The Consequences of Using Different Storage Temperature of 5°C and 10°C in The Identification of Blood Group ABO from Dental Pulp Samples

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Abstract: In forensic dentistry, the examination of the blood group ABO from dental pulp samples can be performed on secretors, which relate to people who have antigens and antibodies in their body tissue constructions. However, this case has several factors that can influence the results of the examination of the blood group from dental pulps which only lasts 180 days. The objective is to identify the result differences in storage treatment between the temperatures of 5°C and 10°C for the blood group analysis from the dental pulp samples. This research analyzes blood by using the absorption-elution technique. The 18 dental pulp samples taken from the ABO blood group. Each blood group consists of 6 samples. The research was performed during the period of April-July 2012. The results indicate that there are similarities between the blood pulp samples stored at 5°C and 10°C with the origin blood types. This is confirmed by using the one-way ANOVA test, with p = .884. The examination of the blood group using the dental pulp in a storage temperature of 5°C indicates an accurate result. However, the accuracy decreases if the storage temperature is 10°C.

1 INTRODUCTION

In the investigation of a criminal act, one must provide scientific physical evidence (Wirasuta, 2010). This evidence helps the investigator to identify the perpetrator. Some techniques could be applied, from simple techniques such as blood type analysis, fingerprint analysis and dental identification to a more sophisticated technique such as DNA (deoxyribonucleic acid) analysis (Lukman, 2016).

In forensic dentistry, the blood type of an individual person's dental becomes a personal identification. Theoretically, the blood type comes from the antigen on the surface of a red blood cell. This antigen can interact with a specific antibody (Ballad, Davis, 2011). The utilization of the blood type in medico-legal examination is the primary method to identify individuals (Shetty, 2010).

The sample of a blood type analysis could be obtained from the dental pulp. There are several steps involved, such as sample extraction, preservation and sample storage. To obtain an optimum result, the analysis should be conducted in a short storage time at 5°C (Lukman, 2006). According to Alcemo (1985), the storage of all specimens could be stored at a low temperature between 4°-10°C. However, if the dental pulp storage duration is too long, it will cause the denaturation of the sample by a chemical and microbiological process which will influence the sample's integrity (Shetty, 2010).

Based on that background, the aim of this research is to discover the different outcome on the conditions of the ABO blood type from the dental pulp samples when stored in temperatures between 5° and 10°C.

2 MATERIALS AND METHODS

Permanent dental pulp was used as a sample in the experiment. This dental pulp originated from dental extraction in Bhayangkara Hospital in Kediri City.

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The research was conducted in April - July 2012. The total respondents were 18 individuals. The respondents were divided into 3 groups of 6 based on their blood type: A, B, and O blood. This study's research used the Absorption-Elution method. For the data processing and analysis, One-way ANOVA was used. Moreover, Post Hoc LSD was applied to find the difference between the groups.

Laboratory analysis (Absorption-Elution method) is conducted as follows (Lukman, 2006):

The tooth that still have pulp tissue was taken, then was ground on iron mortar until it became powder. The tooth powder was placed into 3 test tubes. 3 different antisera (α to tube I, β to tube II, γ to tube III). All tubes were stored in a refrigerator at a certain, temperature for 24 hours (overnight), then the reaction was rinsed with a saline solution 7 times for each test tube.

From each test tube the saline solution was removed, but not the precipitate, and put into the 3 test tubes. 2 drops of aquadest were added. The test tubes were heated up to 56°C for 12 minutes using a microwave, then the test tubes were taken out from the microwave.

The indicator cells A, B, and O were inserted into each test tube with a concentration of 3 - 5% each, then, the test tubes were centrifuged until agglutination occurred, in the end, the tubes were observed for when the agglutination occurred, these steps were conducted in the storage condition of 5°C and 10°C.

3 RESULT

The research was conducted in April - July 2012 in the Hematology Laboratory of Baptist Hospital in Kediri City. The total respondents were 18 individuals. The respondents were divided into 3 groups of 6 based on their blood type, A, B and O.

The result from the two storage conditions (5°C and 10°C) is summarized in Table 1.

Table 1. Blood type examination result

No	Original Blood type Respondent	Blood type from Dental pulp Examination	
		Temp. 5°C	Temp. 10°C
1	A	A	A
2	A	A	B
3	A	A	A
4	A	A	A
5	A	A	-
6	A	A	A
7	B	B	B
8	B	B	B
9	B	B	B
10	B	B	B
11	B	B	-
12	B	B	B
13	O	O	O
14	O	O	O
15	O	O	O
16	O	O	-
17	O	O	O
18	O	O	-

All data including the original blood type of the respondents (research control) and the blood type from the dental pulp examination in both storage temperatures (5°C and 10°C) were processed by the parametrically statistic method (One-way ANOVA). This processing method is used to evaluate if the results are significant enough between the two storage conditions and research control.

A statistical data analysis was performed using SPSS software. In the Normality Shapiro-Wilk Test, the significant value showed .097, .089, and .055 so that p value > 0.05. It means all data was distributed normally. The value in homogeneity of the variance test showed .051 so that, p-value > 0.05. It means all treatments have the same variation in this blood type examination. These results met the requirements to perform a One-way ANOVA test.

The result of the One-way ANOVA test showed the mean square between the groups were .020 and .160 and the p-value of .884. This value is more than 0.05. Therefore, it is concluded that the blood test experiment results are significant enough among all three of the treatments. However the mean result was different between storage in 5°C and 10°C.

4 DISCUSSION

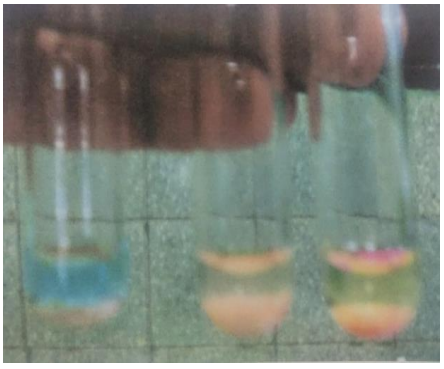

Figure 1. The Result of ABO blood type from dental pulp.

In this research it is known that dental pulp could become the material used in ABO blood type determination by using the absorption-elution technique at a storage temperature of 5°C and 10°C. These findings align with previous research conducted by Inamdar (2011) which showed that the ABO blood type identification was performed by using the same technique. This technique has been applied in Forensic Science for determining the ABO blood type. Moreover, this technique must use a specific temperature.

According to Alfonsius and the research team from Police Medical Laboratory in 1992, ABO blood type examination from a dental pulp sample could be conducted by using the absorption-elution technique which is should be stored at 5°C (Lukman, 2006).

Many research results align with the theory that states dental pulp could be utilized for ABO blood type examination only for 180 days since the date of death. (Shetty, 2010; Ballal and David, 2011).

Based on the blood type examination data, it was known that the dental pulp sample examination after storing it at 5°C resulted in the same result as the original blood type. It was caused by the ability of the temperature to reduce microbiological growth and maintain optimum specimen integrity. In dental pulp there are some types of matrix that produce plasma cell specification such as glycosaminoglycan and fibronectin (Goldberg and Smith, 2004). These substances will not denature so that the blood type analysis and the original blood type sample results end up being the same.

Different findings were encountered when the dental pulp sample was stored at 10°C. This treatment resulted in different blood types from the original blood types of the sample. It is because the growth of decaying organisms becomes faster at 10°C (Biogenex Laboratories, 2006). It will denature the dental pulp specimen through a chemical and microbiological process then the specimen integrity will be altered leading to alteration of the specimen's integrity. These findings align with previous research, conducted by Inamdar (2011). It stated that the inaccuracy of blood type examination was caused by an aerobic microorganism such as gram-negative bacteria (E.coli and S. Maracessens). These bacteria may alter the antigen determination if the dental pulp sample becomes a B antigen which can influence the results. However, according to Ballal and David (2011), the negative results in the blood type examination was caused by an aerobic gram-negative bacteria specimen contamination.

The One-way ANOVA test shows the p-value of .884 which is more than 0.05. It means there is a similarity between the original blood type of the sample and the experimental blood type of the dental pulp sample which was stored at 5°C and 10°C.

5 CONCLUSIONS

From the research results of the ABO blood type examination using a dental pulp sample in the Hematology Laboratory of Baptist Hospital in Kediri City, it is concluded that a blood type can still be determined by using a dental pulp sample stored at either 5°C or 10°C. However, storing a dental pulp sample in the temperature of 5°C gives a result closer to the original blood type compared to storing the dental pulp sample at the temperature of 10°C.

The result of storing the dental pulp sample at 10°C showed 4 undetectable samples, because the specimen can not be maintained well at that temperature. The effect of this is the denaturation of the dental pulp specimen through a chemical and microbiological process which at that point the specimen's integrity is altered. This resulted in the agglutination not occurring.

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