STABILITY TEST OF CONTROL BLOOD WITH ADDITIONAL VARIATION OF ARTIFICIAL ALSEVER CONCENTRATION

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Abstract, Background: Blood examination in the laboratory consits of 3 stages, namely pre-analytical, analytical and post-analytic. In the analytical stage, quality control is carried out using blood control materials which can be assayed and unassayed control materials. There are commercial and artificial blood control materials assayed. The stability of blood control agents depends on the use of preservative media, such as Alsever. Storage of blood in preservative media is related to changes in the metabolic, biochemical, and biomechanical structure of molecules in blood cells, such as changes in shape, deformability, osmotic fragility, ability to aggregation, and intracellular viscosity of blood cells. The most likely damage is cytoskeletal proteins in the blood cell membranes which can cause blood cells to become brittle, increase osmotic fragility and change electrolyte imbalances. A decrease in ATP in blood cells up to 80-90% during storage will cause changes in the morphology of blood cells. This study aims to analyze the appearance of hematological examination of control blood with the addition of variations in the concentration of artificial Alsever solution and determine the stability of control blood by adding variations in the concentration of artificial Alsever solution.

Methods: The research method used is a laboratory experiment.

Results: The results of the study were tested statistically with the GLM test and it can be concluded that the stability of stored blood with the addition of Alsever concentration variations showed no significant differences for the examination of erythrocyte counts, Hb levels, MCV MCH and MCHC but there were significant differences between Alsever 1, 2 and 3 on leukocyte and thrombocyte tests.

Conclusions: Control blood with the addition of artificial Alsever with variations in concentration can be used to calculate the number of erythrocytes, Hb levels, MCV MCH and MCHC, as well as the number of leukocytes and platelets with stability until day 30

Key words: Artificial Alsever, variation of concentration, control blood, stability.

Background

Quality control in the hematology laboratory includes 3 stages, namely pre analytic, analytic and post analytic. For the analytical stage, control materials are generally used which can be assayed and unassayed control materials. Control assayed materials are commercial and some are artificial. Assayed commercial control materials are very practical to use, but they are quite expensive and their stability only lasts 3 weeks. This stability depends on the use of preservative media for blood, such as Alsever's solution.

Storage of blood in preservative media is related to changes in the metabolic, biochemical and biomechanical structure of molecules in blood cells, such as changes in shape, deformability, osmotic fragility, ability to aggregation, and intracellular viscosity of

blood cells, which are collectively referred to as "blood cells". storage lesion or injury" (D'Alessandro, et.al., 2015; Zimring, 2015). The most likely damage is cytoskeletal proteins in the blood cell membrane (Antonelou, et al., 2012). These changes will cause blood cells to become fragile and increase osmotic fragility and changes in electrolyte imbalance. A decrease in ATP in blood cells up to 80-90% during storage and causes morphological changes, a large number of spherocytes become irreversible (Koe, et.al., 2009; Cluitmans, et.al., 2012). However, some of the other irreversible damage specifically include increased osmotic fragility, rigid erythrocytes change from biconcave shape to small echinocytic with protrusion and decreased function, micro vesiculation, and hemolysis (Blasi, et al., 2012).

Proper storage of blood is one way to maintain the quality of control materials (Daryl, et al., 2009). Blood can be stored for about 35-42 days depending on the anticoagulant solution or preservative used to prevent blood clots or coagulation during storage (Deyhim, et al., 2014). Alsever's solution has a function as a blood preservative containing isotonic saline solution to maintain cell balance. The ability of this solution is useful in helping the antigenic life of blood cells, so that the cells can survive for a long time (Sigma-Aldrich).

Ethylendiamine Tetraacetic Acid (EDTA) is the type of anticoagulant that is most often and well used in hematological examinations, but the shelf life of blood is short. Blood storage period with a longer period can use Alsever's solution, because it can prolong the life of erythrocytes (Kiswari, 2014). However, Alsever solution is difficult to obtain because the price is quite expensive, so as an alternative you can make an artificial Alsever solution.

In a previous study in 2018, according to Aulia Hanum, the results showed that hemoglobin, hematocrit, erythrocyte count, MCV, MCH and MCHC values in PRC in commercial and artificial Alsever solutions were stable until the 20th day of storage. In this study, the stability of control blood stored for more than 20 days was carried out with the addition of variations in the concentration of artificial Alsever solution.

This study aims to analyze the appearance of hematological examination of control blood with the addition of variations in the concentration of artificial Alsever solution and to determine the stability of control blood by adding variations in the concentration of artificial Alsever solution.

Methods

This type of research is an experimental laboratory. In this study, the control material was made by adding variations in the concentration of homemade Alsever solution with storage for 0-30 days, then examining the hematological picture every 5 days.

Tools, Materials and Working Method

The tools used include a microscope, tube rack, microtube tube, test tube, beaker, timer, micropipette, pipette tips, weighing bottle, syringe, spatula, dropper, reagent bottle, object glass, balance, measuring flask, tourniquet, measuring cup.
The materials used are normal blood and Alsever's solution which consists of Glucose ($C_6H_{12}O_6$), Sodium Citrate ($C_6H_5Na_3O_7.2H_2O$), Citric Acid Monohydrate ($C_6H_8O_7.H_2O$) and Sodium Chloride (NaCl).

Prosedure :

1. Creation of Artificial Alsever media Alsever's solution was prepared by dissolving 2.05 g of glucose; Sodium citrate 0.8 g; citric acid monohydrate 0.05 g; NaCl 0.42 g; Then add sterile distilled water to the mark on the 100 mL volumetric flask. The solution is homogenized, then transferred to a clean and dry reagent bottle, then label/identify

2. Prepare control blood plus artificial Alsever with various concentrations

   Prepared control blood plus variations in Alsever concentration to 3 concentrations, the first control material was mixed with blood with artificial Alsever (1,5: 1), the second control material was mixed with artificial Alsever (1:1) and the third control material was mixed with blood with artificial Alsever (1:1.5). Each control material was distributed into 30 aliquots and stored in the refrigerator (2-8oC) and checked for hematological profiles every 5 days.

3. Examination of the hematological profile using a hematology analyzer.

   Prepare a hematology analyzer tool, turn it on and do the washing and blanks. Performed a commercial blood control check. The first, second and third artificial control material inspection tests were carried out once every 5 days (Stability test). Record the result and turn off the hematology analyzer after use.

Results

1. Quality control testing with commercial control materials

Tabel 1 Result of Quality control testing with commercial control materials

Parameter	Level	Range	Day						
			0	5	10	15	20	25	30
Erythrocyte (10^{12}/L)	Normal	4,00 – 4,36	4,22	4,15	4,19	4,11	4,16	4,14	4,12
Hb (g/dL)	Normal	11,2 – 12,0	11,6	11,4	11,4	11,4	11,4	11,2	11,3
MCV (fL)	Normal	73,4 – 83,4	77,6	78,8	78,4	79,0	79,4	79,7	80,2
MCH (pg)	Normal	26,3 – 29,3	27,5	27,5	27,2	27,7	27,3	27,0	27,6
MCHC (%)	Normal	32,4 – 38,4	35,5	35,0	34,6	35,0	34,4	33,9	34,3
Leucocyte(10^9/L)	Normal	7,8 – 9,0	8,2	8,0	8,1	8,4	8,3	8,2	7,9
Thrombocyte (10^9/L)	Normal	211 - 271	243	236	241	249	243	211	236

2. Results of Control Blood Examination

The results of the hematological profile examination of control blood without Alsever can be shown in Table 1. The results of the examination of the hematological profile of control blood with the addition of Alsever (1.5:1) can be shown in Table 2

Table 2. Results of Control Blood Examination Without Alsever

Treatment	Hari	R	Eritrosit	Leukosit	Trombosit	Hb	MCV	MCH	MCHC
Kontrol (Tanpa Alsever)	H0	1	4.33	5.3	149	12.3	87.5	29.6	33.7
		2	4.31	5.4	147	12.6	87.2	29.3	33.5
		3	4.28	6.0	128	12.2	87.7	29.3	33.4
		4	4.26	6.1	136	12.5	88.1	29.2	33.2
	Rata-rata		**4.30**	**5.7**	**140**	**12.4**	**87.6**	**29.4**	**33.5**
	H5	1	4.20	5.3	150	12.4	87.8	29.5	33.5
		2	4.28	5.7	145	12.6	87.6	29.4	33.6
		3	4.31	5.8	130	12.6	87.7	29.2	33.3
		4	4.28	6.1	137	12.5	88.0	29.2	33.2
	Rata-rata		**4.27**	**5.7**	**141**	**12.53**	**87.8**	**29.3**	**33.4**
	H10	1	4.27	5.4	165	12.5	88.7	29.2	33.0
		2	4.38	5.4	164	12.5	89.4	28.5	31.8
		3	4.34	5.3	172	12.6	89.2	29.1	32.5
		4	4.21	5.6	184	12.3	89.5	29.3	32.7
	Rata-rata		**4.30**	**5.4**	**171**	**12.48**	**89.2**	**29.0**	**32.5**
	H15	1	4.41	6.0	187	12.6	91.3	28.5	31.2
		2	4.29	6.1	185	12.5	91.7	29.3	31.9
		3	4.34	6.3	173	12.6	92.2	29.1	31.6
		4	4.35	6.1	185	12.5	91.9	28.8	31.3
	Rata-rata		**4.35**	**6.1**	**183**	**12.55**	**91.8**	**28.9**	**31.5**
	H20	1	4.36	6.2	152	12.7	93.1	29.1	31.2
		2	4.34	5.8	129	12.5	92.2	28.9	31.3
		3	4.37	5.7	151	12.7	92.7	29.0	31.3
		4	4.29	5.6	134	12.4	91.9	29.0	31.6
	Rata-rata		**4.34**	**5.8**	**142**	**12.58**	**92.5**	**29.0**	**31.4**
	H25	1	4.32	5.7	127	12.5	92.5	29.0	31.3
		2	4.30	6.1	139	12.4	93.1	28.9	31.0
		3	4.32	5.6	114	12.7	92.7	29.3	31.6
		4	4.27	5.1	124	12.5	92.8	29.2	31.4
	Rata-rata		**4.30**	**5.6**	**126**	**12.53**	**92.8**	**29.1**	**31.3**
	H30	1	4.63	6.0	222	13.4	97.4	29.1	29.8
		2	4.71	5.9	208	13.6	99.0	28.9	29.2
		3	4.31	5.8	200	12.7	98.9	29.6	29.9
		4	5.27	5.5	149	15.1	95.8	28.6	29.9
	Rata-rata		**4.73**	**5.8**	**195**	**13.7**	**97.8**	**29.1**	**29.7**

Table 3 Results of Alsever's Control Blood Examination (1,5 : 1)

Treatment	Hari	R	Eritrosit (10^12/l)	Leukosit (10^9/l)	Trombosit (10^9/l)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Darah Alsever (1,5 : 1)	H0	1	2.35	3.8	24	7.1	84.8	30.4	35.8
		2	2.36	3.8	25	7.2	85.0	30.5	35.9
		3	2.38	3.7	25	7.1	85.2	30.0	35.2
		4	2.42	3.7	25	7.1	85.6	29.7	34.7
	Rata-rata		**2.38**	**3.8**	**25**	**7.1**	**85.2**	**30.2**	**35.4**
	H5	1	2.41	3.2	51	7.0	85.1	29.3	34.5
		2	2.44	3.3	39	7.2	84.6	29.6	35.0
		3	2.41	3.2	36	7.1	84.7	29.7	35.0
		4	2.45	3.4	34	7.2	84.3	29.4	34.9
	Rata-rata		**2.43**	**3.3**	**40**	**7.1**	**84.7**	**29.5**	**34.9**
	H10	1	2.46	2.7	38	7.2	84.2	29.2	34.7
		2	2.33	2.8	32	6.9	84.0	29.8	35.4
		3	2.41	2.9	28	7.2	84.2	29.9	35.5
		4	2.38	2.9	30	7.2	84.5	30.2	35.7
	Rata-rata		**2.40**	**2.8**	**32**	**7.1**	**84.2**	**29.8**	**35.3**
	H15	1	2.40	2.5	29	7.1	84.8	29.6	34.9
		2	2.37	2.4	29	7.1	85.0	30.1	35.4
		3	2.47	2.6	56	7.3	85.6	29.5	34.5
		4	2.44	2.5	55	7.3	85.6	30.1	35.1
	Rata-rata		**2.42**	**2.5**	**42**	**7.2**	**85.3**	**29.8**	**35.0**
	H20	1	2.34	2.5	52	7.0	86.1	30.2	35.1
		2	2.52	2.4	54	7.3	86.3	28.9	33.5
		3	2.44	2.3	49	7.1	85.5	29.3	34.3
		4	2.43	2.4	46	7.1	85.6	29.3	34.2
	Rata-rata		**2.43**	**2.4**	**50**	**7.1**	**85.9**	**29.4**	**34.3**
	H25	1	2.41	2.1	44	7.1	86.3	29.6	34.3
		2	2.39	2.1	52	7.1	85.9	29.9	34.8
		3	2.37	2.2	57	7.0	85.9	29.8	34.6
		4	2.35	2.0	49	7.0	86.0	29.9	34.7
	Rata-rata		**2.38**	**2.1**	**51**	**7.1**	**86.0**	**29.8**	**34.6**
	H30	1	2.37	2.5	82	7.1	88.0	29.8	33.9
		2	2.44	2.4	79	7.1	87.5	29.4	33.6
		3	2.39	2.3	78	7.1	87.5	29.6	33.8
		4	2.47	2.2	75	7.2	87.7	29.2	33.3
	Rata-rata		**2.42**	**2.4**	**79**	**7.1**	**87.7**	**29.5**	**33.7**

The results of the examination of the hematological profile of control blood with the addition of Alsever (1 : 1) can be shown in Table 4 and 5

Table 4 Results of Alsever's control blood examination (1:1)

Treatment	Hari	R	Eritrosit (10^12/l)	Leukosit (10^9/l)	Trombosit (10^9/l)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Darah Alsever (1 : 1)	H0	1	1.97	3.0	17	6.0	72.8	30.4	41.8
		2	1.96	3.3	17	5.9	74.7	30.2	40.4
		3	1.98	2.6	19	5.9	74.0	29.9	40.4
		4	1.94	3.0	16	6.0	74.5	30.9	41.5
	Rata-rata		**1.96**	**3.0**	**17**	**6.0**	**74.0**	**30.4**	**41.0**
	H5	1	1.97	2.6	22	5.9	71.3	30.2	42.4
		2	2.05	2.6	20	6.1	72.8	29.8	40.9
		3	2.06	2.7	20	6.0	72.4	29.3	40.5
		4	2.03	2.6	21	6.0	72.4	29.5	40.8
	Rata-rata		**2.03**	**2.6**	**21**	**6.0**	**72.2**	**29.7**	**41.2**
	H10	1	1.98	2.8	30	6.0	72.0	30.4	42.2
		2	2.02	2.6	26	6.1	72.3	30.3	41.9
		3	2.02	2.6	23	6.0	71.9	29.9	41.6
		4	2.00	2.6	22	6.0	71.9	30.3	42.1
	Rata-rata		**2.01**	**2.7**	**25**	**6.0**	**72.0**	**30.2**	**42.0**
	H15	1	2.00	2.3	25	6.0	72.1	30.1	41.8
		2	1.95	2.5	22	6.0	71.5	31.0	43.4
		3	1.96	2.4	26	6.0	73.3	30.9	42.1
		4	1.98	2.3	27	6.1	73.0	30.7	42.1
	Rata-rata		**1.97**	**2.4**	**25**	**6.0**	**72.5**	**30.7**	**42.4**
	H20	1	1.96	2.2	30	6.0	73.2	30.7	42.0
		2	1.94	2.1	34	6.0	73.0	30.8	42.1
		3	1.95	2.1	28	5.9	72.7	30.5	42.0
		4	1.98	2.1	24	5.9	72.2	30.1	41.6
	Rata-rata		**1.96**	**2.1**	**29**	**6.0**	**72.8**	**30.5**	**41.9**
	H25	1	1.98	2.1	28	6.0	73.0	30.4	41.7
		2	1.94	2.1	31	6.0	72.8	30.9	42.4
		3	1.97	2.1	28	6.0	72.2	30.5	42.3
		4	1.97	1.8	29	5.9	72.2	30.1	41.7
	Rata-rata		**1.97**	**2.0**	**29**	**6.0**	**72.6**	**30.5**	**42.0**
	H30	1	2.01	2.2	39	6.0	74.2	29.8	40.1
		2	1.95	1.9	38	6.0	73.7	30.9	42.0
		3	1.97	2.0	33	6.0	73.3	30.4	41.5
		4	1.96	2.0	38	6.0	73.1	30.8	42.1
	Rata-rata		**1.97**	**2.0**	**37**	**6.0**	**73.6**	**30.5**	**41.4**

Table 5. Alsever control blood test results (1:1.5)

Treatment	Hari	R	Eritrosit	Leukosit	Trombosit	Hb	MCV	MCH	MCHC
Darah Alsever (1 : 1,5)	H0	1	1.58	2.3	14	4.8	56.5	30.4	53.7
		2	1.55	2.2	12	4.7	56.0	30.6	54.6
		3	1.60	2.1	11	4.8	56.2	30.1	53.6
		4	1.56	2.2	12	4.8	55.5	30.7	55.2
	Rata-rata		**1.57**	**2.2**	**12**	**4.8**	**56.1**	**30.5**	**54.3**
	H5	1	1.57	2.2	24	4.8	56.3	30.6	54.3
		2	1.59	2.2	27	4.7	56.0	30.0	53.5
		3	1.59	2.2	22	4.8	56.2	30.5	54.3
		4	1.58	2.2	26	4.8	56.3	30.3	53.8
	Rata-rata		**1.58**	**2.2**	**25**	**4.8**	**56.2**	**30.4**	**54.0**
	H10	1	1.62	2.2	25	4.8	55.7	29.9	53.7
		2	1.57	2.0	24	4.8	55.5	31.0	55.8
		3	1.60	2.1	20	4.8	55.4	30.0	54.2
		4	1.58	2.2	23	4.9	55.6	30.8	55.5
	Rata-rata		**1.59**	**2.1**	**23**	**4.8**	**55.6**	**30.4**	**54.8**
	H15	1	1.56	1.9	22	4.9	55.6	31.5	56.6
		2	1.58	1.9	21	4.8	55.3	30.7	55.5
		3	1.61	1.8	26	4.8	56.0	30.1	53.7
		4	1.59	1.8	23	4.9	56.2	31.1	55.3
	Rata-rata		**1.59**	**1.9**	**23**	**4.9**	**55.8**	**30.9**	**55.3**
	H20	1	1.58	1.6	25	4.8	55.8	30.7	55.1
		2	1.56	1.6	20	4.9	55.7	31.4	56.3
		3	1.57	1.6	21	4.9	55.7	31.3	56.2
		4	1.59	1.5	21	4.9	55.9	30.8	55.0
	Rata-rata		**1.58**	**1.6**	**22**	**4.9**	**55.8**	**31.1**	**55.7**
	H25	1	1.56	1.4	20	4.8	55.9	30.8	55.1
		2	1.58	1.4	21	4.8	56.3	30.6	54.3
		3	1.57	1.5	21	4.9	56.0	31.1	55.6
		4	1.56	1.4	24	4.8	55.9	30.9	55.2
	Rata-rata		**1.57**	**1.4**	**22**	**4.8**	**56.0**	**30.9**	**55.1**
	H30	1	1.62	1.3	22	4.9	55.7	30.2	54.2
		2	1.61	1.3	23	4.8	56.0	30.3	54.1
		3	1.62	1.3	23	4.9	56.2	30.3	53.9
		4	1.56	1.2	26	4.8	55.8	31.2	55.9
	Rata-rata		**1.60**	**1.3**	**24**	**4.9**	**55.9**	**30.5**	**54.5**

4. Statistical Data Processing

The results of the data obtained from this study were tested for normality to find out the data obtained had a normal distribution or not. After obtaining a normal distribution of data, the data was analyzed using the GLM-Repeated Measures test

a. Normality test

The results of the normality test are shown in table 6 below

Table 6 Data Normality Test

Parameter	Value of Sig. on the day						
	0	5	10	15	20	25	30
Erythrocyte count	0.115	0.117	0.131	0.135	0.192	0.106	0.082
Hb Level	0.117	0.106	0.103	0.120	0.128	0.085	0.062
MCV	0.229	0.228	0.301	0.394	0.378	0.347	0.512
MCH	0.762	0.671	0.864	0.916	0.441	0.973	0.994
MCHC	0.370	0.242	0.367	0.369	0.414	0.335	0.381
Leucocyte	0.493	0.208	0.035	0.017	0.032	0.017	0.062
Thrombocyte	0.008	0.072	0.012	0.050	0.106	0.280	0.237

- If the value of Sig. > 0.05, the data is normally distributed
- If the value of Sig. < 0.05, the data is not normally distributed

All measurement data are normally distributed, then the stability test is tested using the General Linear Method – Repeated Measures.

b. Stability Test

Stability test against artificial control materials made from control blood plus artificial Alsever in various concentrations can be shown in the Table 7-13

Table 7. GLM Test Results Erythrocyte Count

Group	Treatment	Sig. Value	Result
day 5 vs day 0	Without Alsever	0,507	P > 0,05
	Alsever 1	**0,027**	**P < 0,05**
	Alsever 2	0,058	P > 0,05
	Alsever 3	0,474	P > 0,05
day 10 vs day 0	Without Alsever	0,895	P > 0,05
	Alsever 1	0,647	P > 0,05
	Alsever 2	0,037	**P < 0,05**
	Alsever 3	0,092	P > 0,05
day 15 vs day 0	Without Alsever	0,126	P > 0,05
	Alsever 1	0,099	P > 0,05
	Alsever 2	0,546	P > 0,05
	Alsever 3	0,368	P > 0,05
day 20 vs day 0	Without Alsever	0,058	P > 0,05
	Alsever 1	0,243	P > 0,05
	Alsever 2	0,796	P > 0,05
	Alsever 3	0,854	P > 0,05
day 25 vs day 0	Without Alsever	0,571	P > 0,05
	Alsever 1	0,935	P > 0,05
	Alsever 2	0,836	P > 0,05
	Alsever 3	0,731	P > 0,05
day 30 vs day 0	Without Alsever	0,126	P > 0,05
	Alsever 1	0,085	P > 0,05
	Alsever 2	0,474	P > 0,05
	Alsever 3	0,103	P > 0,05

Table 8 GLM Test Results Hb level

Group	Treatment	Sig. Value	Result
day 5 vs day 0	Withtout Alsever	0,278	P > 0,05
	Alsever 1	1,000	P > 0,05
	Alsever 2	0,495	P > 0,05
	Alsever 3	0,182	P > 0,05
day 10 vs day 0	Withtout Alsever	0,624	P > 0,05
	Alsever 1	1,000	P > 0,05
	Alsever 2	0,215	P > 0,05
	Alsever 3	0,182	P > 0,05
day 15 vs day 0	Withtout Alsever	0,297	P > 0,05
	Alsever 1	0,391	P > 0,05
	Alsever 2	0,058	P > 0,05
	Alsever 3	0,058	P > 0,05
day 20 vs day 0	Withtout Alsever	0,354	P > 0,05
	Alsever 1	1,000	P > 0,05
	Alsever 2	1,000	P > 0,05
	Alsever 3	0,092	P > 0,05
day 25 vs day 0	Withtout Alsever	0,464	P > 0,05
	Alsever 1	0,058	P > 0,05
	Alsever 2	0,638	P > 0,05
	Alsever 3	0,182	P > 0,05
day 30 vs day 0	Withtout Alsever	0,064	P > 0,05
	Alsever 1	1,000	P > 0,05
	Alsever 2	0,182	P > 0,05
	Alsever 3	0,058	P > 0,05

Table 9 GLM Test Results MCV level

Group	Treatment	Sig.	Result
day 5 vs day 0	Without Alsever	0,297	P > 0,05
	Alsever 1	0,243	P > 0,05
	Alsever 2	**0,001**	**P < 0,05**
	Alsever 3	0,547	P > 0,05
day 10 vs day 0	Without Alsever	0,005	P > 0,05
	Alsever 1	0,391	P > 0,05
	Alsever 2	**0,016**	**P < 0,05**
	Alsever 3	0,100	P > 0,05
day 15 vs day 0	Without Alsever	**0,000**	**P < 0,05**
	Alsever 1	**0,004**	**P < 0,05**
	Alsever 2	0,081	P > 0,05
	Alsever 3	0,497	P > 0,05
day 20 vs day 0	Without Alsever	**0,001**	**P < 0,05**
	Alsever 1	0,121	P > 0,05
	Alsever 2	0,125	P > 0,05
	Alsever 3	0,334	P > 0,05
day 25 vs day 0	Without Alsever	**0,000**	**P < 0,05**
	Alsever 1	**0,033**	**P < 0,05**
	Alsever 2	0,081	P > 0,05
	Alsever 3	0,921	P > 0,05
day 30 vs day 0	Without Alsever	**0,002**	**P < 0,05**
	Alsever 1	**0,002**	**P < 0,05**
	Alsever 2	0,545	P > 0,05
	Alsever 3	0,633	P > 0,05

Table 10 GLM Test Results MCH level

Group	Treatment	Sig.	Result
day 5 vs day 0	Without Alsever	0,638	P > 0,05
	Alsever 1	0,051	P > 0,05
	Alsever 2	0,090	P > 0,05
	Alsever 3	0,703	P > 0,05
day 10 vs day 0	Without Alsever	0,184	P > 0,05
	Alsever 1	0,384	P > 0,05
	Alsever 2	0,492	P > 0,05
	Alsever 3	0,903	P > 0,05
day 15 vs day 0	Without Alsever	0,174	P > 0,05
	Alsever 1	0,294	P > 0,05
	Alsever 2	0,404	P > 0,05
	Alsever 3	0,206	P > 0,05
day 20 vs day 0	Without Alsever	**0,012**	**P < 0,05**
	Alsever 1	0,101	P > 0,05
	Alsever 2	0,635	P > 0,05
	Alsever 3	0,094	P > 0,05
day 25 vs day 0	Without Alsever	0,194	P > 0,05
	Alsever 1	0,213	P > 0,05
	Alsever 2	0,741	P > 0,05
	Alsever 3	0,161	P > 0,05
day 30 vs day 0	Without Alsever	0,238	P > 0,05
	Alsever 1	**0,025**	**P < 0,05**
	Alsever 2	0,701	P > 0,05
	Alsever 3	0,804	P > 0,05

Table 11 GLM Test Results MCHC level

Group	Treatment	Sig.	Result
day 5 vs day 0	Without Alsever	0,495	P > 0,05
	Alsever 1	0,202	P > 0,05
	Alsever 2	0,701	P > 0,05
	Alsever 3	0,625	P > 0,05
day 10 vs day 0	Without Alsever	**0,036**	**P < 0,05**
	Alsever 1	0,881	P > 0,05
	Alsever 2	0,360	P > 0,05
	Alsever 3	0,133	P > 0,05
day 15 vs day 0	Without Alsever	**0,002**	**P < 0,05**
	Alsever 1	0,235	P > 0,05
	Alsever 2	0,138	P > 0,05
	Alsever 3	0,227	P > 0,05
day 20 vs day 0	Without Alsever	**0,002**	**P < 0,05**
	Alsever 1	0,080	P > 0,05
	Alsever 2	0,130	P > 0,05
	Alsever 3	0,100	P > 0,05
day 25 vs day 0	Without Alsever	**0,002**	**P < 0,05**
	Alsever 1	0,090	P > 0,05
	Alsever 2	0,168	P > 0,05
	Alsever 3	0,254	P > 0,05
day 30 vs day 0	Without Alsever	**0,000**	**P < 0,05**
	Alsever 1	**0,004**	**P < 0,05**
	Alsever 2	0,621	P > 0,05
	Alsever 3	0,412	P > 0,05

Table 12 GLM Test Results Leucocyte level

Group	Treatment	Sig.	Result
day 5 vs day 0	Without Alsever	0,824	P > 0,05
	Alsever 1	**0,005**	**P < 0,05**
	Alsever 2	0,125	P > 0,05
	Alsever 3	1,000	P > 0,05
day 10 vs day 0	Without Alsever	0,250	P > 0,05
	Alsever 1	**0,001**	**P < 0,05**
	Alsever 2	0,118	P > 0,05
	Alsever 3	0,215	P > 0,05
day 15 vs day 0	Without Alsever	0,088	P > 0,05
	Alsever 1	**0,000**	**P < 0,05**
	Alsever 2	**0,021**	**P < 0,05**
	Alsever 3	**0,001**	**P < 0,05**
day 20 vs day 0	Without Alsever	0,724	P > 0,05
	Alsever 1	**0,000**	**P < 0,05**
	Alsever 2	**0,010**	**P < 0,05**
	Alsever 3	**0,001**	**P < 0,05**
day 25 vs day 0	Without Alsever	0,858	P > 0,05
	Alsever 1	**0,000**	**P < 0,05**
	Alsever 2	0,011	**P < 0,05**
	Alsever 3	**0,001**	**P < 0,05**
day 30 vs day 0	Without Alsever	0,763	P > 0,05
	Alsever 1	**0,000**	**P < 0,05**
	Alsever 2	**0,011**	**P < 0,05**
	Alsever 3	**0,000**	**P < 0,05**

Table 13 GLM **Test Results Thrombocyte level**

Group	Treatment	Sig.	Result
day 5 vs day 0	Without Alsever	0,604	P > 0,05
	Alsever 1	**0,033**	**P < 0,05**
	Alsever 2	**0,035**	**P < 0,05**
	Alsever 3	**0,002**	**P < 0,05**
day 10 vs day 0	Without Alsever	**0,035**	**P < 0,05**
	Alsever 1	0,056	P > 0,05
	Alsever 2	**0,026**	**P < 0,05**
	Alsever 3	**0,000**	**P < 0,05**
day 15 vs day 0	Without Alsever	**0,001**	**P < 0,05**
	Alsever 1	0,102	P > 0,05
	Alsever 2	**0,008**	**P < 0,05**
	Alsever 3	**0,006**	**P < 0,05**
day 20 vs day 0	Without Alsever	0,870	P > 0,05
	Alsever 1	**0,001**	**P < 0,05**
	Alsever 2	**0,011**	**P < 0,05**
	Alsever 3	**0,001**	**P < 0,05**
day 25 vs day 0	Without Alsever	**0,018**	**P < 0,05**
	Alsever 1	**0,002**	**P < 0,05**
	Alsever 2	**0,002**	**P < 0,05**
	Alsever 3	**0,005**	**P < 0,05**
day 30 vs day 0	Without Alsever	**0,031**	**P < 0,05**
	Alsever 1	**0,000**	**P < 0,05**
	Alsever 2	**0,002**	**P < 0,05**
	Alsever 3	**0,003**	**P < 0,05**

Note:

- Alsever 1 = blood control: Alsever (1,5 : 1)
- Alsever 2 = blood control: Alsever (1 : 1)
- Alsever 3 = blood control: Alsever (1 : 1,5)

Decision making basis:

- H0: There is no significant difference between the average value of the examination results on the day of measurement and the average value of the results on day 0.
- • If p > 0.05, then H0 is accepted
- • If p < 0.05, then H0 is rejected

DISCUSSION

The description of the hematological profile of control blood with the addition of variations in the concentration of artificial Alsever solution can be explained:

The description of the number of erythrocytes in control blood without Alsever and with the addition of artificial Alsever 1, 2 and 3 there is no significant difference, only occurs in Alsever 1 on the 5th day and alsever 2 on the 10th. This is likely due to changes in erythrocyte cells either crenation or lysis that will affect the number of erythrocytes. This also depends on the classification of erythrocyte units, namely young erythrocytes (< 14-21 days) and old erythrocytes (> 21 days), where storage time affects erythrocyte quality, such as the presence of hemolysis and membrane stiffness), which can directly affect transport capacity. gas and erythrocyte clearance, and induces the appearance of molecular patterns associated with erythrocyte cell damage. However, in general the control blood was stable until day 30. Artificial Alsever used with variations in concentrations of 1, 2 and 3, was good enough to stabilize control blood, it's just that the difference in the number of erythrocytes between control blood and control blood was added with Alsever variation. concentrations of 1, 2 and 3. Alsever was able to stabilize control blood until the 30th day, because Alsever's composition contains compounds that are isotonic and function as anticoagulants and preservatives. Alsever can also function as a suspension medium and stabilizer erythrocyte (Lorne). This is also in line with the results of research by Fauziyyah et al (2019), about the stability of erythrocyte morphology in commercial and artificial Alsever solutions.

The results of the examination of Hemoglobin (Hb) levels in control blood without Alsever and control blood with the addition of artificial Alsever 1, 2 and 3 were almost perfect and there were no significant differences in all of them on days 0-30 days. For this reason, Alsever is very well used for Hb control media, because even if there are changes or disturbances in erythrocyte cells, of course, it will still be measured as Hb levels, so Hb levels remain stable until 30th day in all treatments.

The description of MCV index in control blood without Alsever and with the addition of Alsever 1 there was no statistical difference until the 10th day. Control blood with the addition of Alsever 2 was not statistically stable on 5th day. While the control blood with the addition of Alsever 3 was statistically stable until the 30th day. This is influenced by the number of erythrocytes which is also unstable until 5th day. With the addition of Alsever 3 in a ratio of 1:1.5, the blood is more stable and the stability reaches on 30th day.

The MCH index in control blood without Alsever showed no statistical difference until the 15th day and was stable until the 15th day, while on the 20th day it was statistically unstable. Control blood with the addition of Alsever 1 was stable until 25th day, while control blood with Alsever 2 and 3 was stable until 30th day. This was because the hemoglobin level was also stable in almost all treatments, either stored blood without Alsever or with the addition of Alsever 1.2 and 3.

The description of the MCHC index in control blood without Alsever showed a statistically significant difference on the 10th day of measurement, so it was stable only until the 5th day. Control blood with the addition of Alsever 1 had a statistically significant difference on the 30th day meaning it was stable until the 25th day, while the control blood with the addition of Alsever 2 and 3 was stable until the 30th day of storage. This is due to the effect of Hb levels on the calculation of the MCHC Index, where Hb levels are very stable.

The description of the Leukocyte Count in control blood without the addition of Alsever there was no statistically significant difference during 30 days of storage, the leukocyte count was stable until the 30th day, while in control blood with the addition of Alsever 1 there was a statistically significant difference on the 5th day, meaning that it was already unstable until 5th day.

Control blood with the addition of Alsever 2 and 3 there was a statistically significant difference on the 15th day and was stable until the 10th day. From these results, it can be seen that stored blood without Alsever was more stable, the number of leukocytes was stable until the 30th day, while the addition of Alsever caused the blood to become unstable and the leukocytes were significantly different on the 5th day. This is partly because the stored blood can undergo degenerative changes. These changes are not only caused by the presence of anticoagulants, but also because the blood is distorted (Dacie et al., 1994).

In the results of the study, it can be seen that the description of the Platelet Count in the control blood without Alsever and Alsever 2 and 3 shows that there is no statistically significant difference until the 5th day so that it is still quite stable until the 5th day, while the control blood with Alsever 1 has no difference. which was statistically significant until day 15, meaning that it was stable until 15th day. Alsever 1 with a ratio of 1.5 and 1 showed more stable platelet count results, because the ratio of blood counts was higher than Alsever's solution. This can be explained that Alsever's solution with a certain ratio with blood has a function as anti-platelet aggregation (Grandiosa, et al, 2018) so that it can achieve long-lasting stability and can last up to the 15th day.

Conclusions

Control blood with the addition of artificial Alsever with variations in concentration can be used to calculate the number of erythrocytes, Hb levels, MCV MCH and MCHC, as well as the number of leukocytes and platelets with stability until day 30

Acknowledgements

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