〈Brief Note〉

Method for Rh blood group testing using a general-purpose automated biochemical analyzer

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Summary Background: In this study, a high-throughput and high-precision Rh blood typing screening method that utilizes a general-purpose biochemical analyzer to perform direct red blood cell sampling was investigated.

Methods: The blood group antisera used were Monoclonal anti-D Wako and Rh control serum (FUJIFILM Wako Pure Chemical Corporation). Polyethylene glycol (Ortho Clinical Diagnostics) was used as a reaction enhancer. The general-purpose biochemical analyzer employed was the TBA-120FR HbA1c measurement unit (Canon Medical Systems).

Results: The Rh blood group of the patient samples was determined based on the relative values to the mean changes of ten healthy volunteers. The repeatability was CV5% or lower, and the testing of 628 patient samples showed 100% agreement between the results obtained using the proposed method and those from the tube test method.

Conclusions: With the use of the proposed method, Rh blood typing can be performed with ease promptly, and with a high degree of precision.

Key words: Rh blood typing, Biochemical analyzer, TBA-120FR

1. Introduction

Three kinds of blood typing systems are known: ABO, Rh, and HLA. We reported a high-precision ABO blood group measurement method using a General-Purpose Automated Biochemical Analyzer[1]. Rh blood typing is frequently used similar to ABO blood typing. It is particularly significant for the confirmation of compatibility between the blood

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donor and recipient at the time of blood transfusion as known as transfusion side effects. Additionally, the blood typing is performed manually by examining for the presence of a blood group substance (blood group antigen) on the surface of red blood cells.

The general Rh blood group typing and screening methods are the tube test (TT) method and the column agglutination technology (CAT) method[2-4]. The TT method requires highly-trained personnel to perform the test and interpret results, whereas the CAT method requires a specialized analyzer and reagents. Here, we report a high-throughput and high-accuracy Rh type blood group study using a general-purpose biochemical analyzer (TBA-120FR HbA1c measurement device, Canon Medical Systems, Otawara City, Tochigi, Japan) capable of direct red blood cell sampling.

2. Materials and Methods

Blood group analysis reagents

The blood group antisera used were Monoclonal anti-D Wako Serum (anti-D) and Rh Control Serum (Rh-Cont) (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan). Polyethylene glycol (PEG) (Ortho Clinical Diagnostics, Tokyo, Japan) was used as a reaction enhancer[5].

anti-D and Rh-Cont were prepared by diluting in a 5-fold dilution of PEG (20% PEG–PBS (phosphate-buffered saline)) included with 10 mmol/L PBS.

General-purpose biochemical analyzer

TBA-120FR Sora Edition HbA1c measurement unit was used as the general-purpose automated biochemical analyzer. Specifically, it can dispense either whole blood or post-centrifuged red blood cells and process 200 HbA1c test samples per hour.

Reagents and control methods

This study was approved by the Institutional Review Boards of Kagawa Prefectural University of Health Sciences and Kinashi Obayashi Hospital (number 187). EDTA-2Na blood samples, which are no weak grades and subgroups, was used. They were obtained from patients examined at the Multiphasic Health Screening Department of Kinashi Obayashi Hospital after obtaining informed consent. Furthermore, the control method utilized the standard TT method in accordance with the Transfusion & Transplantation Testing Techniques issued by the Japanese Association of Medical Technologists.

Rh blood group measurement method

The patient samples were centrifuged at 2,000 rpm (800 G) for 5 min. The centrifuged blood cells were then diluted to 4% with physiological saline in the TBA-120FR. Next, 10 μL of the 4% patient blood cell solution was reacted in 100 μL of both anti-D and Rh-Cont diluted solutions, and the change in absorbance at 660/804 nm due to agglutination for 10 min was measured.

Methods for determining the Rh blood group

The changes in absorbance in anti-D and Rh-Cont solutions were confirmed using 4% blood cell suspension from 10 healthy adult Rh-positive volunteers. The mean change in absorbance of anti-D of the ten Rh-positive volunteers was set at 100%, and the changes in absorbance of the patient blood cells were compared and quantified. Furthermore, patient data were collected, and after setting the Rh-positive and Rh-negative cutoff values, Rh blood typing was performed.

3. Results

Antiserum concentrations for anti-D and Rh-Cont

Both anti-D and Rh-Cont were diluted with 20% PEG–PBS, and the changes in absorbance were determined for the Rh-positive and Rh-negative blood cells. The results showed that the 10-fold dilutions provided good reactivity in both anti-D and Rh-Cont. The time course of the absorbance changes in Rh-positive and Rh-negative blood cells are shown in Fig. 1.

Rh-positive cutoff values

The blood cells from 100 blood group

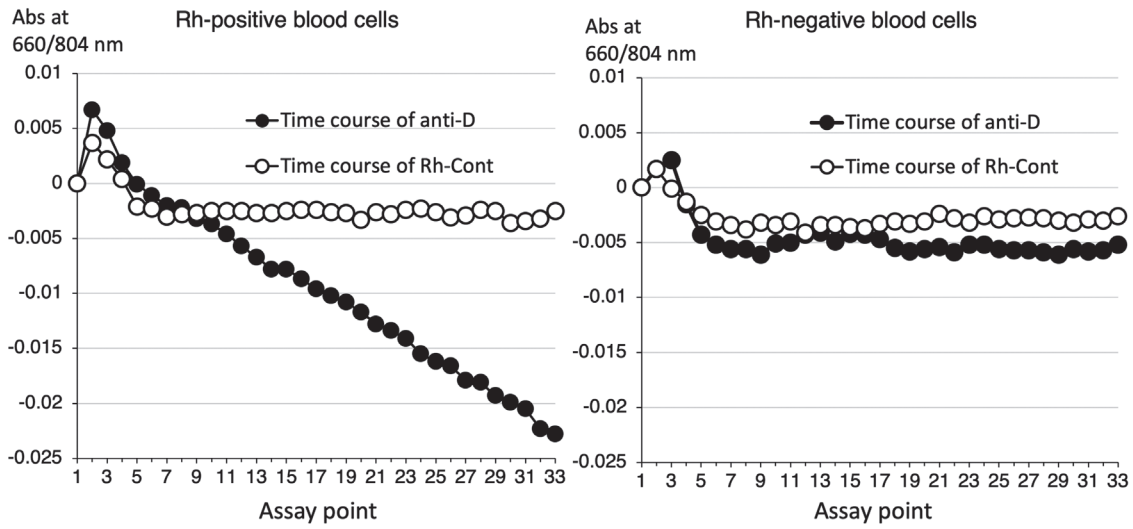

Fig. 1 Time course of Rh blood type measurement using the TBA-120FR Sora Edition HbA1c Unit
Using the proposed method, we diluted the Monoclonal anti-D Wako Serum(anti-D) and Monoclonal anti-D Wako Rh control Serum(Rh-Cont)10 times with 20% PEG-PBS and then used the blood cells from the healthy volunteers to determine the response over time.

Table 1 Rh typing results of the patient samples (n = 628)

Rh type group (by TT method)	anti-D (%)	Rh-Cont (%)
Rh-positive (n = 624)	74.0 ± 18.5	1.9 ± 3.1
Rh-negative (n = 4)	1.5 ± 1.7	1.0 ± 1.5

Test results of 628 clinical samples (mean ± SD)

Rh-positive patients were measured to determine the Rh-positive cutoff value. The result (mean ± SD) for anti-D was 76.0% ± 19.0%. In addition, the Rh-positive cutoff value (mean − 3SD) was 19.0%. It means that values ≥19.0% indicate a positive result. A similar examination was repeated for Rh-Cont, and the result (mean ± SD) was 2.1% ± 2.7%.

Rh-negative cutoff values

The Rh-negative cutoff values were investigated in the Rh-negative blood samples from 4 patients. The result (mean ± SD) and Rh-negative cutoff value (mean + 3SD) for anti-D were 1.5% ± 1.7% and 6.6%, respectively. Based on these results, the value ≤7.0% obtained using this method means negative result. Furthermore, the value that were above the Rh-negative cutoff value and below the Rh-positive cutoff value should be considered indeterminate.

Repeatability

The repeatability of the proposed method was investigated by using Rh-positive blood samples obtained from healthy volunteers. The result (mean ± SD) of 20 measurements was 80.8 ± 3.88, indicating reproducibility (CV%) values of 4.80%.

Comparison with the TT method

The proposed method and TT method were compared using 628 patient blood samples (Rh-positive, 624; Rh-negative, 4; Table 1). The

results revealed a 100% agreement between both methods.

4. Discussion

The Rh blood type testing method mainly utilizes the TT method, which is performed manually, and the CAT method. The TT method requires certain skill and training for performing the test and interpreting the presence of aggregation, and individual differences in skill can lead to erroneous interpretations. On the other hand, in the CAT method, the sampling, reagent dispensing, centrifugation, and determination can be performed fully automatically using a specialized analyzer, but a specialized device and column reagent are required. In the proposed method, a general-purpose Rh blood group antiserum can utilize as same as TT method. Moreover, using a general-purpose biochemical automatic analyzer, a 4% blood cell suspension was automatically prepared and reacted with the antiserum in a thermostat bath at 37°C. Subsequently, the agglutination reaction was measured over time, making it possible to easily measure Rh blood type.

The quantification of this reaction was possible by setting the mean absorbance changes of anti-D of 10 Rh-positive volunteers as 100% and comparing and quantifying the absorbance changes of the patient blood cells. The result of the Rh-negative participants and Rh-Cont showed almost no agglutination reaction, but 624 Rh-positive participants had results of 74.0 ± 18.5%. These results were presumed to be because of the differences in the reactivity between the patient blood cells and anti-D serum.

An ORTHO VISION analyzer turnaround time of >20 min has been reported[6]. The reaction time of the proposed method was 10 min, and rapid testing of Rh blood group was possible because the TBA-120FR Sora Edition HbA1c measurement unit has the ability to process 200 samples per hour. Additionally, an assessment of the repeatability confirmed a high accuracy of less than 5% CV, and our study of 628 patient samples showed 100% consistency between this method and the TT method. Therefore, with this method, Rh blood group screening was performed with high throughput and sensitivity, and it seems useful for routine Rh blood group testing.

However, because the proposed method is only able to perform Rh blood group screening test. Thus, examining cases such as serological weak D phenotypes and special cases in which the agglutination reaction of Rh control serum is observed[7-9] is necessary, and further studies to evaluate them are essential.

5. Conclusion

With the use of a General-Purpose Automated Biochemical Analyzer which can dispense either whole blood or post-centrifuged red blood cells, the blood cell suspensions can be automatically prepared and the Rh blood typing can be performed easily, rapidly, and precisely. Therefore, it is a potential alternative testing method for Rh typing, but further evaluation of the other essential aspects of this typing is necessary.

Conflicts of interest

The authors have no conflicts of interest.

Ethical Approval

This study was approved by the Institutional Review Boards of Kagawa Prefectural University of Health Sciences and Kinashi Obayashi Hospital (number 187).

Contributorship

ST, SK, AM, and MT researched the literature and conceptualized the study. SK was involved in the data analysis. ST and SK wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved its final version.

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