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## **The quality of Haemoglobin examination results with variations in incubation time using the Cyanmethemoglobin method**

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### **Abstracts**

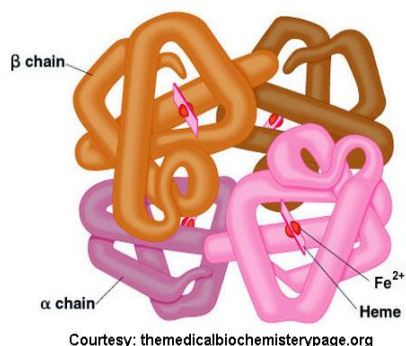
Anaemia is a condition where blood red blood levels or haemoglobin is lower than normal values. More than 50% of cases of anaemia are spread throughout the world. One of checks to diagnose anaemia is examination of haemoglobin levels. Examination of haemoglobin levels can be determined by several methods, namely the Sahli's method, the Cyanmethemoglobin method manually and by automatic method. Examination of haemoglobin levels with a of more than 5 minutes incubation time leads to inaccurate results. The purpose of this research is to determine the effect of incubation time on the results of haemoglobin level examination of the Cyanmethemoglobin method. This type of research is experimental research to determine the effect of the incubation time on the results of haemoglobin level examination of the Cyanmethemoglobin method. The population in this research is outpatients with a diagnosis of kidney failure at Gatoel Hospital, Mojokerto, in March 2019. The number of examination samples is 9 samples which is carried out at the Gatoel Hospital Laboratory, Mojokerto. The results of this research show that the average haemoglobin incubation rate of 5 minutes is 7,52 g/dl, incubation of 20 minutes is 7,18 g / dl, and incubation of 30 minutes is 7,04 g / dl. The conclusion of this research is that there is no effect on the delay in incubation time on the results of the haemoglobin level examination of the Cyanmethemoglobin method.

**Keywords:** haemoglobin, cyanmethemoglobin, anaemia

## 1. Introduction

The development of technology among the community is in line with the expected demands, including in the health sector, especially in its services. Quality assurance of laboratory examinations requires proper diagnosis, including sample handling time [1]

Haemoglobin is a red pigmented protein found in red blood cells that functions to transport oxygen from the lungs to the tissues and transport carbon dioxide from the tissues to the lungs [2]. In the laboratory, haemoglobin examination is included in a routine blood test that is often carried out. Haemoglobin levels in the blood can be observed by several manual methods including the tallquist method, the sahli method and the Cyanmethemoglobin method and can also be done automatically [3]. Circulation of haemoglobin in the blood that is below normal 12-15 g/dl can cause a person to develop anemia. The prevalence rate of anemia in Indonesia is 11.9 g/dl [4].



**Figure 1.** Haemoglobin Molecule and Heme Structure (Source: themedicalbiochemistry.org)

Anemia is a condition where the level of red blood substance or haemoglobin is lower than the normal value. According to the Basic Health Research reference [5] more than 50% of cases of anemia spread throughout the world are directly caused by iron deficiency. Iron deficiency can cause interference with the growth of both body cells and brain cells. In Indonesia, nutritional deficiency anemia is still one of the main nutritional problems. The prevalence of anemia in Indonesia in 2007 was

11.9%. For clinical purposes, the Cyanmethemoglobin method is easy to perform and the results are more accurate than the Sahli method. The Cyanmethemoglobin method is a reference method for haemoglobin estimation, all types of haemoglobin can be measured except sulphaemoglobin [3]

Almost all laboratories use a spectrophotometer to check haemoglobin using the Cyanmethemoglobin method. And we often find that in the laboratory, officers often ignore the time for complete blood tests and the incubation time of the examination, for example in the examination of haemoglobin levels which is caused by several factors, namely the number of patients in the laboratory so that the officers ignore the incubation time of approximately 20-30 minutes [6]. By ignoring the incubation time which is too long in haemoglobin examination, the Cyanmethemoglobin method is feared to get inappropriate results [7].

Knowing the importance of haemoglobin levels in the blood for the prevention and treatment of a disease, the researchers conducted research on the quality of haemoglobin examination results with variations in incubation time using the Cyanmethemoglobin method.

## 2. Experiments Procedure

This type of research is experimental, which is to find out the influence of incubation time on the results of haemoglobin levels of the Cyanmethemoglobin method. The process of tabulation and analysis of data using the SPSS program with statistical tests used is analysis of variance (ANOVA) to find out whether there is an influence of incubation time on the results of haemoglobin levels examination Cyanmethemoglobin method.

## 3. Result and Discussion

### Result

After examining the effect of incubation time on the results of the examination of haemoglobin levels using the Cyanmethemoglobin method at the Gatoel

Mojokerto Hospital Laboratory in March, 27 samples obtained the following results:

**Table 1.** Average Haemoglobin Levels with Differences in Incubation Time

Sample	Haemoglobin Level g/dl		
	Incubation 5 minutes	Incubation 20 minutes	Incubation 30 minutes
Average	7,52	7,18	7,04
SD	1,017	1,057	1,035

Based on the table, it can be seen that the average haemoglobin for 5 minutes incubation was 7.52 g/dl, 20 minutes incubation was 7.18 g/dl and 30 minutes incubation decreased to 4.05.

After getting the results of the examination of haemoglobin levels at the incubation time, then proceed with the normality test of the data using the Shapiro-Wilk Test. If the results of the data are normally distributed, then proceed with the Anova test. It is said to be significant if the value of  $\text{sig} > 0.05$  then the data is normally distributed, if the value of  $\text{sig} < 0.05$  then the data is not normally distributed.

The results of the normality test using the Shapiro-Wilk Test on haemoglobin level examination data with a delay in incubation time obtained a significance of 5 minutes = 0.132, 20 minutes = 0.089, 30 minutes = 0.106. So that the data is normally distributed. This is indicated by a significant level, the value of  $\text{sig} > 0.05$ . After the data is known to be normally distributed, it is continued using the Anova test.

From the results of the ANOVA test, the examination of haemoglobin levels using the Cyanmethemoglobin method was found to have a significant value of  $0.611 > 0.05$ , so that the results showed that there was no effect of incubation time on the results of the examination of haemoglobin levels using the Cyanmethemoglobin method.

Thus, it can be concluded that  $H_a$  is rejected,  $H_0$  is accepted, which means that there is no effect of incubation time on the results of the examination of haemoglobin levels using the Cyanmethemoglobin method with a time of 5, 20, and 30 minutes.

## Discussion

Based on the results of the examination of haemoglobin levels at 5 minutes incubation, it was obtained an average of 7.52 g/dl. Incubation of 20 minutes obtained an average of 7.18 g/dl. Incubation of 30 minutes obtained an average of 7.04 g/dl. From these results, it can be seen that the 5-minute incubation was higher than the 20-minute incubation, the lowest 20-minute incubation, and an increase in the 30-minute incubation delay.

The data from the examination were then statistically tested using the ANOVA statistical test with SPSS program, and the test results showed a number of 0.611 ( $\alpha > 0.05$ ), which means that the results of haemoglobin examination using the Cyanmethemoglobin method with a delay of incubation time of 5, 20, 30 minutes are significant. This means that  $H_0$  is accepted, which means that there is no significant effect based on the incubation time delay on the results of the examination of haemoglobin levels using the Cyanmethemoglobin method. It is said that there is no significant effect because there is a provision if the value is significant  $> 0.05$ , then  $H_0$  is accepted, which means there is no effect.

From the results of the ANOVA statistical test, it did not show a significant difference. However, from the study of the effect of incubation time on the results of the examination of haemoglobin levels with the Cyanmethemoglobin method, which was carried out for 5 minutes incubation, 20 minutes incubation and 30 minutes the average results for the 3 times still showed differences. The difference was due to the incubation process that was not in accordance with the procedure because the perfect reaction process only occurred within 5 minutes, the color formed was very stable and could be measured by a spectrophotometer [8].

In the examination of haemoglobin levels, the Cyanmethemoglobin method shows that the difference between incubation for 5 minutes, 20 minutes, and 30 minutes is only 0.5 g/dl to 1 g/dl, which means the difference is very small and not significant. In this examination, the researcher found a problem, namely that one of

the hospital laboratories in Mojokerto did not use a standard solution for the examination of haemoglobin Cyanmethemoglobin, even though it did not use a standard solution when the examination of haemoglobin Cyanmethemoglobin was replaced using control blood.

When the researchers conducted a haemoglobin examination using the Cyanmethemoglobin method at one of the hospitals in Mojokerto, the researchers used a room temperature of 20-25°C so that the results of haemoglobin levels were still stable at a temperature of 20-25°C. Haemoglobin examination needs to be considered several factors that affect the stability of blood samples so that there are no deviations in the results of the examination. These factors are temperature, blood pipetting, and contamination [9].

Although the results of statistical tests did not show results that had a significant effect, as laboratory personnel, they still had to carry out examinations according to procedures because after statistical tests were carried out there were still differences in the average of the three incubation times, although only slightly and not significant. This difference in average will affect the results of inaccurate haemoglobin levels released.

#### 4. Conclusion

Based on the results of the research on the effect of haemoglobin incubation time with the Cyanmethemoglobin method, it can be concluded that there is no effect of incubation time on the results of the examination of haemoglobin levels using the Cyanmethemoglobin method and the average 5-minute incubation haemoglobin level is 7.52 g/dl. The 20 minutes incubation haemoglobin level was 7.18 g/dl. The 30 minutes incubation haemoglobin level was 7.04 g/dl.

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