The Effects of Environmental Temperature Exposure of Blood Spatter Towards Protein and Agglutinations Level in ABO Blood Typing

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Abstract: The time needed for gathering evidence of a crime is an obstacle in the process of identifying the blood type in the blood spots on the cloth. The blood will dry out after contact with the outside air within 3-5 minutes and change the color from red to dark brown. Hence, the aim of this study was to investigate the effect of exposure to environmental temperature on the levels of protein and agglutination in ABO blood typing of blood spots after 0 (1 hour after blood drops on cloth), 5, 10, 15, and 20 days. Time series design was used in this study using 30 blood spots on cotton from 1 individual subject (blood type A). Blood samples were incubated at room temperature (23-24º C) (15 samples) and other ones were incubated at ambient temperature (exposed to sunlight and rain). Determination of protein level was performed with a UV spectrophotometer using trizole reagent. The agglutination level was examined by elution absorption method using antisera A and read macroscopically. One Way ANOVA and Kruskal Wallis test were performed in this trial and conclude that there was no significant effect to protein level (p>0.01). Based on the trial ABO blood typing can still be performed for all blood ages (0, 5, 10, 15, 20 days).

1 INTRODUCTION

Murder cases in Indonesia tended to increase from 1,277 cases in 2014 to 1,491 cases in the next year (BPS, 2016). Examining the crime scene, the evidence commonly found in cases of murder is blood spots. Blood spots on the objects around the victim are often disguised or even removed by the perpetrator by throwing the victim's clothes away. Hence, the blood spots could become vague or unseen (Yudianto, 2013). The blood will dry out within 3-5 minutes after contact with the outside air. Once blood dries up, the color changes from red to dark brown (Princess and Ketut, 2015). In the forensic investigation, one of the most common blood spot examinations is determining the blood type (Knight, 2001). Blood typing can be done quickly and cheaply; however, it can provide accurate data to assist an investigation process (Yudianto, 2013).

The human blood type is grouped according to several blood typing systems. In 1900, the man who first discovered the ABO blood typing was Landsteiner. With the development of medical science, many blood typing systems are found, namely Rhesus (Rh), M and N, Kell, Duffy, and Lewis (Knight, 2001). ABO blood type examination of a sample of blood spots is still an important technique for the identification of corpses in criminal cases. Blood type examination in blood spots can be done by direct agglutination or elution absorption method (Nishi et al., 2005). The former method will become more difficult when the red blood cells lysis because of the sunlight exposure. However, as long as the antigen in the blood spots is still attached to the clothes, the antigen can still be detected.

Different levels of protein in dried blood over periods of time can be affected by several factors such as the occurrence of microbes that lead to cell degradation, UV exposure of the sun, and ambient temperature. Putri and Ketut (2015) stated that the

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decreasing protein level in dried blood can occur due to the activity of microorganisms as time goes by. Protein in the dried blood can stand up to four months, but there is no further data regarding the effect of environmental temperature exposure to the dried blood. Hence, the aim of this study was to determine the effect of the exposure on the level of protein and agglutination in ABO blood typing of blood spots after 0 (2 hours after blood drops on a cloth and exposed to the room temperature), 5, 10, 15, and 20 days. The maximum exposure time of 20 days was chosen based on Article 24 paragraph (1) of Indonesian Criminal Procedural Code, which regulates the detention duration of a suspect in the interest of the examination shall only be valid for a maximum period of twenty days.

2 MATERIAL AND METHOD

This study was laboratory experiments using control time series design. In this study, the independent variable is defined as the exposure time of 0 days (1 hour after blood drops on the cloth), 5 days (5x24 hours), 10 days (10x24 hours), 15 days (15x24 hours) and 20 days (20x24 hours), while the dependent variable is the protein and agglutination level in ABO blood typing. The confounding variable is room temperature (23–24ºC) and the ambient temperature (exposed to sunlight and rain). Samples of 30 blood spots on cotton were obtained from one individual subject (blood type A). Blood samples were incubated at room temperature (15 samples) and the others were incubated at ambient temperature. This study was conducted at Human Genetic Laboratory Institute of Tropical Disease Center Airlangga University Surabaya. Samples which had been exposed to room or ambient temperature at a given period of time were then tested for the protein level with a UV spectrophotometer using trizole reagent. While the agglutination level was examined by elution absorption method using antisera A and read macroscopically. The data from samples incubated at room temperature was analyzed using One Way ANOVA, while the others were analyzed using Kruskall Wallis.

3 RESULT AND DISCUSSION

Measurement of protein concentration using UV spectrophotometer from sample of blood spatter of both room (23-24ºC) and climate exposure (exposed to sun and rain) for 0 days (1 hour after blood drip on cloth), 5th day, 10th day, 15th day and 20th day. Shapiro Wilk normality test is performed to determine whether the data is normally distributed or not.

Table 1: Means of protein concentration at room temperature. (23 – 24ºC)

No	Days	Protein (mg / ml)
1	0	4.77
2	5	3.85
3	10	2.01
4	15	1.86
5	20	1.52

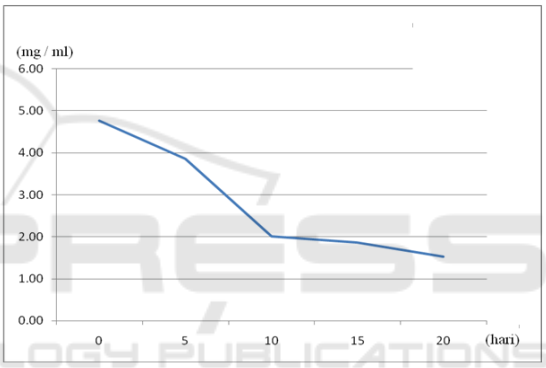

Figure 1: Protein concentration graphic at room temperature. (23 – 24ºC)

Based on Shapiro Wilk normality test, results obtained all times of exposure significance (0 = 0.484, 5 = 0.888, 10 = 0.618, 15 = 0.502, 20 = 0.619) variables show the significance value (Sig)> 0.01, all the is normally distributed

Based on the homogeneity test, the value of significance (sig) was 0.109> 0.01, it can be concluded that the protein content from the sample at room temperature (23-240C) from all times of exposure (0, 5, 10, 15, 20 days) fulfills the assumption of homogenity in One Way Anova test.

The results of One Way Anova test, prove that the significance value (sig) is 0.119> 0.01. Alternative hypothesis is rejected, it can be concluded that there is no effect of long exposure at room temperature (23-24ºC) significantly to protein concentration for all time variables (0, 5, 10, 15, 20 days).

Table 2: Means of Agglutination level at room temperature (23-24° C)

No	Days	Aglutination
1	0	+4
2	5	+3
3	10	+1
4	15	+1
5	20	+1

Shapiro Wilk normality test is performed to determine whether there is correlation of exposure time's effect on the sample of blood spatter on the cotton fabric left at ambient temperature (exposed to sun and rain) to the protein content.

Table 3: Means of protein concentration at ambient temperature (exposed to sun and rain)

No	Days	Protein (mg / ml)
1	0	2.50
2	5	2.90
3	10	2.06
4	15	1.86
5	20	1.76

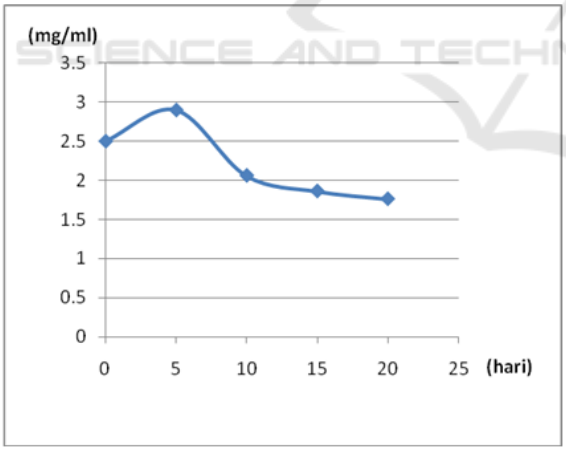

Figure 2: concentration graphic at ambient temperature (exposed to sun and rain)

Shapiro Wilk normality test shows all the variable exposure times value significance (sig)> 0.01, (0 = 0.054, 5, 10 = 0.172, 15 = 0.605, 20 = 0.301). The data distribution of this research is normally distributed.

Based on homogeneity test, the value of significance (sig) is 0.003. Protein level from the sample of blood spatter left at ambient temperature (exposed to sunlight and rain) at 0, 5, 10, 15, 20 days is heterogeneous. The assumption of homogenicity in One Way Anova test is not fulfilled. The results of protein concentration were tested by non-parametric test using Kruskall Wallis test.

Kruskall Wallis test obtained significance value (sig) of 0.833> 0.01, then null hypothesis is accepted and alternative hypothesis is rejected, it can be concluded that there is no effect of exposure time at ambient temperature (exposed to sunlight and rain) protein levels in the blood spots on the cloth left within 0, 5, 10, 15, 20 days.

Table 4: Means of agglutination level at ambient temperature (exposed to sun and rain)

No	Days	Aglutination
1	0	+4
2	5	+1
3	10	+1
4	15	+1
5	20	+1

The result show that the protein level of blood spots exposed to the room temperature slightly decreased over the given period of time. However, a statistical test of One Way Anova did not show significant difference (p > 0,01). It means that protein denaturation process can occur at room temperature very slowly; therefore, the decrease will not be significant indeed.

On the other hand, the other samples, which are exposed to the environmental temperature depicted a fluctuation level of protein. Thus, Kruskall Wallis statistical test yielded no significant difference (p > 0,01), which means there is no correlation between exposure time and the protein level of blood spots. The uncertain environmental temperature in the presence of exposure to sunlight and rain can accelerate the process of decay. The most optimal degradation process occurs at a temperature of 70-100°F or equivalent to 21-38°C. At those temperatures, the chemical breakdown process of tissue and the development of microorganisms will help the occurrence of decay. At temperatures below 50°F (0°C) or above 100°F (45°C) the decomposition process becomes slower due to inhibition of microorganism growth (Aziz, 2014).

4 CONCLUSIONS

From the discussion above, we can conclude that the room temperature exposure did not affect the protein level of the blood spots significantly. Furthermore, there is no correlation between ambient temperature exposures to the blood spots. The blood type of the samples can still be detected after 20 days of exposure both at room and ambient temperature.

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