

## Shaking Red-Cap Blood Collection Tube without Additive Substances is Recommended to Accelerate The Blood Clotting Process

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### Abstract

Many blood collection tube manufacturers do not recommend shaking the red tube. Shaking the red tube to avoid the intensity of the changed of blood that interacts with the glass surface of the tube will trigger the contact path in the coagulation cascade. Generally, the blood takes 30-60 minutes to clots at room temperature without shaking. The aim of this study was to determine the effectiveness of shaking the red-cap blood collection tube in producing serum volume. The method of this study was experimental As much as 5 mL of blood was taken and put into 3 tubes with a volume of 1mL each tube. The first and the second tube were shaken 8 times. The first tube was incubated for 10 minutes while the second tube was incubated for 25 minutes. Meanwhile, the third tube (as a control) was not shaken but was incubated for 40 minutes. The tube were centrifuged at 3000 g for 10 minutes. The serum volume was measured using micropipette and collected into Eppendorf tube. The results showed that there were a difference in the number of serums formed after tube shaking by time variation ( $P = 0.002$ ), the results of the Post Hoc Test using Bonferroni test while showed that the second tube did not have a difference in serum volume with control ( $P = 0.751$ ). It can be concluded that the red-cap blood collection tube, which was shaken 8 times and incubated for 25 minutes long could accelerate the coagulation process.

### Keywords

Shaking, Blood Collection Tube, Blood Clotting

## INTRODUCTION

Many blood collection tube manufacturers do not recommend shaking the red-cap blood collection tubes (not containing additives). The purpose of shaking blood collection tubes is to mix additives with blood such as anticoagulants (1). Red-cap collecting tubes are generally used for clinical chemistry and immunoserology to obtain serum specimens and to incubate the specimen allowed to clot in this tube (2).

Shaking the red-cap blood collection tube increase the interaction of blood to the glass surface of tube, then it can trigger the contact path which causes activation of factor XII (FXIIa) and leads to the blood clotting cascade (3–5). Activating the blood-clotting cascade through contact factors may accelerate the blood clotting process in the red-cap blood collection tube, then the specimen can be performed immediately in the pre-analytic stage.

Generally, the unshaken red-cap blood collection tube takes 30-60 minutes to clot the blood at room temperature in order to obtain serum with a good quality (6). Therefore, the aim of this study was to determine the effectiveness of shaking the red-cap blood collection tube in producing serum volume. This research was carried out experimentally on 30 volunteers.

## MATERIALS AND METHODS

### Design, samples, and techniques

The research was conducted experimentally on August 2018. As much as 5 mL blood samples were collected from 30 students of Medical Laboratory Technology Study Program, Faculty of Health, Nahdlatul Ulama University of Surabaya. The research was conducted in B Tower of laboratory of Nahdlatul Ulama University of Surabaya.

The blood samples were drawn about 5 mL using syringe, then put into 3 blood collection tubes without additives 1 ml for each tube (red lid), tubes 1 (first tube) and 2 (second tube) are used as test tubes and tube 3 (third tube) as a control. The test tubes were immediately shaken 8 times, except the control tube. Incubation was performed to give the chance for blood to clot, The first tube was incubated for 10 minutes, the second tube was incubated for 25 minutes and third tube was incubated for 40 minutes.

Incubated blood samples were immediately centrifuged with Thermo Scientific SL 16 R R (Thermo Fisher Scientific Inc, Langenselbold, Germany) at 3000 g for 10 minutes. The serum volume was measured using micropipette and collected into Eppendorf tube.

### Ethical Approval

Ethics Commission of Nahdlatul Ulama University of Surabaya, East Java, Indonesia, approved this research. Informed consents

were obtained from all patients before drawing blood specimens.

### Statistical analysis

Significant test was used in this study; ANOVA test was used to determine the effect of shaking and incubation time in formation of serum volume compared to the control. Post Hoc analysis was used to determine the same treatment with the control and determine the optimum treatment that can be used. P value <0.05 was stated to be statistically significant.

## RESULTS

### Characteristics of Respondents

As much as 30 respondents were enrolled in this study after obtaining individual approval and Institutional informed consent. Respondents were active students in the Medical Laboratory Technology Study Program, Faculty of Health, Nahdlatul Ulama University of Surabaya. The range of age was 20 to 23 years old with an average of 20.7 years old. The ratio of gender between men and women was 1:27.

### Serum Volume

The obtained blood samples were treated based on research methods. The first treatment was given by shaking the tube 8 times and by incubating for 10 minutes. The second treatment was shaken 8 times and incubated for 25 minutes. Furthermore, the control was not carried out by shaking. However it was incubated for 40 minutes.

The characteristics of formed serum volume presented in Table 1.

**Table 1.** Characteristics of serum volume in various treatments

| PARAMETER     | MEAN<br>( $\mu$ L) | SD     |
|---------------|--------------------|--------|
| 1st Treatment | 308                | 103.54 |
| 2nd Treatment | 368                | 77.07  |
| Control       | 390                | 88.49  |

### The effect of Shaking on Serum Volume

The ANOVA test result revealed that the probability/sig results was 0.002. Since the probability the probability value/sig was <0.05, it can be concluded that there was a difference in the amount of formed serum after shaking the tube by time variation.

Post Hoc Test was used to these three homogeneous group variants ( $P = 0.243$ ) then using Bonferroni test to determine serum volume compared to the control. Significant value of shaken blood tube incubated for 10 minutes was 0.002. It means that there was a difference in serum volume with control. Meanwhile, significant value of another shaken blood tube, which incubated for 25 minutes, was 0.751, which means that there was no difference in serum volume compared with control.

## DISCUSSION

Vacuum tube or commonly known as vacutainer is vacuum containers used for blood collection (7). There are two types of

the red-cap vacuum tubes, which are made from glass and plastic.

Red Plastic tube contains the blood clotting activator while red glass tube does not contain any additives. Both tubes are used to obtain serum for clinical chemistry or immunoserology tests. Most vacuum tube manufacturers suggest to not shaking red glass tube due to excluded from additives (2,7).

The surface of glass on collecting tube is negative charge, and then it easily triggers the contact path in coagulation cascade during the blood clotting process. Zymogen factor XII (FXII) is activated into FXIIa after contact with the glass tube surface and activated multistep cascades, while thrombin (FIIa) is formed as an activator forming fibrin threads (5). It means that the shaking of the red-cap blood collection tube is recommended to increase contact of blood with the tube surface and to activate the blood clotting process. Hence, blood-clotting process is formed faster.

Shaking test of two blood collection tubes incubated in 10 and 25 minutes produced significant different in serum volume ( $P=0.002$ ). The serum volume formed in the first treatment was significantly different compared to the control ( $P = 0.002$ )

and serum volume was less than that in the control treatment. The differences in serum volume formed could be due to incomplete blood hemostasis, because blood clot retraction process was not optimal when serum was trapped by imperfect blood clot, thus producing less serum volume (8).

The serum volume formed in the second treatment was not significantly different compared to the control ( $P=0.751$ ). This means that the shaking of the red-cap tube incubated for 25 minutes had the same volume obtained from the commercial technique (control), while in this study the control was incubated for 40 minutes, thereby saving 15 minutes.

## CONCLUSIONS

The red-cap blood collection tube without additives is recommended with 8 times shaking and 25 minutes incubation. The limitations of this study were measuring serum volume without considering debris that might be carried in serum.

## CONFLICT OF INTEREST

There are no conflicts of interest.

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