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Comparative study of two automated pre-transfusion testing systems (microplate and gel column methods) with standard tube technique

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ABSTRACT

Aims: Clinically significant antibodies may become undetectable and still provoke transfusion reactions. Hemolysis has been reported among transfusion recipients with anti-Kidd that was undetectable by gel column but detectable by other methods. Methods: We compared two automated technologies - microplate (MP) and gel column (GC) methods - with manual methods in ABO/RhD typing, irregular antibody screening, identification, titration, and detection threshold of mixed-field agglutination. Results: Automated systems agreed generally with tube results in 98% or more of ABO forward and RhD groupings, but showed weaker reactions in ABO reverse testing against A1 (K=0.88 with MP and K=0.77 with GC) and B cells (K=0.66 with MP and K=0.68 with GC) and failed to detect some anti-A (2 of 273 samples with MP) and anti-B (2 of 273 with MP and 1 of 272 with GC). MP missed 2 (anti-E and –Fy^b) of 8 antibodies and GC missed 5 (2 anti-E and 1 each of -Fy^b, -Jk^a and –Le^a) of 10 antibodies, which manual PEG-IAT detected. Among 11 known alloantibodies, MP detected 7 antibodies at higher dilution than tube PEG-IAT, whereas GC showed lower scores in 7 samples than tube PEG-IAT and missed 2 clinically significant antibodies (anti-C and anti-Fy^b). Conclusion: Automated systems, comparable to manual tube technique for forward grouping of ABO and RhD, are less sensitive in ABO reverse testing. As GC more often failed to detect clinically significant antibodies of low-titer, users should be especially mindful of possible post-transfusion hemolysis.

Keywords: ABO grouping, Gel column method, Irregular antibody, Microplate method, Tube technique

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INTRODUCTION

Automated pre-transfusion testing has gained favor to overcome drawbacks of tube technique, which is subject to human error even in laboratories well-staffed with dedicated transfusion specialists. Studies comparing standard tube technique with automated pre-transfusion testing have focused on the sensitivity of irregular antibody detection [1–5]. Automated microplate (MP) systems have been reported to be more sensitive than standard tube technique [4]. Conversely, a collaborative cohort found gel column (GC) systems to be less sensitive than low-ionic strength solution-enhanced indirect antiglobulin tests in detecting clinically significant irregular antibodies [6]. In a recent series of 17 patients with post-transfusion hemolysis, anti-Jk^a was detectable by MP but not by GC [7]. In this study, we compare two desk-top automated systems, MP (ECHO, Immucor) and GC (WADiana Compact, Grifols), with standard tube technique for ABO grouping, RhD typing, irregular antibody screening, titration of known irregular antibodies, and detection threshold of mixed-field agglutination.

MATERIALS AND METHODS

Samples

To compare ABO/D blood grouping between a direct agglutination method on a microplate and a standard tube method, and to antibody screening between a solid phase (SP) method and a standard tube method, we processed 273 consecutive routine samples during October 2012. Then, to compare blood grouping and antibody screening between a gel column (GC) method and standard tube method, we processed 272 consecutive routine samples during December 2012. Samples of newborns were excluded from this study.

Standard tube method

For determining the ABO group of red blood cells (RBC), 1 drop of each reagent (Bioclone anti-A, Bioclone anti-B, Ortho-Clinical Diagnostic, Tokyo, Japan), and 1 drop of 3–5% patient's RBCs were added to appropriately-labeled tubes, mixed, centrifuged, resuspended and examined for agglutination. For the ABO reverse test, 2 drops each of patient's plasma and 1 drop of reagent cells (AFFIRMAGEN A_1 or B cells, Ortho-Clinical Diagnostics) were mixed, centrifuged, resuspended and examined for agglutination.

For D typing, 1 drop of reagent (Bioclone anti-D, Ortho-Clinical Diagnostics) and 1 drop of the corresponding control (Rh-hr Control, Ortho-Clinical Diagnostics) and 3–5% patient RBCs were mixed, centrifuged, resuspended and compared for agglutination. If there was no agglutination of the RBCs in both the reagent and control tubes, the weak D test was performed using an indirect antiglobulin (IAT) procedure. The RBCs were incubated at 37°C for 30 minutes, then washed 3 times with saline. A rabbit-source antihuman reagent (Ortho Anti-IgG (Rabbit), Ortho-Clinical Diagnostics), was added to each tube, centrifuged, resuspended and examined for agglutination. IAT Control cells coated with IgG (prepared in-house) were added to each negative test to confirm antihuman reactivity.

The antibody screen test was performed using a, polyethylene-glycol (PEG)-IAT method. Two drops of patient's plasma and 1 drop each of a 3-cell screen set (SURGISCREEN, Ortho-Clinical Diagnostics) and a special screen cell (Di(a+), Ortho-Clinical Diagnostics) were mixed, centrifuged, resuspended and examined for direct agglutination. To each tube, 2 drops of 20% PEG (prepared in-house)[8] were added as a enhancer and incubated at 37°C for 15 minutes, washed 4 times with saline, and 2 drops of antihuman reagent (Ortho Anti-IgG (Rabbit), Ortho-Clinical Diagnostic) were added. After incubation, and centrifugation, the tubes were examined for agglutination. The in-house IgG-coated RBCs were added to each negative test [7].

If any patient sample reacted with any screen cell, antibody identification was performed using an eleven-cell panel (RESOLVE Panel A and B, Ortho-Clinical Diagnostics) and/or sixteen-cell panel (PANOCELL-16, Immucor K.K., Tokyo, Japan) with PEG-IAT.

Solid phase method using an automated instrument

For ABO/D grouping, performed by direct agglutination method on a microplate 20 µL of patient's red cell suspension and 40 µL of murine monoclonal ABO reagents (anti-A, anti-B, Immucor K.K.,) and monoclonal Rh reagent and control (Gamma-clone anti-D and Monoclonal Control, Immucor K.K.) were pipetted onto a microwell plate (Galileo Echo, Immucor K.K.), centrifuged, resuspended and agglutination was interpreted by the automated algorithm and camera. For ABO reverse typing, 15 µL of each of the reverse grouping cells (Referencell, Immucor K.K.) and 30 µL of patient's plasma were mixed, centrifuged, resuspended and examined for agglutination.

For the solid phase antibody screening, 25 µL of patient's plasma and 50 µL of additive (Capture LISS, Immucor K.K.) were added to the plate wells containing the RBC monolayers (Capture-R Ready-Screen(3), Immucor K.K.), The automated analyser (Galileo Echo, Immucor K.K.) completed the testing, added the indicator cells (Capture-R Ready Indicator Cells) and interpreted the results. All negative reactions, were checked visually for confirmation per the Manufacturer's Operators' Manual.

When any potential antibody was suspected, identification was performed using the manual PEG-IAT method.

Gel column method using an automated instrument

For ABO/D grouping, 10 µL of patient's RBC suspension was added to the appropriate column on the gel card. The gel cards are prepared with antisera (DG Gel ABO/Rh (2D) Card, Kainos Laboratories, Tokyo, Japan) already added. The ABO reverse test was performed using 50 µL of 0.8% RBC (DG Reverse-Cyte A_1,B 0.8%, Kainos Laboratories) All pipetting and processing is performed by an automated analyzer (WADiana Compact, Kainos Laboratories). After centrifugation, the cells pellet either at the top (positive reaction) or bottom (negative reaction) of the matrix. The results are read automatically by the instrument and can be confirmed visually

For the antibody screen, 50 µL of patient's plasma were mixed with 25 µL each of 3 screen cells (Search-Cyte TCS 0.8%, Kainos Laboratories), incubated at 37°C for 15 minutes, centrifuged and interpreted by the instrument. Reactions could be read by the instrument and interpreted visually.

Antibody identification, if needed, was performed using the manual PEG-IAT method.

Dilution detection sensitivity of antibodies

Antibody detection sensitivity of the three methods was compared using 11 frozen samples including one each of anti-D, -E, -C, -Jk^a, -Fy^b, -Le^a, -S, -Di^a,-Jr^a, and two anti-M. In order to minimize the influence of freezing and thawing, frozen samples were thawed once at 37°C, divided into two aliquots, and cryopreserved again at -30°C until testing. After re-thawing, serially diluted samples were measured by two automated methods and by tube PEG-IAT. Subsequently, agglutination strengths and antibody titration was scored according to the AABB Technical Manual [9].

Part of this study, comparing MP with tube PEG-IAT, was a previous brief report [10].

Detection thresholds of mixed-field agglutination

Thresholds of mixed-field agglutination were compared among three methods by using the samples prepared with group A and group O red blood cells from healthy donors, adjusting the mixing ratios in 10% increments.

Statistical analysis

For ABO, RhD grouping and indirect antiglobulin screening for irregular antibodies, Kappa statistics quantified the agreement of reaction strength between the two automated systems and the tube technique. Kappa (K) values of K(0.81–1.0) indicate near perfect agreement; K(0.61–0.8), substantial agreement; K(0.41–0.6), moderate agreement; K(0.21–0.4), fair agreement; and K(0.0–0.2), slight agreement. Titration score was analyzed by the Friedman test and the nonparametric Wilcoxson's signed rank test. Differences with a p value of 0.05 or less were considered statistically significant. All statistical analyses were performed with SPSS Statistics version 24 (IBM Corporation).

RESULTS

ABO grouping and RhD typing

In ABO forward grouping of 273 samples, MP concordance with standard tube technique was 99.3% (K = 0.985) for A and 98.9% (K = 0.976) for B in agglutination strength (Table 1A and Table 1B). Four samples containing fibrin clots were indeterminate by MP. GC concordance was 100% (K = 1.0) both for A and B of 272 samples.

In ABO, reverse testing (Table 1A and Table 1B), MP was concordant with tube technique in 273 samples at 93.8% (K = 0.884) for agglutination strength against A_1 cells and 79.5% (K = 0.663) against B cells. Gel column method was concordant with tube technique in 272 samples at 86.1% (K = 0.769) against A1 cells and at 79.2% (K = 0.675) against B cells. MP missed 2 samples of anti-A and 2 samples of anti-B, and showed weaker reactions than tube technique in 54 samples of anti-B. Similarly, GC missed one anti-B, and had weaker reactions than tube technique in 30 anti-A and 54 anti-B. MP required re-examination and/or detailed examination due to indeterminate ABO reverse grouping in 14 (5.1%) samples; in contrast only four samples (1.5%) by tube technique needed such examinations. Four samples in GC were indeterminate due to slightly unclear blood cell borders, and one sample was false positive due to the influence of strong chyle. Gel column method required visual confirmation and re-examination for 7 (2.6%) samples; in contrast, tube technique had no indeterminate results.

In RhD typing (Table 1A and Table 1B), both MP and GC showed good agreement with tube technique: 98.9% (K = 0.75) and 99.6% (K = 0.80) concordance, respectively.

Irregular antibody screening

In routine irregular antibody screening, MP and GC methods showed substantial concordance with tube technique, at 98.9% (K = 0.79) and 97.8% (K = 0.61), respectively (Table 2). However, MP missed one anti-E and one anti-Fy^b out of 8 alloantibodies, which tube PEG-IAT detected. Similarly, GC failed to discover 5 irregular antibodies (two anti-E and one each of anti-Fy^b, -Jk^a, and -Le^a) out of 10 clinically significant antibodies, which showed weak agglutination by tube PEG-IAT. One anti-E was ultimately detected by GC re-examination using enzyme (papain)-treated RBCs. Conversely, tube PEG-

Table 1 A Comparison of reaction strength between microplate method and tube technique in ABO forward/reverse grouping and RhD typing

ABO forward grouping (n = 273)

a

Anti-A		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	149	2[*1]				
	w+						
	1+						
	2+						
	3+						
	4+						122

K = 0.985

- Reactivity concordance rate 99.3% (271 of 273 samples)

b

Anti-B		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	178	2[*1]				
	w+						
	1+						
	2+						
	3+						1
	4+						92

K = 0.976

- Reactivity concordance rate 98.9% (270 of 273 samples)

ABO reverse grouping (n = 273)

c

A_1 blood cell		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	119	2[*1]				
	w+		1[*2]				
	1+	1[*3]					
	2+					1	
	3+				1	1	5
	4+	1[*3]				6	135

K = 0.884

- Reactivity concordance rate 93.8% (256 of 273 samples)
- 3.3% (9 samples: below gray diagonal) showed weaker reactivity by microplate method than tube technique.
- 2.2% (6 samples: above gray diagonal) showed weaker reactivity by tube technique than microplate method.

d

B blood cell		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	92					
	w+						
	1+	1[*4]					
	2+		1[*5]				
	3+	1[*6]	1[*6]		9	4	2
	4+		1[*7]	1	15	24	121

K = 0.664

- Reactivity concordance rate 79.5% (217 of 273 samples)
- 19.8% (54 samples: below gray diagonal) showed weaker reactivity by microplate method than tube technique.
- 0.73% (2 samples: above gray diagonal) showed weaker reactivity by tube technique than microplate method.

RhD typing (n = 273)

e

Anti-D		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	3					
	w+						
	1+		1[*8]				
	2+						
	3+					1	2
	4+						266

K = 0.746

- Reactivity concordance rate 98.9% (270 of 273 samples)
- 0.37% (1 sample: below gray diagonal) showed weaker reactivity by microplate method than tube technique.
- 0.73% (2 samples: above gray diagonal) showed weaker reactivity by tube technique than microplate method.

[*1] Contamination of fibrin clots, confirmed to be negative by re-examination.

[*2]Rouleaux formation, confirmed to be weak positive by tube technique with no enhancer.

[*3]Confirmed to be weak positive by microplate method re-examination, but identified as group B by tube technique.

[*4]Cold-reactive antibody

[*5]Confirmed to be indeterminate by microplate method re-examination, but identified as group O by tube technique (6 months old infant)

[*6]Confirmed to be negative/indeterminate by microplate method re-examination, but identified as group A by tube technique.

[*7] 2+ by microplate method re-examination.

[*8] Possibility of chimera

Re-examination by microplate method for 14 samples: *1,*2,*3,*5,*6,*7, and *8

Re-examination by the tube technique for 4 samples: *2,*3,*4, and *8

IAT missed one autoantibody, which MP detected, and one cold-reactive anti-M, which GC detected.

Table 1B Comparison of reaction strength between gel column and tube technique in ABO forward/reverse grouping and RhD typing

ABO forward grouping (n = 272)

a

Anti-A		Microplate Method					
		0	Indeterminate	1+	2+	3+	4+
Tube Technique	0	149	2*1				
	w+						
	1+						
	2+						
	3+						
	4+						127

K = 1.0

- Reactivity concordance rate 100%

b

Anti-B		Gel Column Method					
		0	+/-	1+	2+	3+	4+
Tube Technique	0	185					
	w+						
	1+						
	2+						
	3+						
	4+						87

K = 1.0

- Reactivity concordance rate 100%

ABO reverse grouping (n = 272)

c

A_1 blood cell		Gel Column Method					
		0	+/-	1+	2+	3+	4+
Tube Technique	0	122	4*1	1*2			
	w+						
	1+						
	2+						
	3+					2	1
	4+					30	112

K = 0.769

- Reactivity concordance rate 86.1% (236 of 272 samples)
- 10.9% (30 samples: below gray diagonal) showed weaker reactivity by gel column method than tube technique.
- 0.36% (1 sample: above gray diagonal except for *1,*2) showed weaker reactivity by tube technique than gel column method.

Sensitivity of two automated systems and the tube technique in the detection of red cell alloantibodies

Microplate detected at higher dilutions than tube PEG-IAT and GC a total of six antibodies (anti-E, -Jk^a, -Jr^a, -Di^a, -D, and anti-C) among 8 clinically significant irregular antibodies (MP versus PEG-IAT: $p<0.05$, MP versus GC: $p<0.05$); PEG-IAT was superior to GC ($p<0.05$) in identifying eight clinically significant antibodies. Anti-C, which MP and tube PEG-IAT detected, was missed by

d

B blood cell		Gel Column Method					
		0	+/-	1+	2+	3+	4+
Tube Technique	0	87					
	w+						
	1+						
	2+		1*3				
	3+	1*4		4	4	13	1
	4+				1	43	117

K = 0.675

- Reactivity concordance rate 79.2% (217 of 272 samples)
- 19.7% (54 samples: below gray diagonal) showed weaker reactivity by gel column method than tube technique.
- 0.37% (1 sample: above gray diagonal) showed weaker reactivity by tube technique than gel column method.

RhD typing (n = 272)

e

Anti-D		Gel Column Method					
		0	+/-	1+	2+	3+	4+
Tube Technique	0	2					
	w+						
	1+						
	2+						
	3+						1
	4+						269

K = 0.799

- Reactivity concordance rate 99.6% (271 of 272 samples)
- 0.36% (1 sample: above gray diagonal) showed weaker reactivity by tube technique than gel column method.

*1Blood cell-reagents interface was slightly unclear.

*2Strong chylous sample

*3± by gel column re-examination, but identified as group A by the tube technique.

*4Confirmed to be negative by gel column re-examination, but identified as group A by tube technique.

Gel column weak positive by visual confirmation for 5 samples: *1, and *2

Performed gel column re-examination for 2 samples; *3, and *4

Table 2: Comparison of indirect antiglobulin test for irregular antibody screening

(A) Microplate method vs tube technique

Indirect Antiglobulin Test (n=270)		Microplate Method	
		Neg	Pos
Tube Technique	Neg	261	1*
	Pos	2**	6

K = 0.794

- Concordance rate 98.9% (267 of 270 samples).

*Autoantibody: negative by tube technique, but positive by microplate method.

**Anti-E and -Fy^b which could not be detected by microplate method. The reaction strengths by tube technique were 1+ and w+, respectively.

(B) Gel column method vs tube technique

Indirect Antiglobulin Test (n=270)		Gel Column Method	
		Neg	Pos
Tube Technique	Neg	259	1#
	Pos	5##	5

K = 0.614

- Concordance rate 97.8% (264 of 270 samples).

#A cold-reactive anti-M was not detected by tube technique, but detected by gel column method.

##Two anti-E, 1 each of -Fy^b, -Jk^a, and -Le^a, among 10 antibodies which were detected by tube technique, could not be detected by gel column method. One anti-E could be detected by gel column method using enzyme (papain)-treated red cells.

GC. However, anti-Fy^b, which tube PEG-IAT detected, was missed by MP and GC. Among three clinically less significant antibodies, GC was most sensitive, detecting two anti-M samples; MP did not detect an anti-Le^a that the other two methods detected (Figure 1).

Detection threshold of mixed-field agglutination

Tube technique was able to identify mixed-field agglutination with the best resolving power using artificially composed chimeras of group O and A_1 cells in ratios of 1:9 through 9:1, whereas MP identified chimeras at 60–80%, but not when O cells comprised 10%, 50%, and 90% of a sample. Gel column method did not identify chimeras at 10–30% of group O cell composition, but identified them at 40–90% (Table 3).

DISCUSSION

The tube technique and two automated systems showed good concordance, with rates of 98% in ABO forward grouping and RhD typing. Contamination with small fibrin clots caused indeterminate results in MP.

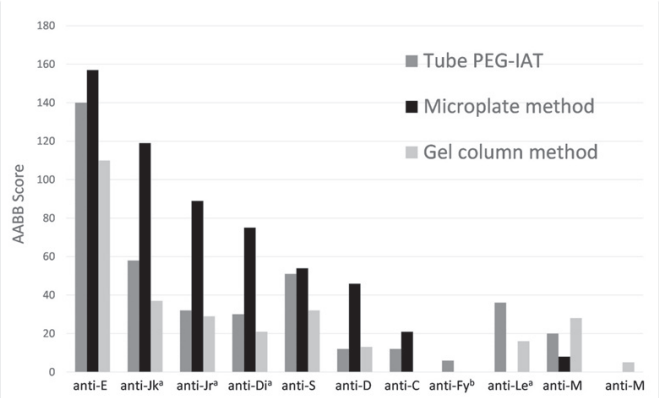

Figure 1: Six antibodies, anti-E, -Jk^a, -Jr^a, -Di^a, -D, and -C, could be detected up to the highest dilution magnification by microplate method. The most sensitive antibodies by the PEG-IAT included anti-S, -Fy^b, and -Le^a. Anti-Fyb was detected only by the PEG-IAT. The most sensitive antibodies by gel column method were two anti-M samples. Anti-C was not detected by gel column method.

In ABO reverse grouping, MP showed better agreement with tube technique than GC, but reaction strength in MP was weaker than that in tubes. Such slightly weaker reactions of GC at detecting anti-B iso-hemagglutinins have been reported [11]. Weaker reactions may originate from the technological principles differentiating the three methods. Gel column method subjects red blood cells to ordinary gravity over a period of time, so when antigen-antibody affinity is low, an antibody could dissociate and ultimately settle out on a bottom layer, even as red blood cells crosslink. On the other hand, tube technique forms pellets by centrifugal force, and can detect weak reactivity enhanced by breaking contact with the tube wall. Microplate dispenses samples and reagent, centrifuges after the reaction occurs, shakes (refloating and shaping the blood cells), and finally uses a reader to measure agglutination. The shaking operation is stronger than that of the tube technique, and may cause a weaker agglutination than that in tubes.

In addition, weaker reactivity to B blood cells than A_1 blood cells could be explained by having fewer antigen-binding sites. The number of antigens expressed on A_1 adult blood cells is about 1×10^6/cell versus about 0.7×10^6 for group B red blood cells [12]. This difference may explain why mis-transfusion of A_1 red cells, compared with B cells, to group O recipients is more likely to be fatal [13]. It is also reported that the major causes of mismatch between the result of forward and reverse ABO blood grouping is caused by weak reactivity of anti-B [11]. Discrepancies arise from other causes, such as weak subgroups of A or B and ABO non-identical hematopoietic stem cell transplantation. Thus the numerical difference in expression of A and B antigen, and the difference in antibody titers between anti-A and anti-B in patient plasma are consistent with the current results.

Table 3: Comparison in the detection of chimera/mixed-field agglutination with three methods

Red Cell Ratio	Tube technique		Microplate method		Gel column method		
A_1 cell : O cell	anti-A	anti-B	anti-A	anti-B	anti-A	anti-B	anti-A,B
100 : 0	4+	0	4+	0	4+	0	4+
90 : 10	mf	0	4+	0	4+	0	4+
80 : 20	mf	0	4+	0	4+	0	4+
70 : 30	mf	0	4+	0	4+	0	4+
60 : 40	mf	0	3+	0	dp	0	dp
50 : 50	mf	0	3+	0	dp	0	dp
40 : 60	mf	0	indeterminate	0	0	0	dp
30 : 70	mf	0	indeterminate	0	0	0	dp
20 : 80	mf	0	indeterminate	0	dp	0	dp
10 : 90	mf	0	0	0	0	0	dp
0 : 100	0	0	0	0	0	0	0

mf: mixed-field, dp: double population

- Microplate method showed positive reactivity when the group O RBC ratios were between 10% and 50%, and were indeterminate when the group O ratios were between 60% and 80%.
- Gel column method showed double population when the group O ratios were between 40% and 90%.

In irregular antibody detection study for routine screening, MP missed 2 and GC missed 5 clinically significant antibodies, all of which tube PEG-IAT detected. Our modified PEG-IAT (2 drops PEG vs. 4 drops in original [9]) in PEG-IAT may have enhanced detection of weak antibodies, by avoiding over-dilution. Conversely, MP detected one autoantibody and GC detected one cold-reactive anti-M that tube PEG-IAT missed. It has been reported that warm-autoantibodies are more likely to be detected by solid phase versus column [2]. Column methods and solid phase methods usually use anti-IgG antibodies for their antiglobulin test, and they show low detection sensitivity for antibodies of low clinical significance, such as IgM-type antibodies [14,15], but there are cases of IgM-type antibodies being detected. By warming and washing in tube methods, IgM antibodies may dissociate from red blood cells, and form agglutination in a few cases [3]. One patient having anti-E required a blood transfusion before completion of antibody testing, but no acute hemolysis, delayed hemolytic transfusion reaction (DHTR), or delayed serological transfusion reaction (DSTR) was observed, possibly the transfused red cells may have been E-negative.

There have been reports of antibodies undetectable by automated systems [7, 16, 17]. Anti-Jk^a, most notorious for transient detection and subsequent acute hemolytic transfusion reaction and clinically overt DHTR after transfusion of Jk^a-positive red blood cells, was significantly missed by GC in 30.5% (32/105) of patients, which a solid-phase adherence test could discover, and, importantly, 17 recipients developed DHTR (6), possible DHTR (3), or DSTR (8) following Jk^a antigen-unknown red cell transfusion [7]. Conversely, GC is reported to be comparable to tube PEG-IAT in irregular antibody detection and resulted in a lower incidence of DHTR and DSTR [5]. A fully automated solid-phase red cell adherence system (ABS 2000) is reported to be discordant with tube technique (using an albumin or low-ionic-strength saline additive) in 245 (3.3%) of 7510 samples; 19 manual method false-negatives (1 anti-C^w, 6 anti-E, 3 anti-Jk^a, and 7 warm autoantibodies), 9 solid-phase false-negatives (1 anti-D, 2 anti-K, 4 anti-M, and 2 anti-Le^a), 2 manual method false-positives and 7 solid-phase false-positives [2]. Similarly, in the detection of irregular antibodies, two solid-phase microplate assays (Capture-R and Solidscreen) are reported to be more sensitive by 18–22% compared with PEG-IAT tube method [4].

The detection rates of patients with irregular red cell antibodies at our institute ranged between 113/10,000 by tube albumin-IAT and 125/10,000 by PEG-IAT [8]. In this survey, the detection rates were better than our historical records: 259/10,000 by MP, 220/10,000 by GC, and 333/10,000 by PEG-IAT. However, of note, antibodies of low strength but clinically significant were missed in 25% of cases by MP and 50% by GC.

Microplate generally showed higher AABB agglutination score by titration studies using frozen antibody-positive samples (Figure 1). Anti-Fy^b with weak reactivity by tube PEG-IAT was not detectable by MP and GC. Anti-C failed to be detectable by GC. One of two anti-M samples was not detected by MP using Capture-R Ready-Screen (III), in which M antigen was heterozygous (Figure 1), but was detected by subsequent re-examination using a homozygous reagent. This indicates that blood group antigens having a dosage effect also affect detection sensitivity. In fact, it is reported that less sensitivity in automated detection of antibodies against antigens related to the Duffy and Kidd blood group systems compared to the tube technique seems

to be related to the reagent's zygotic status [3]. When screening clinically important antibodies, corresponding homozygous red blood cells need to be included in screening panels. One anti-Le[a] and two anti-M currently used for comparison of the detection sensitivity among three methods were not able to distinguish whether they were IgG-type or IgM-type due to weak agglutination reactivity. Though samples used in this study had been previously positive by PEG-IAT, repeated freezing and thawing might influence the reactivity.

The detection sensitivity of MP was higher than PEG-IAT in this study, but there were antibodies negative by MP which showed weak agglutination reaction by PEG-IAT. This phenomenon implies generally high sensitivity of the MP method, but it cannot detect all clinically important antibodies.

The mixed-field agglutination, or red cell chimera, results of this study clearly showed that tube technique was the most sensitive in discovering mixed chimeras composed of A_1 and O cells, maybe because visual confirmation can easily distinguish double populations. However, automated MP and GC may not be adequate for clinical samples such as post-ABO mismatched hematopoietic stem cell transplantation, and post-ABO non-identical red cell transfusion for emergency.

CONCLUSION

Two automated systems investigated in this report concord well with tube technique in ABO forward and RhD typing. However, they appeared to be less sensitive than tube technique in detecting isohemagglutinin anti-A/anti-B in ABO reverse testing and in identifying ABO chimeras. Automation is efficient and can prevent some kinds of human errors, but in regard to the detection of low-titer, clinically significant antibodies, GC may fail to detect those that can endanger patients with post-transfusion hemolysis.

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Author Contributions

Takako Ono – Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Hitoshi Ohto – Substantial contribution to conception and design, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Hiroyasu Yasuda – Substantial contribution to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Rie Hikichi – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Kinuyo Kawabata – Substantial contribution to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Keiji Minakawa – Acquisition of data, Drafting the article, revising it critically for important intellectual content, Final approval of the version to be published
Satoshi Ono – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Masami Kikuchi – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Akiko Sugawara – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Shunnichi Saito – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Nozomi Takano – Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Kenneth E. Nollet – Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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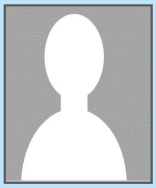

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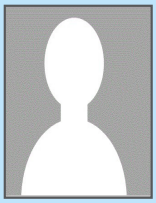

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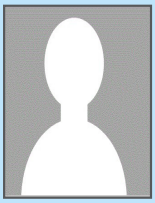

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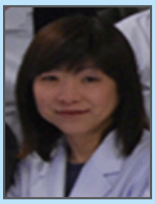

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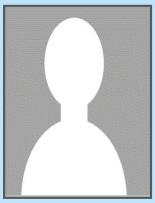

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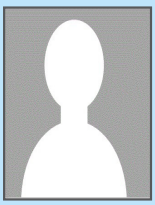

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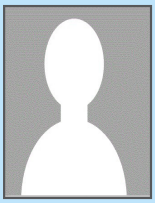

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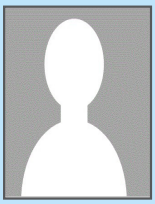

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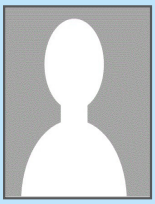

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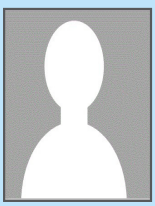

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