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Comparison of blood group testing accuracy using EldonCard® and tube method in blood transfusion unit at Prof. I.G.N.G Ngoerah General Hospital



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ABSTRACT

Background: Blood group testing is essential before performing blood transfusions. The EldonCard® is a modified slide method that utilizes a dry antibody reagent to agglutinate red blood cells with the corresponding antigen. Evaluating the compatibility of blood group results between the EldonCard® and the tube method is essential. This study aimed to assess the level of agreement between blood type and rhesus factor results obtained using the EldonCard® and the tube test.

Methods: This cross-sectional study was conducted at the Blood Transfusion Unit of Prof. I.G.N.G Ngoerah Hospital in Denpasar from March to April 2023. A total of 44 blood samples were collected in K2EDTA tubes from donors through consecutive sampling. Blood type and rhesus factor were determined using both the EldonCard® and the tube method. Results were compared and analyzed statistically with Microsoft Excel 2019 and SPSS Version 26, applying Cohen's Kappa Coefficient to evaluate the level of agreement.

Results: Based on demographic characteristics, the age range of donors was 18 to 57 years, with a median age of 32 years. Most participants were male (35 individuals), with blood type O positive being the most common. There was excellent concordance between the EldonCard® and the tube method ($\kappa = 0.932$; $p = 0.00$).

Conclusion: This study demonstrates that the EldonCard® shows very high agreement with the tube method for blood type testing.

Keywords: Blood group, EldonCard®, tube test.

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INTRODUCTION

Blood is a bodily fluid responsible for transporting oxygen and nutrients throughout the body and removing metabolic waste from tissues. Knowing an individual's blood type is crucial for medical purposes, particularly for preparing blood transfusion procedures. Errors in determining blood type for either recipients or donors can lead to morbidity and mortality due to transfusion reactions.¹⁻³

Blood group testing can be conducted manually or automatically. Manual blood typing methods include the slide, tube, and microplate methods, all based on the principle of agglutination, which involves the reaction between agglutinogens (antigens) on the surface of red blood cells and agglutinins present in serum or

plasma, forming agglutination.²⁻⁵

The slide test is a simple, rapid method with a test duration of five to ten minutes. Advantages of this method include requiring minimal reagents and being highly effective in urgent cases where immediate blood transfusion is necessary. However, the slide test is less sensitive than the tube method and may fail to detect weakly or rarely expressed antigens. Furthermore, drying of the reaction mixture can cause aggregation, leading to false-positive results.²⁻⁵

ELDON, a health technology company, has developed a modified slide method product called the EldonCard®. This card includes a dry antibody reagent that agglutinates red blood cells containing the matching antigen.⁶

The tube method, a commonly used and more sensitive technique than the slide

test, offers several benefits. For example, the incubation process prevents drying, as seen in the slide test, and centrifugation aids in detecting weak antigen-antibody reactions. Additionally, it requires less reagent, and agglutination can be read more easily. Due to these advantages, the tube method is the reference standard for blood typing. However, limitations include a longer testing time for high volumes, the need for specific skills and result-reading techniques, and storage and archiving challenges.²⁻⁵

The tube method is considered the gold standard for blood type identification due to its ability to detect the D antigen using monoclonal IgM/IgG anti-D antibodies, thereby identifying weak titers. This sensitivity makes the tube method safer for transfusion, although it requires centrifugation and expertise to interpret

results accurately.^{4,7-11}

Blood type information is essential not only for personal identification but also for ensuring compatibility in transfusions. This is especially critical in emergencies requiring immediate blood transfusions. In urgent situations, a method that is simple, fast, instrument-free, affordable, and does not require specialized skills is preferred. Many blood banks opt for the slide test as a rapid screening tool.^{3,6-8} This has led to the development of alternative blood typing methods, such as the EldonCard®, which uses the slide method with dry reagents.

Based on those mentioned above, this study aims to evaluate the concordance between the EldonCard® and the tube method as a reference standard.

METHODS

Time and place of research

This cross-sectional study was conducted at the Blood Transfusion Unit of Prof. I.G.N.G. Ngoerah General Hospital, Denpasar, from March to April 2023. A total of 44 blood samples were collected from donors using a consecutive sampling technique. The blood samples were collected in K2EDTA tubes, and blood group testing was performed on the same samples using both the EldonCard® and the tube method.

Tube method examination

The tube method examination uses the principle of agglutination. Red blood cells can be autologously suspended with serum, plasma, or saline or require washing before resuspending in saline. The sample type is adjusted according to the recommendations of the reagent kit insert used. The reagents contain anti-A, anti-B, and optionally anti-AB. This test is also performed on serum samples, requiring additional reagents in the tube method, such as A1, A2, B, and O cell suspensions at 2-5%.^{2,4,5}

The initial procedure involves testing red blood cells (cell grouping) and serum grouping. The result is considered positive if strong agglutination occurs and negative if there is no agglutination after resuspension. When blood typing results are macroscopically inconclusive using the tube method, a drop of the

tube mixture is placed on a slide and examined under a microscope. Very weak agglutination reactions can be detected microscopically.^{2,4,5}

The procedure for rhesus blood type testing with the tube method involves adding one drop of anti-D and one drop of control reagent into a second tube. Then, one drop of a 2-5% red cell suspension is added to each tube and centrifuged at 1000 rpm for one minute. The cell sediment at the bottom of the tube is gently resuspended to check for agglutination. The result is interpreted as D-positive if there is positive agglutination in the tube with anti-D and negative agglutination in the control. A negative result is recorded if no agglutination occurs in the anti-D or control tubes. For patient samples, a negative result is considered Rh-negative. For donor samples with a negative result, further testing is required to determine the presence of weak D antigen. Positive agglutination in the control tube indicates an invalid result.^{2,4,5}

Examination using the EldonCard®

The EldonCard® is a dry reagent format card based on direct hemagglutination reaction. Antibody reagents are embedded on the card and will agglutinate red blood cells that contain the corresponding antigen. The absence of an agglutination reaction in a specific circular area on the card indicates the absence of the corresponding antigen reagent.⁶

The examination procedure includes writing the name and identity on the card and then adding a drop of clean water to each circular area. Add 5 µL of blood to each area and stir clockwise for about 10 seconds to mix the reagent thoroughly. To ensure even agglutination, position the EldonCard® vertically and rotate its sides in the 12, 6, 3, and 9 o'clock positions, allowing 10 seconds for each side. The result is considered positive if strong agglutination occurs and negative if no agglutination is observed.⁶

Data analysis

The research results were statistically analyzed using Microsoft Excel 2019 and SPSS version 26 software. The test results were evaluated using Cohen's Kappa Coefficient to determine the concordance

level between methods. The range of Cohen's Kappa Coefficient values is as follows: 0–0.20 (poor), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (good), and 0.81–1 (very good).¹² The concordance rate for blood typing results between the EldonCard® and the tube method was assessed.

RESULTS

This study was conducted in March and April 2023 on 44 blood donors undergoing blood typing. Based on demographic characteristics, the youngest was 18 years, and the oldest was 57 years, with a median age of 32 years. The majority were male, with 35 individuals. The most common blood type determined by the EldonCard® was blood type O, with 18 individuals having positive Rh, while the tube method identified 20 individuals with blood type O and positive Rh (Table 1).

The results in Table 2 show a very good agreement in blood typing between the EldonCard® method and the tube method, with significant concordance ($\kappa = 0.932$; $p = 0.000$).

DISCUSSION

The EldonCard® test uses a card containing dry antibody reagents that directly agglutinate red blood cells with antigens matching those antibodies. This test takes an average of 1 to 2 minutes, faster than the tube method. In this study, there was an excellent agreement between blood typing results using the EldonCard® and the tube method, with a Kappa value of 0.93. The rhesus testing also showed no difference between the EldonCard® and tube methods. These findings are consistent with a study by Ravichandram et al. in 2016, which tested blood type and rhesus using three methods: EldonCard®, slide method, and tube method, achieving 100% concordance. This result also aligns with a multicentre study conducted by Eldon Biological in Europe in 2004, where ABO and rhesus blood typing achieved a 99.9% concordance rate.^{13,14}

There were two samples in this study with discordant results between the EldonCard® and the tube method: blood type B on EldonCard® but type O in the tube test. This finding is similar to Eldon

Table 1. Blood donor demographics

Variables	n (%)	Median (min-max)
Gender		
Male	35 (81)	
Female	9 (19)	
Age (Years)		32 (18-57)
Blood Type Using EldonCard® Method		
A	11 (25)	
B	14 (32)	
O	18 (41)	
AB	1 (2)	
Rhesus +	44 (100)	
Rhesus -	0 (0)	
Blood Type Using Tube Method		
A	11 (25)	
B	12 (27)	
O	20 (46)	
AB	1 (2)	
Rhesus +	44 (100)	
Rhesus -	0 (0)	

Table 2. Concordance Results of Blood Typing Using EldonCard® and Tube Method with Kappa Value

Variables	Kappa Value	p
Blood Type Using EldonCard® and Tube Method	0.933	0.000*

*Statistically significant if p-value <0.05

Biological's multicenter study, where two out of 2990 samples had false-positive results. This could occur if the reaction mixture dries and forms aggregates, leading to a false-positive result, or if slight reagent contamination from an adjacent area affects the neighboring field.^{13,14}

Several advantages of using the EldonCard® are that the test can be performed quickly, stored at room temperature, is portable, easy to use, and does not require routine quality control. The EldonCard® is also recommended for emergency blood typing in military emergencies requiring immediate transfusion.^{6,13,15}

While the EldonCard® offers many advantages, it has limitations compared to the tube method. For instance, it cannot identify subgroups (A1, A2, A1B, and A2B) or rare groups such as the Bombay blood type. Long-term storage at high temperatures may also adversely affect the test kit's use and accuracy.^{6,13,15}

Although this study shows that the EldonCard® has some advantages over conventional methods, there are limitations that future studies can address,

such as evaluating blood typing in patients with hematologic malignancies, who are more prone to blood type changes. This would allow for further examination of the consistency of ABO and rhesus blood typing results in patients with special conditions.

CONCLUSION

This study shows that blood grouping and rhesus testing with the EldonCard® agree very well with the tube method. The practicality, speed, and lack of need for specialized skills make the EldonCard® advantageous compared to the tube method. The EldonCard® examination is also highly useful for the general public in unexpected emergencies that require immediate blood type identification.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

AUTHOR CONTRIBUTION

Each author made an equal contribution to this study.

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None.

ETHICS CONSIDERATION

This study received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University, with approval number 1152/UN14.22.VII.14/LT/2023.

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