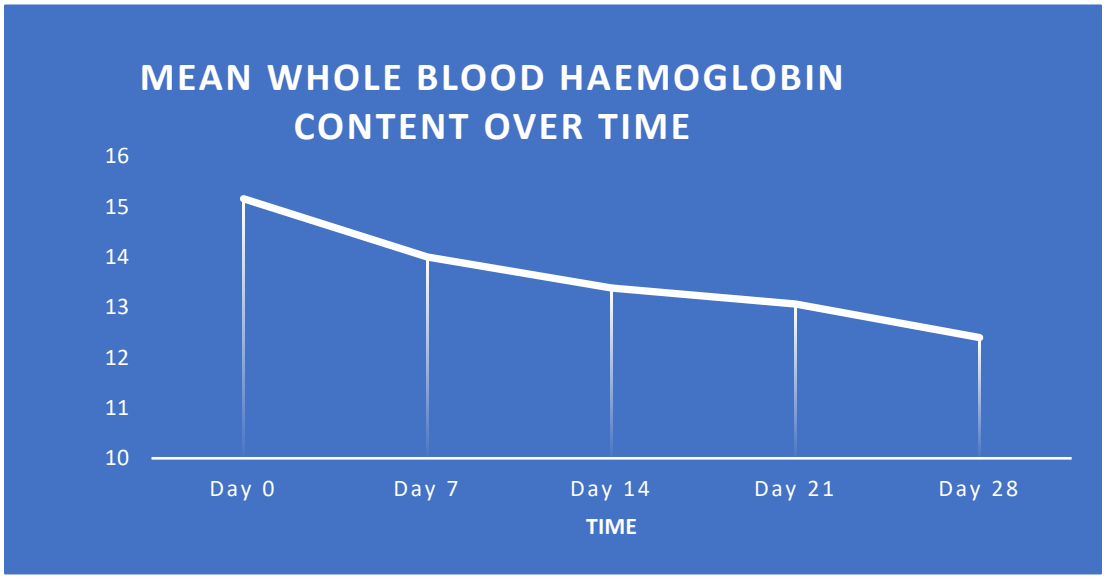

Figure 1: Graphical presentation of the variation of whole blood hemoglobin content over a storage period of 28 days.

4.3 Testing of Hypothesis for Hb concentration

A repeated measure one-way ANOVA was run at the 5% significance level ($\alpha = 0.05$) to test the hypothesis and results are presented on table 3 below. Results indicate statistical significance (p-value < 0.05); we therefore reject the null hypothesis. Hence, at the 5% level of significance ($\alpha = 0.05$), whole blood stored in CPDA-1 under the Regional Hospital Blood bank storage conditions experienced a statistically significant (p-value = 0.000) gradual decrease in hemoglobin level over time

Table 3: Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value
Time	Wilks' Lambda	0.044	32.733[b]	4.000	6.000	0.000

a. Design: Intercept

s Within Subjects Design: Time

b. Exact statistic

c. Computed using alpha = .05

Table 4 depicts a pairwise comparison of whole blood Hb content over a storage period of 28 days. For all pair-wise comparisons, a statistically significant (p-value < 0.05) decrease in whole blood hemoglobin level was observed except for day 14 vs 21 where the observed decrease in whole blood hemoglobin level was not statistically significant (p-value = 0.717). Hence, except for day 14 vs 21, there was a significant decrease in whole blood Hb every week.

Table 4: Pair-wise comparison of whole blood hemoglobin content over a storage period from 0 to 28 days

Storage time (days)	**p-value[b]**
0 vs 7	0.036 [S]
0 vs 14	0.001 [S]
0 vs 21	0.000 [S]
0 vs 28	0.000 [S]
7 vs 14	0.002 [S]
7 vs 21	0.007 [S]
7 vs 28	0.000[S]
14 vs 21	0.717[NS]
14 vs 28	0.000 [S]
21 vs 28	0.002 [S]

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level and below.

b. Adjustment for multiple comparisons: Bonferroni.

S. Significant

NS. Non-significant

4.4 Statistical Presentation of Variation in Blood Glucose Concentration for a period of 28days.

Table 5 shows descriptive statistics for blood glucose concentration over a storage period of 0 to 28 days. The highest mean concentration of blood glucose was recorded on day 0 while the least was receded on day 28. Measurements of blood glucose level for the 45 donors were more similar on day 14 as indicated by a relatively low standard deviation of 41.70817 while measurements were more dissimilar on day 0 as indicated by a relatively high standard deviation of 59.32291.

Table 5: Descriptive statistics for blood glucose concentration over a storage period of 0 to 28days

Storage time	Mean	Std. Deviation	N
Day 0	503.5760	59.32291	45
Day 7	404.3500	54.29390	45
Day 14	340.4180	41.70817	45
Day 21	265.0820	45.77481	45
Day 28	229.4770	43.40234	45

Figure 2 is a graphical presentation of the variation of blood-glucose content over a storage period of 28 days. Going by figure 2, blood glucose level was found to decrease with increase in storage time as indicated by the negative slope of the graph.

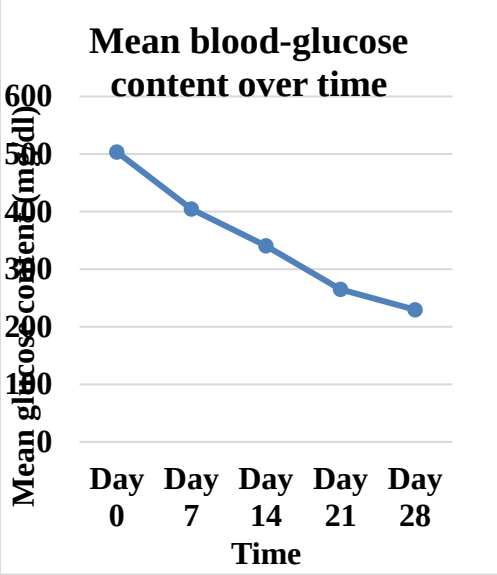

Figure 2: Graphical presentation of the variation of blood-glucose content over a storage period of 28 days

4.5 Testing of Hypothesis for blood glucose concentration

A repeated measure one-way ANOVA was run at the 5% significance level ($\alpha = 0.05$) to test the hypothesis and results are presented on table 6 below. Results indicate statistical significance (p-value < 0.05); we, therefore, reject the null hypothesis. Hence, at the 5% level of significance ($\alpha = 0.05$), blood glucose stored in CPDA-1 under the Hospital Blood bank storage conditions experienced a statistically significant (p-value = 0.000) gradual decrease in glucoselevel over time.

Table 7 shows a pairwise comparison of whole blood glucose content over a storage period of 28 days. In accordance with table 6, for all pairwise comparisons, a statistically significant (p-value < 0.05) decrease in blood glucose level was observed. Hence there was a significant decrease in blood glucose level every week.

Table 7: Pairwise comparison of glucose content in blood over a storage period from 0 to 28 days

Storage time (days)	p-value[b]
0 vs 7	0.000 S
0 vs 14	0.000 S
0 vs 21	0.000 S
0 vs 28	0.000 S
7 vs 14	0.000 S
7 vs 21	0.000 S
7 vs 28	0.000 S
14 vs 21	0.000 S
14 vs 28	0.000 S
21 vs 28	0.015 S

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

S. Significant

CONCLUSIONS

Based on this research, there is a decrease in blood hemoglobin and glucose concentration in blood donor sample that has been stored for four weeks more especially from day 7, this indicates that the use of CPDA-1 does not completely stop the changes that occur in RBC as there are several changes occurring in stored blood collectively known as storage lesion causing the red cell viability to be reduce, our findings re-enforce the need to put in place measures to reduce RBC storage lesion which may be influenced by several factors such as changes in pH, temperature, asymptomatic infections, and longer period of storage

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