

Full Length Research Paper

Forensic Identification via ABO Blood Grouping of Exfoliated Deciduous Teeth

Inne S. Sasmita^{1*}, Fahmi Oscandar² and Novita H. Effendi³

¹Department of Pediatric Dentistry, Faculty of Dentistry, Padjadjaran University, Indonesia.

²Department of Radiology, Faculty of Dentistry, Padjadjaran University, Indonesia.

³Dentistry Department, Faculty of Dentistry, Padjadjaran University, Indonesia.

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This study investigated the feasibility and accuracy of blood types identification through deciduous teeth, using absorption-elution. A total of 15 deciduous teeth extracted from 15 children were used to identify blood types which were compared with the blood types of the children's parents. The blood types of 5 (of 5) samples were fully-identified whose agglutination process took place less than a week. The blood types of four samples (of 5 samples) were identified if the agglutination process took place more than a week. The blood types of two samples (of 5 samples) were identified if the agglutination process took place more than a month. Taken together, our results suggest that deciduous teeth can be used to identify blood types using absorption-elution method for forensic odontology and the accuracy of blood types identification depends on the duration of agglutination.

Key words: Absorption-ellusi, blood type, deciduous teeth, agglutination.

INTRODUCTION

Indonesia is geologically situated at the confluence of the three major tectonic plates (Eurasian, Indo-Australian and the Mediterranean) and demographically composed of a variety of ethnic, religious, social and cultural backgrounds. Indonesia is located in one of the hot spot area of the most active disaster (Simarmata, 2009), and these circumstances indicate that Indonesia as a high risk country is prone to natural disasters such as the occurrence of earthquakes, tsunamis, floods, volcanoes, hurricanes and landslides, as well as some disaster caused by human activities, such as bomb explosions and accidents (for example transport plane crashed, or the ship sinks). Not

the least of these disasters can cause mass disaster which claimed many victims (Puspongoro, 2006). The incidence of disasters in Indonesia, which claimed many lives, is increasing. National Disaster Management Agency (BNPB, 2012) has had a data distribution of disasters in Indonesia starting from the year 1815 to 2012, and the incidence of disasters have increased in the last ten years.

As many as 60% of children in the world were victims of a natural disaster (The data is the final output of the United Nations International Strategy for Disaster, UNISDR). In addition, the high number of child victims becomes important issues being discussed by the countries

*Corresponding author. E-mail: innesuhernasasmita@yahoo.co.id.

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in the world (National Geographic, 2012).

One way to identify the victim is the method of forensic odontology. Every health-care facilities are appropriately prepared to anticipate disasters in the region, or assist other health services in the affected areas (Ministry of Health, 2005). Therefore, forensic odontology is very important to understand its role.

Data examination results were then classified into primary data and secondary data as follows (Atmadja, 2004):

- (1) Primary: Fingerprints, dental profile, DNA.
- (2) Secondary: Visual, photography, property victims, medical-anthropology (height, race, blood type).

Identification of the gear used was done when other common methods such as observation and fingerprint methods are not able to produce the expected results, or as an additional identification when needed. Ideally, positive identification (successful) should be based on the identification of two or more (Wikipedia, 2013).

ABO blood group antigen system, usually found on the walls of red blood cells, and if it meets with the appropriate antiserum, will bind and cause agglutination reaction. This reaction occurs as a result of the engagement between the red blood cells to one another. Another case of the teeth, which are not, contained red blood cells, the agglutination reaction may not be visible, so as to determine the type of antigen must be done in an indirect way (Quendangen and Siswosaputro, 1993). Methods of blood type, indirectly there are three kinds (Budiyanto et al., 1997), namely absorption method-inhibition, absorption-elution and mixed granulation.

MATERIALS AND METHODS

The type of research conducted is pre-experimental (Notoatmodjo, 2005). The population in this study was children who come to the health center (RSKGM) having revocation indication (Pasundan) with their teeth.

Samples were 15 subjects with extraction of primary teeth from Pasundan Health Center. For comparison, the results of the blood group of the teeth and the patient's blood group was also asked from the parents. Criteria taken were primary teeth caries and non- caries criteria:

- (1) All kinds of incisive deciduous teeth, canines and molars, which had an internal resorption;
- (2) Blood type through the new primary teeth removed;
- (3) Examination of the blood group of primary teeth that have decayed.

Data collection techniques

In this study, the authors conducted observations through direct type in blood grouping. After that is done, experiment with absorption-elution method was carried out. The new teeth were extracted and had long suffered decomposition, divided into groups above detection, and then grouped according to detection to be

studied. After which it was used for the determination of anti-blood group A, B, AB. The result is two: macroscopic and microscopic.

Research variables

The variables of this study are (1) Variables: Method of absorption-elution, children's teeth; (2) Dependent variable: Blood type through your child's teeth; (3) Omitted variables: Gender, age, nutrition.

The research procedure is as follows (Figure 1):

1. Writer classifying each type of primary teeth caries and non-carious primary teeth are divided into (a) 7 pieces of non carious primary teeth, (b) 8 pieces of carious primary teeth;
2. Teeth is crushed in the iron plate so crushed into a powder. The powder is put into a test tube which is divided into 3 tubes;
3. Then each was given antisera: (a) antisera A to the first tube, (b) Antisera B to tube II, (c) Antisera H to tube III;
4. Third straight tube inserted/stored in the refrigerator 5°C for 24 h day and night.
5. After 24 h, the three tubes were washed with saline solution as much as 7 times;
6. Disposed of saline solution from the tube, and sediment taken.
7. Spilled each tube, 2 drops of distilled water with a pipette.
8. Then heated at a temperature of 56°C for 12 min.
9. The three tubes given indicator cells A, B, and O with a concentration between 3 to 5%.
10. Then centrifuged tube with three spinners in order to occur agglutination (clumping).
11. Newsletter can be seen where the clot tube (agglutination).

On the tube is a visible clumping of blood group identification results of the laboratory analysis. If the result is as follows: if there is clumping (agglutination), then it is said to be a positive test result (+), while if there is no clumping (agglutination) then the result is said to be negative (-). The research process to get the results is as shown in Figure 2.

Processing and presentation of data

Overview of research results macroscopically in visible agglutination (looks coarse grains) and no agglutination (appears as a homogeneous solution). The reading of the results of research carried out is represented in Table 1.

RESULTS

From the research conducted was found several factors that affect the success rate of identifying blood types using the absorption method, one of which is the elution time period between the revocation time of conducting the research. To obtain the antigen and antibody binding optimal, it can take as long as overnight (Quendangen and Siswosaputro, 1993). If a period of more than one night, then the association of antigen and antibody becomes less than optimal (Graph 1). This happens because of the longer distance after the revocation period and the less antigen tooth powder which reacts with the antiserum. Table 2 shows the visible results of the research that has been done. It turns out that the results are not in accordance with the information and blood type directly (manually) extracted from the extraction patients;

Table 1. Way reading research.

A antisera	Antisera B	Blood Teeth
+	+	O
+	-	B
-	+	A
-	-	AB

there were 3 people (20.00%). As for the number of checks in accordance with the results of direct examination, there are 8 people (53.33%). There are only 4 people (26.67%) who cannot be checked because the agglutination reaction does not occur. So it can be described as well in details of the number of samples studied. Sample kinds are shown in Table 3.

In Table 3, there appears a number of details of the studied samples of primary teeth, showing that of the 15 samples of primary teeth present, 8 (53.33%) can be identified as blood type and 4 (26, 67%) cannot be identified. But there is also a dubious result of 3 persons (20.00%).

DISCUSSION

Based on Table 4, the number of the most positive reactions in blood grouping is at a distance of less than one week (100.00%), while for a distance of one week to two weeks only about 4 were positive (80.00%) of 5 samples. At least for the positive reaction of the blood grouping of more than one month, only 2 positive reactions (40%) of 5 samples were studied.

In Table 4, interval revocation was less than a week, giving an accuracy of 100%. This suggests that in the survivors, until death, it is less than one week blood group identification through primary teeth which can still be checked and determined in the appropriate blood type. Results was at an interval of 1 to 2 weeks, giving 80% accuracy, indicating that the time had passed more than a week's quality, thereby diminishing the accuracy of the blood groups through the primary teeth. Although within 1 to 2 weeks it was still able for us to identified blood type, because it is most likely to get the expected results then. At a time interval of one month, there showed accuracy of only about 40%. It should be noted for those who will perform forensic identification, which would result in a negative reaction which is quite large and had traced the cause.

According Quendangen and Siswosaputro (1993), association between antigens with antisera in this study can be influenced by several factors, among others:

1. Comparison between antisera are given to the tube with the antigen in the tooth powder. Comparison between

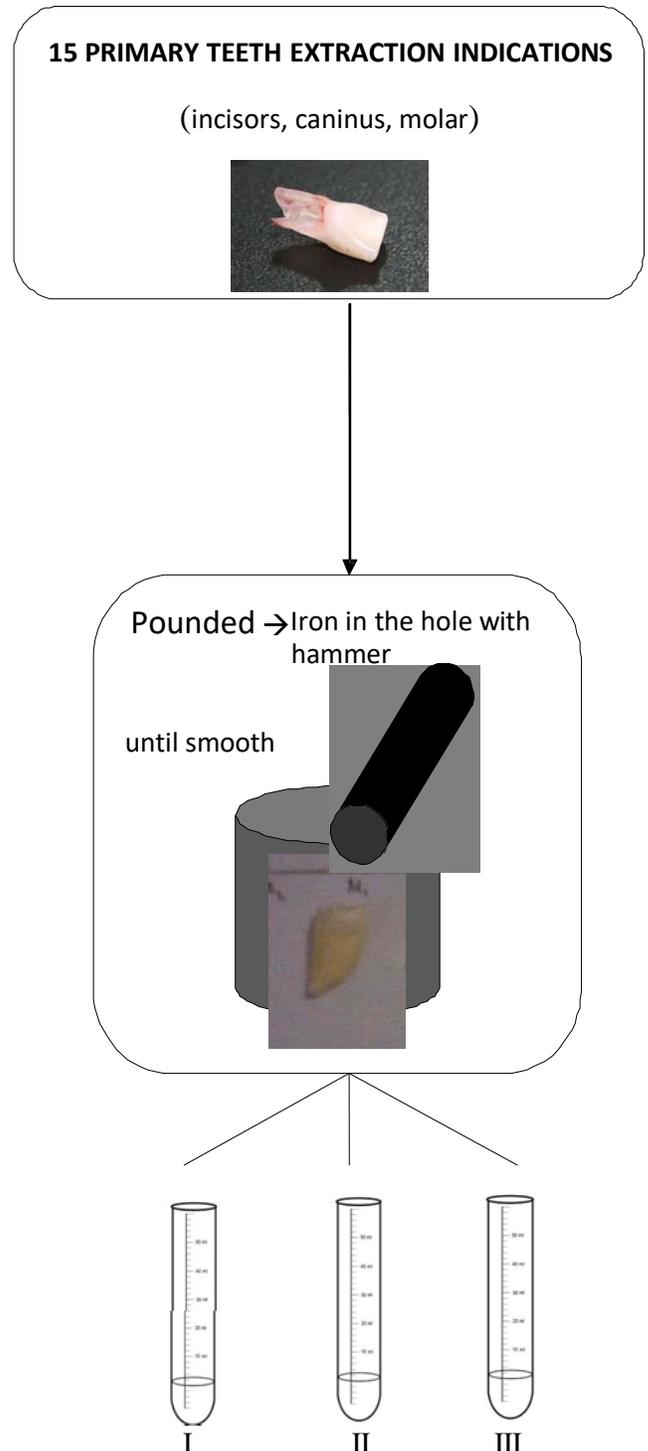


Figure 1. Schematic procedure research.

the antisera with the antigen is to be balanced and proportional. So when bonding occurs antigen with antisera is expected, given the whole antisera bind to the antigen. On the contrary, the positive reaction will not occur or the agglutination reaction will not be visible.

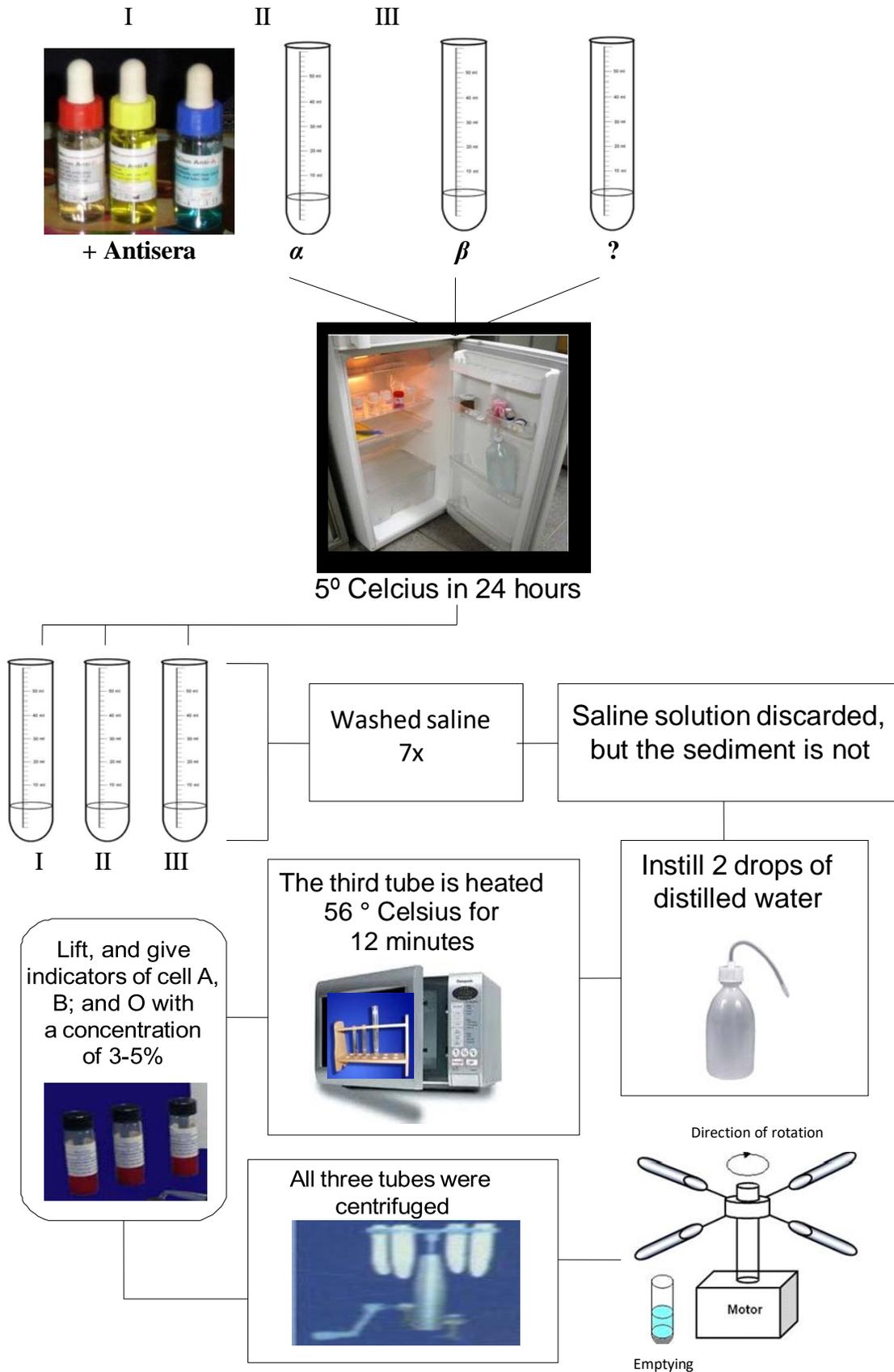


Figure 1 Contd. Schematic procedure research.

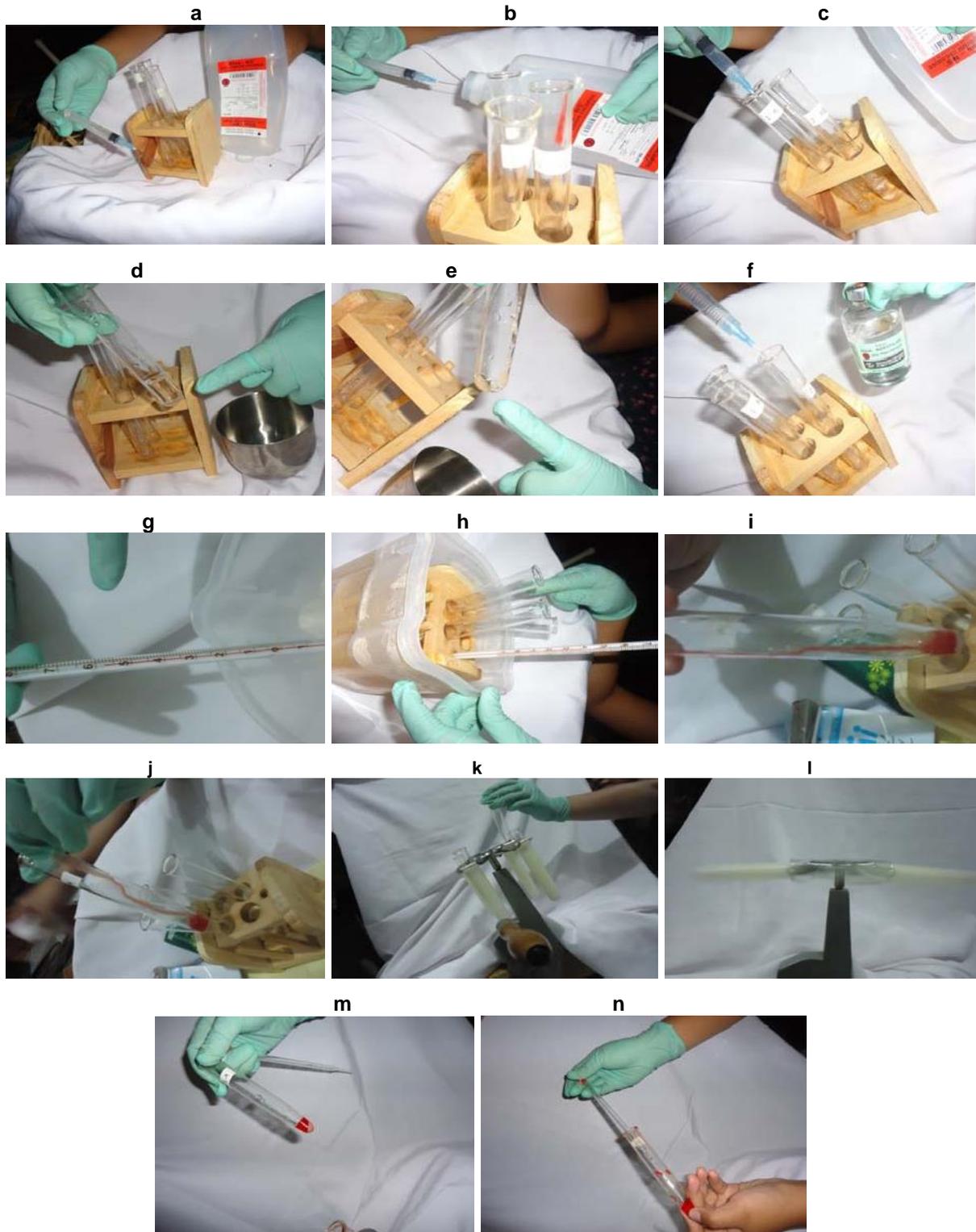


Figure 2. Research process to get results. (a) Stage 1 (after dental pounded and antisera during the night); (b) Stage 2 (saline solution 0.9% or NaCl); (c) Stage 3 (inserted into each tube - 7x); (d) Stage 4 (discard each laundering, NaCl until it colored transparent); (e) Stage 5 (discard NaCl, sediment remains); (f) Stage 6 (give distilled in each tube to taste); (g) Stage 7 (heat temperature with temperature $\pm 56^{\circ}\text{C}$); (h) Stage 8 (enter third tubes, the temperature of 56°C for 12 min); (i) Stage 9 (ABO erythrocytes rate indicator cells); (j) Stage 10 (beat up mixes); (k) Stage 11 (Log into centrifugal tube); (l) Stage 12 (rotate tool centrifugal); (m) Stage 13 (see the results of agglutination); (n) Stage 14 (see the separate pipette case of agglutination); (o and p) Stage 15 and 16 (record results which do not undergo agglutination and blood group identification according to the existing system and connect with the blood type in the sample).

Table 2. Dissertation sample according to examination results.

Result	Number	Percent
Can't be checked	4	26.67
Right to information and direct examination	8	53.33
Not right to information and direct examination	3	20.00
Total Number of	15	100.00

Table 3. Through blood type examination results obtained through dental eldest experiments conducted.

No	Teeth	Agglutination of red blood cells against antisera trial results			Blood group	Information
		A	B	AB		
1	I	(+)	(+)	(+)	O	O
2	C	(-)	(+)	(-)	B	B
3	C	(+)	(-)	(-)	A	A
4	M	(-)	(+)	(+)	B	B
5	I	(-)	(+)	(-)	B	B
6	I	(+)	(+)	(+)	O	O
7	M	(-)	(-)	(+)	Ab	B (*)
8	I	(?)	(?)	(?)	?	B
9	M	(+)	(-)	(+)	A	B (*)
10	I	(+)	(+)	(+)	O	A (*)
11	I	(-)	(+)	(-)	B	B
12	M	(?)	(?)	(?)	?	O (*)
13	C	(?)	(?)	(?)	?	A (*)
14	I	(+)	(+)	(+)	O	O
15	I	(?)	(?)	(?)	?	A (*)

(+): Experiencing agglutination; (-): Not Experiencing agglutination; (?): Agglutination Doubt; (*): Not available. I= incisor, C = canine, M = molar.

Table 4. Relations positive reaction (agglutination) with distance time revocation.

Distance time after revocation (exposure to room temperature)	Total number	Reaction agglutination	Percent
<1 week	5	5	100.00
1 - 2 weeks	5	4	80.00
More than 1 month	5	2	40.00

2. Effect of heating temperature must be precise to obtain bonding optimal antigen and antibody, and also it should be overnight incubation period.

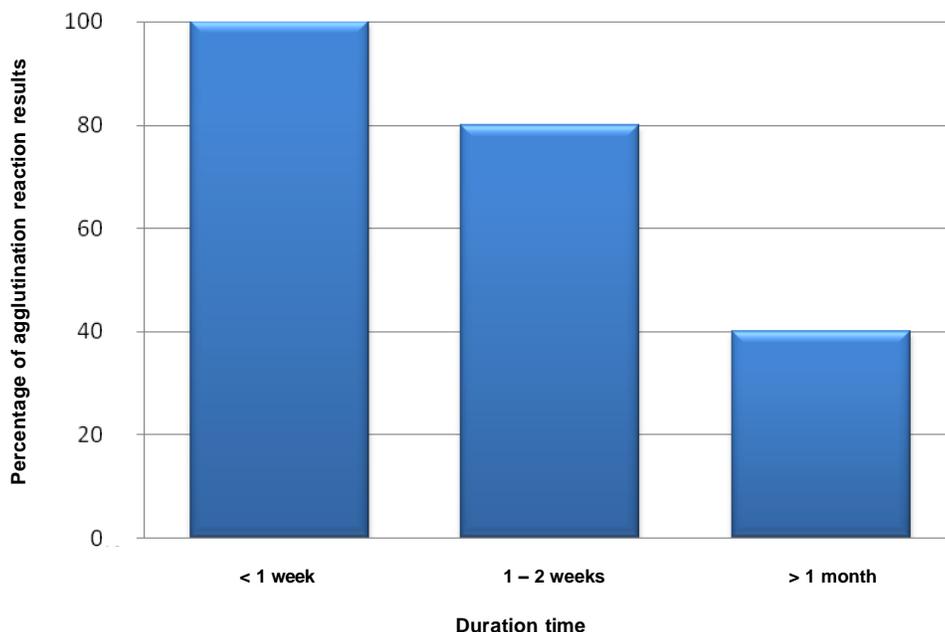
3. The influence of air humidity on the reaction of antigen and antisera during storage greatly affect blood levels of classification accuracy. Antisera also should be stored in a temperature of 4°C to prevent damage.

4. Dilution should also be good in order to minimize errors in the mixing of antigen and antisera to prevent clotting.

5. If there is no tooth powder, anti-H or anti-H is negative; then the teeth are no antigens, so there will be no reaction between antigens with antisera.

6. Erythrocytes can be checked as deciduous teeth with a material known only in approximately more than 1 month from the day of death, if more than that, then the accuracy will decrease.

Tables 2 and 3 show the results of a sample dissertation according to the results of the examination. It turns out



Graph 1. Relations positive reaction with distance time revocation.

that the results are not in accordance with the information, and blood type taken directly from the patients, ranging from 20 and 26.67% cannot be checked because the agglutination reaction is expected to occur, which cannot be seen clearly (false). This is because patients who come to the health center still lack knowledge about the blood type they have, so there is still only one class of their blood. Besides that, while this is appropriate to the direct examination of blood taken from the patient, the results are there that do not fit, because the comparison of antisera with teeth samples are not comparable, and even then that does not conform to caries in primary teeth and have experienced resorption. The results cannot be identified due to the influence of improper storage and erythrocytes can be checked or known by the dental material which is only about 131 days since death (Budyanto et al., 1997).

Conclusion

Based on the research results of the identification of blood groups in primary teeth through absorption methods by elution, the obtained results included the following information: Identification of blood groups through the method of absorption. Elution can be done on primary teeth as identification information for forensic odontology.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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