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ORIGINAL ARTICLE

Method for ABO Blood Group Testing Using a General-Purpose Automated Biochemical Analyzer

Satoshi Tada [1], Shoichi Kanayama [2], Akemi Miyagawa [3], Koji Murao [4]

[1] *Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu City, Kagawa, Japan*
[2] *Canon Medical Systems Corporation, Otawara City, Tochigi, Japan*
[3] *Department of Clinical Laboratory, Kinashi Obayashi Hospital, Takamatsu City, Kagawa, Japan*
[4] *Department of Clinical Laboratory, Faculty of Medicine, Kagawa University, Kita-Gun, Kagawa, Japan*

SUMMARY

Background: **We investigated a high-throughput and high-precision forward ABO blood typing screening method that utilizes a general-purpose biochemical analyzer to perform direct red blood cell sampling.**
Methods: **The blood group antisera used were Ortho® BioClone® Anti-A Serum and Ortho® BioClone® Anti-B Serum. AFFIRMAGEN® Reagent Red Blood Cells (Ortho Clinical Diagnostics) were used for AB standard red blood cells. The general-purpose biochemical analyzer employed was the TBA™-120FR HbA1c measurement unit (Canon Medical Systems).**
Results: **ABO blood group of patient samples was determined based on values relative to amount of change in the AFFIRMAGEN® response. Repeatability was CV5% or lower, and testing of 1,112 patient samples showed 100% agreement between the results obtained using the proposed method and those obtained using the tube test method.**
Conclusions: **The proposed method allows ABO blood typing to be performed simply, quickly, and with a high degree of precision.**
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Correspondence:
Satoshi Tada
Department of Medical Technology
Kagawa Prefectural University of Health Sciences
281-1 Hara Mure-Cho
Takamatsu City, Kagawa 761-0123
Japan
Phone: +81 87-870-1264
Fax: +81 87-870-1202
Email: tada@chs.pref.kagawa.jp

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KEY WORDS

ABO blood typing, biochemical analyzer, TBA™-120FR

INTRODUCTION

There are many blood group antigen systems, including the ABO blood group system, the Rh blood group system, and the human leukocyte antigen (HLA) system. The ABO blood group is particularly important in determining the compatibility of blood donors and blood recipients in order to avoid transfusion reactions when performing blood transfusions, and ABO blood typing is therefore commonly and widely performed. The ABO type is determined by physically comparing the results of forward typing, which tests for blood substances (blood antigens) on the surface of red blood cells, with those of reverse typing, which tests for blood group antibodies in serum.

The main blood group typing and screening methods are the tube test (TT) method and the column agglutination technology (CAT) method [1,2]. The TT method requires a high degree of proficiency in the test operations and in making determinations based on the results. The CAT method, on the other hand, requires specialized reagents. In this article, we report on our evaluation of a high-throughput and high-accuracy forward blood typing screening method using a general-purpose biochemical analyzer.

MATERIALS AND METHODS

Blood group analysis reagents

The blood group antisera used were Ortho® BioClone® Anti-A Serum (Ortho Clinical Diagnostics, Tokyo, Japan) (A) and Ortho® BioClone® Anti-B Serum (Ortho Clinical Diagnostics) (B). Both A and B were diluted with 10 mM phosphate buffered saline (PBS). AFFIRMAGEN® Reagent Red Blood Cells (Ortho Clinical Diagnostics, Tokyo, Japan) were used for A and B group standard blood cells.

General-purpose biochemical analyzer

The general-purpose biochemical analyzer used was the TBA™-120FR Sora Edition HbA1c measurement unit (Canon Medical Systems, Otawara City, Tochigi, Japan). This analyzer is a general-purpose automated biochemical analyzer that can dispense either whole blood or post-centrifuged red blood cells. It can process 200 HbA1c test samples per hour.

Reagents and control methods

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

ABO group measurement method

The AFFIRMAGEN® was used without alteration. The patient samples were centrifuged at 2,000 rpm (800 G) for 5 minutes. The centrifuged blood cells were then diluted to 4% in physiological saline in the TBA™-120-FR Sora Edition HbA1c measurement unit. Then, 10 µL of AFFIRMAGEN® and 10 µL of the 4% patient blood cell solution were reacted in 100 µL of both A and B diluted solutions, and the change in absorbance at 660/804 nm caused by agglutination for 10 minutes was measured.

Methods for determining the ABO blood group

Using AFFIRMAGEN® A blood cells and AFFIRMAGEN® B blood cells as the standard blood group cells and with the absorbance values for the reactions of A and B set as 100%, we compared the absorbance values of the patient blood cells with those of AFFIRMAGEN® A blood cells and AFFIRMAGEN® B blood cells. Patient data were also compiled in order to determine the A and B group positive and negative cutoff values, with the blood groups subsequently determined based on these values.

RESULTS

Antiserum concentrations for A and B

A and B were diluted with PBS, and the changes in absorbance were determined for AFFIRMAGEN® A and AFFIRMAGEN® B blood cells. The results showed that 10-fold dilutions provided good reactivity in both A and B. AFFIRMAGEN® A and AFFIRMAGEN® B blood cells showed changes in absorbance (Figure 1).

Negative cutoff values

One hundred subjects with blood group O were studied. The results (mean ± SD) for A and B were 4.7% ± 2.6% and 4.8% ± 2.7%, respectively. The negative cutoff value (mean + 3SD) was 12.4% for A and 13.0% for B. These results allowed us to conclude that values ≤ 15% obtained using this method indicate a negative result.

Positive cutoff values

To determine the positive cutoff value for A, measurements were obtained for the blood cells from 100 blood group A patients. The result (mean ± SD) for A was 62.9% ± 5.3%. The positive cutoff value (mean - 3SD) was 46.8% for A, indicating that values ≥ 46.8% indicate a positive result.

To determine the positive cutoff value for B, measurements were obtained for blood cells from 100 blood group B patients. The result (mean ± SD) for B was 71.1% ± 6.4%. The positive cutoff value (mean - 3SD) was 51.8%, indicating that values ≥ 51.8% indicate a positive result.

The result of A positive and B negative was determined as type A, the result of A negative and B positive as type B, the result of A negative and B negative as type O, and the result of A positive and B positive as type AB. In addition, results that were above the negative cutoff value and below the positive cutoff value were considered indeterminate.

Repeatability

We investigated the repeatability of the proposed method using blood obtained from healthy volunteers with blood type A and blood type B. The results of 20 measurements for each sample were 69.9 ± 2.51 and 70.6 ± 1.31 (mean ± SD), respectively, indicating reproducibility (CV%) values of 3.59% and 1.86%, respectively.

Table 1. Results for ABO typing of patient samples (n = 1,112).

Blood Type Group (by TT method)	Anti-A (%) *	Anti-B (%) **
Group A (n = 464)	63.8 ± 5.6	5.1 ± 2.4
Group B (n = 220)	5.0 ± 2.8	71.2 ± 6.5
Group O (n = 328)	4.7 ± 5.1	2.5 ± 2.7
Group AB (n = 100)	61.3 ± 5.2	59.8 ± 5.4

*** - Relative % with A blood cells set to 100%.**
**** - Relative % with B blood cells set to 100%.**
Test results for 1,112 clinical samples (mean ± SD).

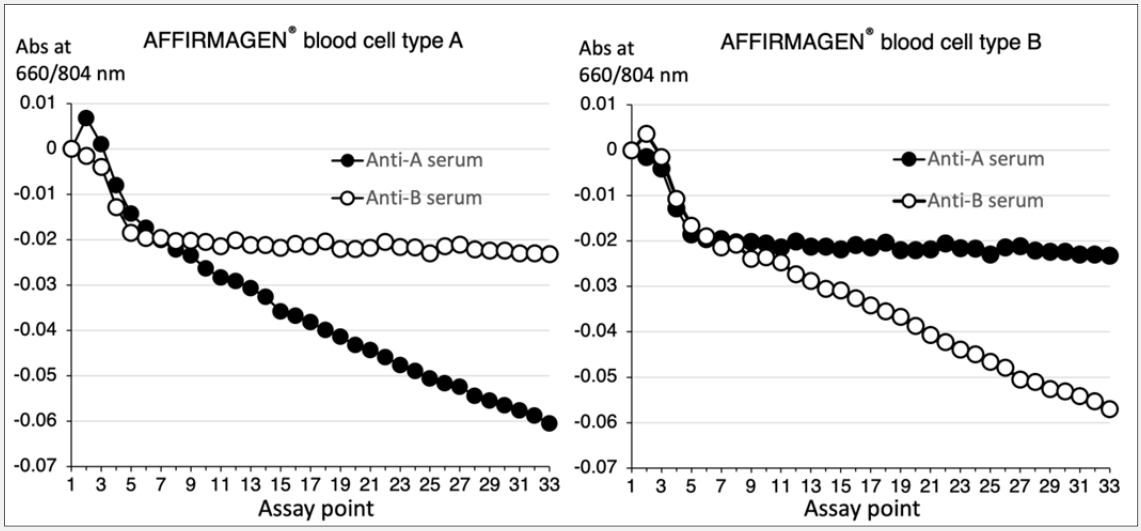

Figure 1. Time course of blood type measurement using the TBA™-120FR Sora Edition HbA1c Unit.

Using the proposed method, we diluted the Anti-A and Anti-B sera 10-fold with PBS and then used AFFIRMAGEN® standard blood cells to determine the response over time.

Comparison to the TT method

The proposed method and the TT method were compared using 1,112 patient blood samples (type A: 464 samples, type B: 220 samples, type O: 328 samples, type AB: 100 samples; Table 1). The results showed 100% agreement between the proposed method and the TT method.

DISCUSSION

The main blood group typing and screening methods are the TT and CAT methods. The TT method requires a high degree of proficiency in the operation of the test and in determining the result. There are individual differences in the agglutination screening method which may sometimes lead to incorrect results. The CAT method, on the other hand, requires the use of a special analyzer and specialized reagents to automatically sample and dispense the reagents and make determinations. The proposed method utilizes the same type of general blood type serum as used in the TT method and a gener-

al-purpose biochemical analyzer TBA™-120FR Sora Edition HbA1c unit. This allows a 4% blood cell suspension to be prepared, reaction with antisera to be performed in a constant-temperature thermostatic chamber at 37°C, and the progress of the agglutination reaction to be measured over time. The entire process is conducted automatically, and it is therefore a simple method for determining blood groups. In addition, it allows us to convert patient blood cell and antiserum reactions to numerical values in comparative measurements based on absorbance ratios with AFFIRMAGEN® A blood cells and AFFIRMAGEN® B blood cells set to 100%. The negative cutoff vales are relatively similar while the positive cutoff values are quite different. We speculate that this discrepancy could be due to differences in reactivity of patient blood cells and A/B antibodies. It has been reported that the turnaround time for the ORTHO VISION® analyzer is at least 20 min [3]. The response time using the proposed method is 10 minutes, and the TBA™-120FR Sora Edition HbA1c unit can process 200 samples per hour. This means that blood group screening can be performed quickly. Our results for repeatability showed high sensitivity, at under CV5%, and our investigation of 1,112 patient samples showed 100% agreement between the proposed method and the TT method. Thus, the proposed method allows blood group screening to be performed with high throughput and high sensitivity, which we believe should make it useful as a blood group screening test for use in general clinical settings. However, because the proposed method is only able to perform forward screening, further studies must be conducted to evaluate its applicability to reverse screening and Rh testing. Furthermore, it is necessary to evaluate the limitation of subgroup testing and detection of mixed fields.

CONCLUSION

ABO blood group testing using the TBA™-120FR HbA1c measurement unit allows the automated preparation of blood cell suspensions as well as simple, fast, and precise forward ABO blood typing screening. As a result, it has potential to be an alternative testing method for ABO typing. However, further evaluation for the other essential aspects of ABO typing is needed.

Ethical Approval:
This study was conducted after approval was received from the Institutional Review Boards of Kagawa Prefectural University of Health Sciences and Kinashi Obayashi Hospital (number 187).

Author Contributions:
ST, SK, AM, and KM researched the literature and conceived of 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 the final version of the manuscript.

Declaration of Interest:
The authors declare no competing interests.

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