

Assessment of RBC Antibody Frequencies and Comparison of Screening and Identification Techniques Used in a Tertiary Hospital in the Philippines

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ABSTRACT

Background. Pre-transfusion testing is done to avoid transfusion morbidity from unexpected RBC antibodies. Available commercial kits from Western brands may not consider racial differences in antibody frequencies between East/Southeast Asians and Western populations. The limited number of blood banks in the Philippines precludes research on RBC antibody screening and identification in the country.

Objective. This study aimed to compare RBC antibody screening and identification methods in patients at a tertiary hospital in the Philippines, assess the frequency of major blood group antibodies using both techniques, and review clinical histories of discrepant and nonspecific cases.

Methodology. Retrospective review showed 118 cases with both screening and identification tests using both conventional tube-based technique and column agglutination or gel-based technique. Antibody frequencies and discrepant or nonspecific results were recorded. Concordance rates were calculated, and differences between the two methods were analyzed using 95% confidence interval (95% CI). Clinical histories of discrepant and nonspecific cases were also reviewed.

Results. The most frequent major blood group was Rh (41 cases or 34.7%), followed by MNS (34 cases or 28.8%) and Kidd (15 cases or 12.7%). The most common antibody was Anti-E (24 cases or 20.3%), followed by Anti-Mi^a (19 cases or 16.1%), and Anti-M and Anti-c (12 cases each, or 10.2% each). The concordance rate for screening was statistically significant at 72%. Concordance rate for identification was 59.3%, with significant difference in identifying Anti-Mi^a. Clinical histories for discrepant or nonspecific cases showed previous transfusions, pregnancy, lymphoproliferative conditions, and certain medications.

Conclusion. Statistically significant differences between the two methods were found, with the gel-based technique identifying more Anti-Mi^a cases. Negative results from the tube-based method do not fully exclude Anti-Mi^a. These discrepancies highlight the benefit of using both methods for comprehensive RBC antibody screening and identification, done as a complement to the other.

Key words: blood bank, blood transfusions, blood grouping, antibody

ISSN 2507-8364 (Online)

Printed in the Philippines.

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Received: 16 October 2024.

Accepted: 19 November 2024.

Published online first: 28 November 2024.

<https://doi.org/10.21141/PJP.2024.13>

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INTRODUCTION

A common cause of transfusion morbidity is unexpected RBC antibodies.^{1,2} To avoid this, pre-transfusion testing through screening and identification of major blood group antibodies is done. Different test kits implore the use of reagent RBCs or screening cells, where their reaction to patient serum leads to agglutination or hemolysis.

Two methods used for the detection and identification of RBC antibodies include the tube-based method and the gel-based method, each with its own advantages and disadvantages. The conventional tube technique is based on the visible macroscopic aggregates of hemagglutination seen. It offers flexibility to test at different phases, as well as an option to use additive solutions. While it has been considered the reference method,^{3,4} it has unstable endpoint and interobserver variability.⁵ On the other hand, the column agglutination technique or gel-based method is based on differential migration of RBC agglutinates through a small microcolumn containing a dextran



acrylamide size exclusion gel column. Compared to the previous technique, it allows for a more standardized and reproducible approach. However, it has a higher incidence of false positives and has been shown to enhance serologic reactivity that may not be clinically significant.^{5,6}

Available commercial kits utilizing these two techniques are often manufactured in Western countries, and are often based on American or European demographics.⁷ Issues may arise, as there have been recorded racial differences in the frequencies of RBC antibodies in East and Southeast Asians compared to Western populations.⁷⁻⁹ Literature on Asian countries show that the frequently seen antibodies are: E, D, M, and Mi^a in Chinese; Lea, E, Mi^a and Le^b in Southeast Asians; and Mi^a and E antibodies in Eastern Taiwanese.⁹ Antibodies against MNS blood group were also the most common in Malaysia and Taiwan.⁷ This is in contrast to frequencies seen in Western populations, where the most frequent antibodies identified in Americans are E, Le^a, K, D, Le^b, M, P₁, Fy^a, C, and c.⁹ With existing commercial kits being often based on Western data where the kit was manufactured, this may be disadvantageous for Asian countries that rely on such kits for routine use.⁷ It cannot be dismissed that some antibodies may not be represented in the panels that are utilized in these kits.¹⁰ For example, while antibodies to MNS antigens are common in South and East Asian populations, these are often missed in standard screening cells.¹¹ Unfortunately, due to the limited number of blood banks that offer such services in the country, there is yet to be a study exploring RBC antibody frequencies, as well as screening and identification techniques used in the Philippines.

This study aimed to compare the two (2) antibody screening and identification methods for the major blood group antibodies in patients who underwent antibody screening and identification in the blood bank of a tertiary hospital in the Philippines. Additionally, it aimed to assess the frequency of major blood group antibodies, and to review clinical histories of discrepant and nonspecific cases.

METHODOLOGY

This is a retrospective, descriptive-analytical, and cross-sectional study approved by the Institutional Ethics Review Committee (IERC) of St. Luke's Medical Center – Quezon City (SLMC-QC), which abided by the Principles of the Declaration of Helsinki (2013) and conducted along the Guidelines of the International Conference on Harmonization - Good Clinical Practice (ICH-GCP) on privacy and confidentiality. The two kits used for antibody screening were the Panoscreen I, II and III 3-vial set by Immucor, Inc. (tube-based method) and the ID-Diacell I-II-III Asia by Bio-Rad (gel-based method). On the other hand, the two kits used for antibody identification were the Panocell-10 12-vial set by Immucor, Inc. (tube-based method) and the ID-DiaPanel by Bio-Rad (gel-based method).

Patient selection

Of 1703 cases reviewed from the Blood Bank and Transfusion Medicine Section of the Institute of Pathology laboratory information systems and manual records, from January 2012 to October 2023, only those included in this

study were patients who had complete data on both their RBC antibody screening and identification results using both methods for each test, leaving a total of 118 cases. Cases with incomplete records were excluded in this study.

Data analysis

Data gathered from medical records included the following: age, sex, antibody screening result with corresponding type of kit used, antibody identification result with corresponding type of kit used, and clinical history. Frequency by percentage was used for the descriptive analysis of the study. The concordance rate in percentage was calculated between the two screening and two identification methods. Analysis of the significance of the difference was done using 95% CI.

RESULTS

This study included 118 patients out of 1703 cases reviewed, of which 79 were female and 39 were male. The mean age of our sample population was 57.42 (SD 20.9 years). In terms of major blood groups, the most frequent were antibodies to variants of Rh (41 samples or 34.7%), followed by MNS (34 samples or 28.8%), and Kidd (15 samples or 12.7%). The less frequent blood groups were Lewis (5 samples or 4.2%), Lutheran (2 samples or 1.7%), Kell and Duffy (1 sample each, or 0.8%) (Figure 1). Regarding specific RBC antibody, the most common was Anti-E (24 samples or 20.3%) followed by Anti-Mi^a (19 samples or 16.1%), Anti-M and Anti-c (12 samples or 10.2% each), and Anti-Le^a (11 samples or 9.3%). The less common antibodies in the sample population were Anti-Jk^b (8 samples or 6.8%), Anti-Jk^a (7 samples or 5.9%), Anti-P₁ (5 samples or 4.2%), Anti-Le^b (3 samples or 2.5%), Anti-Lu^a, Anti-e and Anti-C (2 samples or 1.7% each), and Anti-s, Anti-S, Anti-N, Anti-Fy^a, Anti-K, and Anti-C^w (1 sample or 0.8% each) (Table 1, Figure 2).

Table 1. Frequency of RBC antibodies

Antibody	n (%)
<i>Anti-D</i>	0 (0)
<i>Anti-C</i>	2 (1.7)
<i>Anti-c</i>	12 (10.2)
<i>Anti-E</i>	24 (20.3)
<i>Anti-e</i>	2 (1.7)
<i>Anti-C^w</i>	1 (0.8)
<i>Anti-K</i>	1 (0.8)
<i>Anti-k</i>	0 (0)
<i>Anti-Fy^a</i>	1 (0.8)
<i>Anti-Fy^b</i>	0 (0)
<i>Anti-Jk^a</i>	7 (5.9)
<i>Anti-Jk^b</i>	8 (6.8)
<i>Anti-Le^a</i>	11 (9.3)
<i>Anti-Le^b</i>	3 (2.5)
<i>Anti-M</i>	12 (10.2)
<i>Anti-N</i>	1 (0.8)
<i>Anti-S</i>	1 (0.8)
<i>Anti-s</i>	1 (0.8)
<i>Anti-Mi^a</i>	19 (16.1)
<i>Anti-H</i>	0 (0)
<i>Anti-P₁</i>	5 (4.2)
<i>Anti-Lu^a</i>	2 (1.7)
<i>Anti-Lu^b</i>	0 (0)
Autoantibodies present	36 (30.5)
Nonspecific antibody/ies	15 (12.7)

Based on our findings, the gel-based screening method was able to detect more cases with RBC antibodies (117 samples or 99.2%) compared to the tube-based screening method (86 samples or 72.9%). Concordant positive results were 72% (85 samples). The difference between the two methods was statistically significant based on their non-overlapping computed 95% CI, with the gel-based method having a 95% CI of 96.1 – 99.9, and the tube-based method having a 95% CI of 64.4 – 80.3 (Table 2). In the discrepant screening results, the majority of cases were positive in the gel-based method and negative in the tube-based method (32 samples or 97%), with only one case being positive in the tube-based method and negative in the gel-based method. Among these, Anti-Mi^a was the most identified antibody (16 samples or 48.5%) (Table 3, Figure 3). On the other hand, comparing the tube-based versus the gel-based methods in antibody identification, concordance was 59.3% (70 samples) (Table 4). The difference in identifying

Anti-Mi^a antibody was notably statistically significant between the two methods based on their non-overlapping 95% CI, with the gel-based having a 95% CI of 9.6 – 22.5, and the tube-based having a 95% CI of 1.2 – 7.9. There was no statistical difference seen in the other antibodies (Table 5, Figure 4).

Of the 48 discrepant identification results, clinical review showed history of transfusion (30 cases or 62.5%), past pregnancy (22 cases or 45.8%), malignancy (16 cases or 33.3%), history of a volume expander (11 cases or 22.9%), infection (4 cases or 8.3%), and autoimmune disease (2 cases or 4.2%) (Table 6, Figure 5). The clinical history of the 15 cases who were deemed to have antibodies with no specificity were also reviewed, which showed history of transfusion (10 cases or 66.7%), past pregnancy (10 cases or 45.8%), malignancy (8 cases or 53.3%), history of a volume expander (7 cases or 46.7%), infection (5 cases or 33.3%),

Table 2. Concordance between gel-based and tube-based methods

Screening kit, n=118	n (%)	95% CI
Gel-based*		
Positive	117 (99.2)	96.1 - 99.9
Negative	1 (0.8)	0.1 - 3.9
Tube-based*		
Positive	86 (72.9)	64.4 - 80.3
Negative	32 (27.1)	19.7 - 35.6
Concordance	85 (72.0)	
* ID-Diacell I-II-III Asia (Bio-Rad)		
** Panoscreen I, II and III 3-vial set (Immucor, Inc.)		

Table 3. Frequency of identified antibodies from the discrepant cases

Antibody identified	n (%)
Anti-E	2 (6)
Anti-Jk ^a	1 (3)
Anti-Le ^a	1 (3)
Anti-Le ^b	1 (3)
Anti-M	2 (6)
Anti-N	1 (3)
Anti-Mi ^a	16 (48.5)
Anti-Lu ^a	1 (3)
Autoantibodies present	9 (27.3)
Nonspecific antibody/ies	6 (18.2)

Table 4. Concordance between the gel-based and tube-based identification kits

Identification kit, n=118	n (%)
Concordance	
Yes	70 (59.3)
No	48 (40.7)

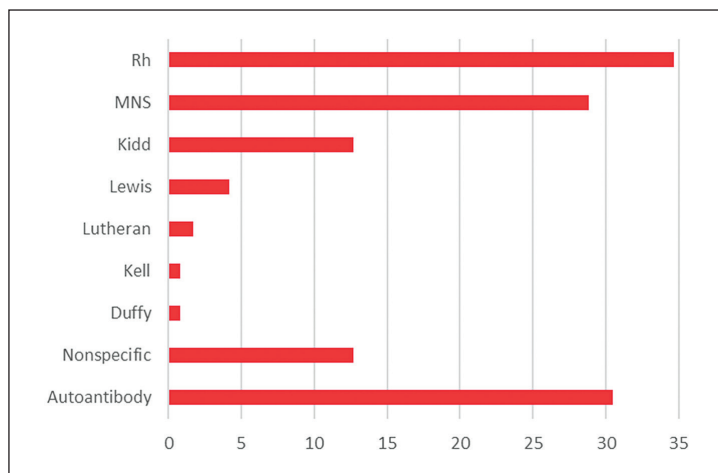


Figure 1. Frequency of RBC major blood group identified.

Table 5. Frequency of identified antibodies per method

Antibody identified	Gel-based, n (%)	95% CI	Tube-based, n (%)	95% CI
Anti-D	0 (0)	-	0 (0)	-
Anti-C	2 (1.7)	0.4 - 5.3	1 (0.8)	0.1 - 3.9
Anti-c	9 (7.6)	3.8 - 13.5	6 (5.1)	2.1 - 10.2
Anti-E	18 (15.3)	0.6 - 22.5	17 (14.4)	9 - 21.6
Anti-e	1 (0.8)	0.1 - 3.9	2 (1.7)	0.4 - 5.3
Anti-C ^v	2 (1.7)	0.4 - 5.3	1 (0.8)	0.1 - 3.9
Anti-K	1 (0.8)	0.1 - 3.9	1 (0.8)	0.1 - 3.9
Anti-k	0 (0)	-	0 (0)	-
Anti-Fy ^a	0 (0)	-	0 (0)	-
Anti-Fy ^b	0 (0)	-	0 (0)	-
Anti-Jk ^a	2 (1.7)	0.4 - 5.3	2 (1.7)	0.4 - 5.3
Anti-Jk ^b	5 (4.2)	1.6 - 9	5 (4.2)	1.6 - 9
Anti-Le ^a	9 (7.6)	3.8 - 13.5	7 (5.9)	2.7 - 11.3
Anti-Le ^b	1 (0.8)	0.1 - 3.9	1 (0.8)	0.1 - 3.9
Anti-M	15 (12.7)	7.6 - 19.6	13 (11)	6.3 - 17.6
Anti-N	0 (0)	-	0 (0)	-
Anti-S	2 (1.7)	0.4 - 5.3	0 (0)	-
Anti-s	0 (0)	-	1 (0.8)	0.1 - 3.9
Anti-Mi ^a	18 (15.3)	9.6 - 22.5	4 (3.4)	1.2 - 7.9
Anti-H	0 (0)	-	0 (0)	-
Anti-P ₁	2 (1.7)	0.4 - 5.3	4 (3.4)	1.2 - 7.9
Anti-Lu ^a	1 (0.8)	0.1 - 3.9	0 (0)	-
Anti-Lu ^b	0 (0)	-	0 (0)	-
Pan-agglutination	20 (16.9)	11 - 24.5	20 (16.9)	11 - 24.5
Non-specific	19 (16.1)	10.3 - 23.5	24 (20.3)	13.8 - 28.3

and Daratumumab medication (1 case or 6.7%) (Table 7, Figure 6). It can also be noted that many cases in the sample population showed the presence of autoantibodies (36 samples or 30.5%) (Table 1), as well as in cases with discrepant results (6 or 18.2%) (Table 3).

DISCUSSION

Our findings show similarities with other Asian countries in terms of frequency of each RBC antibody. Anti-E had a frequency of 20.3% in our sample, which is comparable to Southeast Asians (17.3%) and Taiwanese (15.6%); it is more prevalent among Chinese (53.1%). Our sample's Anti-E antibody frequency is also similar to Americans (20.3% versus 20.8%). Next, the frequency of Anti-Mi^a antibody in our sample (16.1%) is also comparable with the frequencies in Chinese (10.9%) and Southeast Asians (12.5%); it is more prevalent among Taiwanese population (44.4%).⁹ The Anti-Mi^a antibody belong to the Miltenberger (Mi) subsystem associated with the MNS blood group. It is rarely reported in the Western population and is rare

Table 6. Clinical history of cases with discrepant results

	n (%)
<i>Autoimmune Disease</i>	2 (4.2)
<i>Infection</i>	4 (8.3)
<i>Volume Expander*</i>	11 (22.9)
<i>Malignancy</i>	16 (33.3)
<i>Past Pregnancy</i>	22 (45.8)
<i>Previous Transfusion</i>	30 (62.5)

*Volume expander including (dextran, gelatin derivatives, hydroxyethyl starch, and human albumin solutions)

Table 7. Clinical history of cases signed out as “antibody of no specificity”

	n (%)
<i>Autoimmune disease</i>	0 (0)
<i>Infection</i>	5 (33.3)
<i>Volume expander*</i>	7 (46.7)
<i>Malignancy</i>	8 (53.3)
<i>Past pregnancy</i>	10 (66.7)
<i>Previous transfusion</i>	10 (66.7)
<i>Daratumumab</i>	1 (6.7)

*Volume expander including (dextran, gelatin derivatives, hydroxyethyl starch, and human albumin solutions)

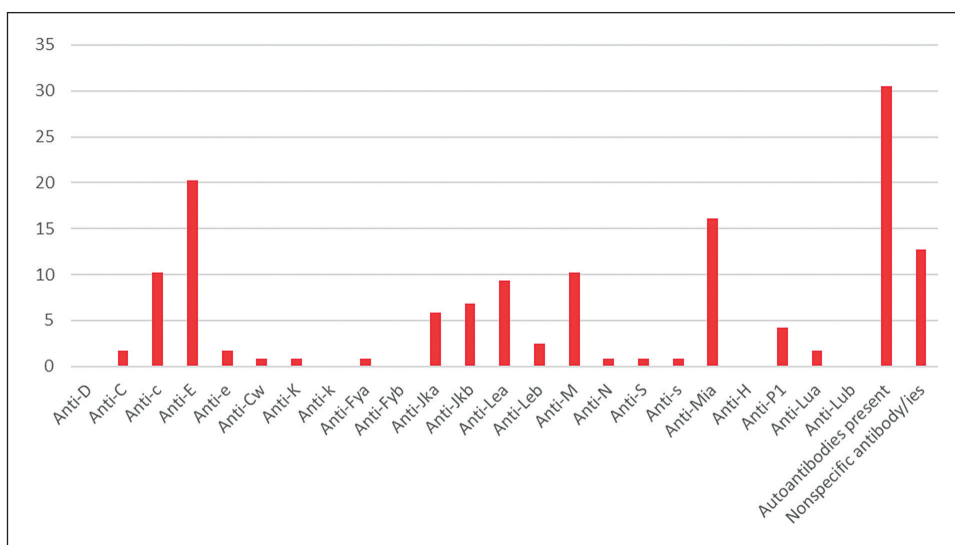


Figure 2. Frequency of RBC antibodies.

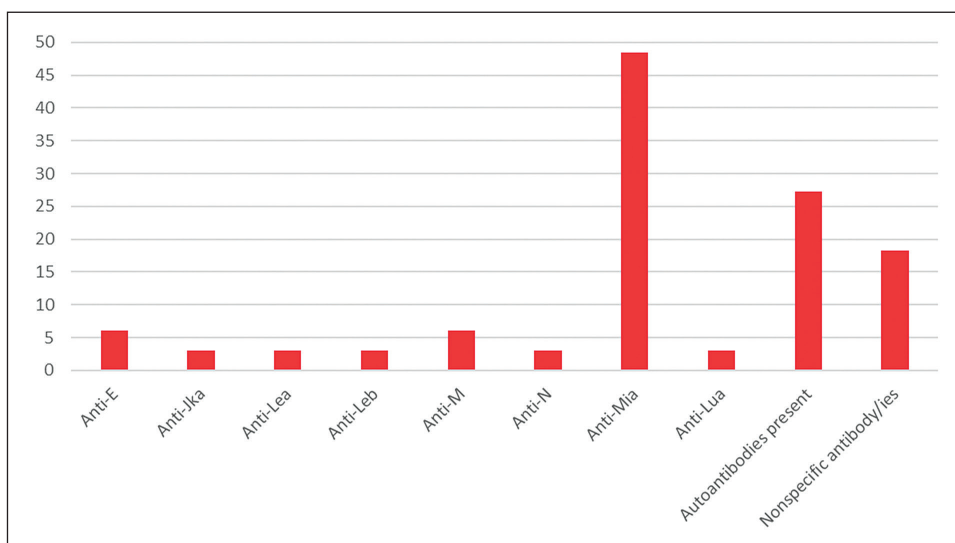


Figure 3. Frequency of antibodies in discrepant cases.

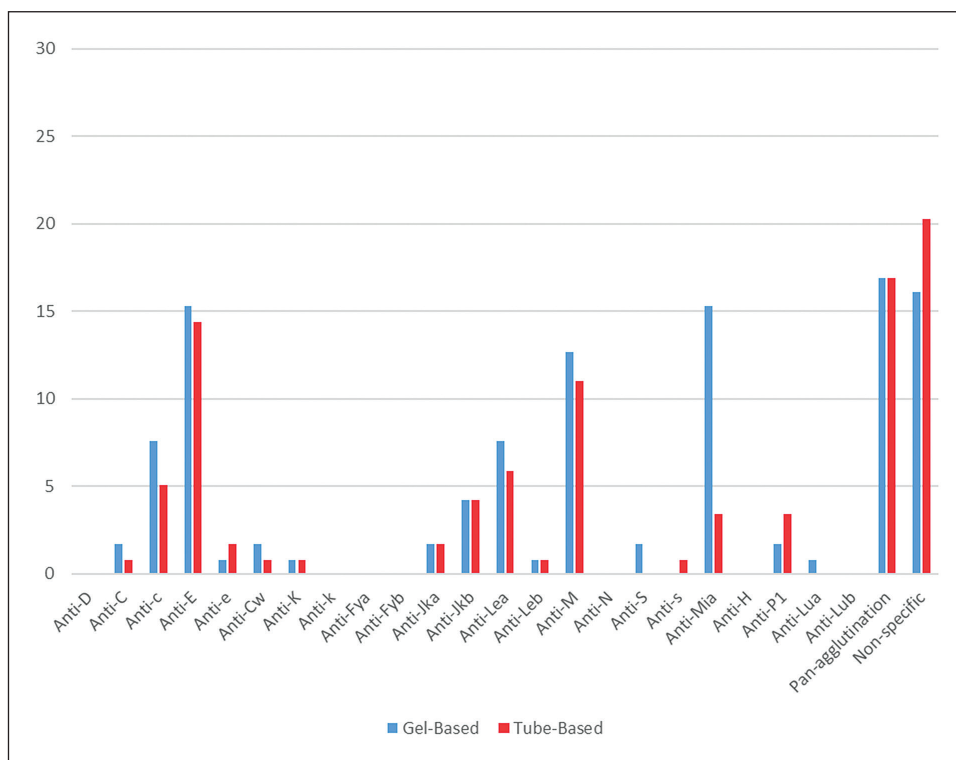


Figure 4. Frequency of identified antibodies per method.

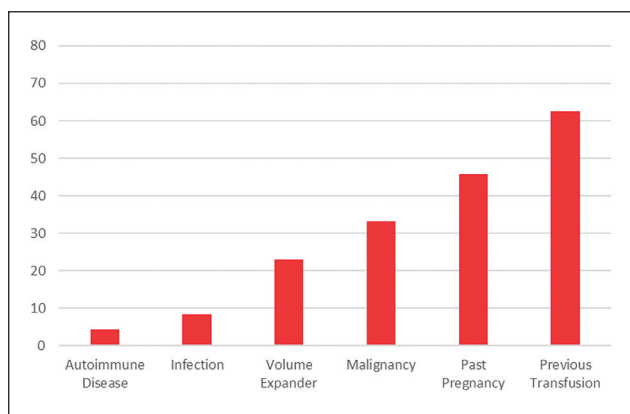


Figure 5. Clinical history of cases with discrepant results.

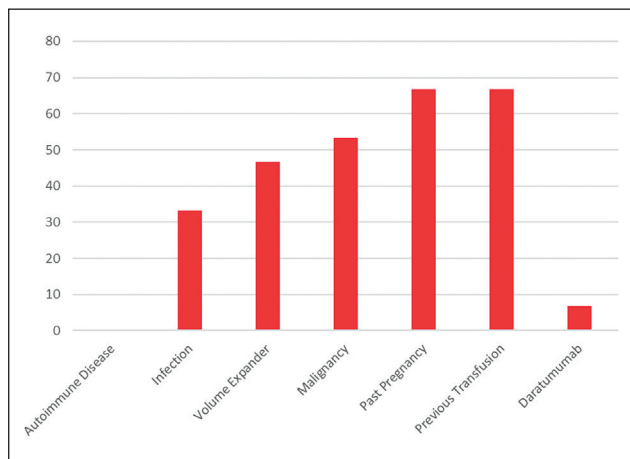


Figure 6. Clinical history of cases signed out as antibody of no specificity.

in Europeans and Africans, but is commonly found in Chinese and Southeast Asians, particularly Taiwanese, Hong Kongese, Thai, and Malaysians.¹⁰ Lastly, the Anti-K antibody is noted to be low in our sample population, which coincides with its lower frequency in Southeast Asian populations compared to European or Caucasian counterparts.⁸

Concordance rates between the two methods from English literature were as low as 73% and as high as 98.68%.¹² It can be noted however that these studies mostly compared similar gel-based kits to one another, in contrast to our study. From our data, it is evident that the gel-based method was able to detect and identify Anti-Mi^a antibody more frequently. The gel-based kit contains at least one red cell reagent that is positive for Anti-Mi^a, which is lacking based on the standard RBC reagents used in the tube-based kit. As the antibodies identified using red cells in the gel-based kit are naturally occurring IgM antibodies, Syed Azim et al., theorized that some of the Anti-Mi^a antibodies identified by the said kit could well be IgM only antibodies.⁷ As IgM antibodies tend to react at cold or room temperature compared to the IgG antibodies that react at body temperature, they rarely cause hemolysis in vivo and are therefore considered less significant. It can be argued that kits that detect only the clinically significant antibodies are preferred, since they preserve scarce personnel resources and minimize delay in the provision of compatible blood components.¹³ Nevertheless, proper detection of Anti-Mi^a antibodies is still important during pre-transfusion testing especially among Asian populations, as there are some studies that show that Anti-Mi^a antibodies are IgG reactive to 37°C and have been implicated in causing hemolytic disease of newborn and hemolytic transfusion reactions.^{10,14,15}

Nonspecificity in antibody identification during pre-transfusion testing is uncommon, where a positive antibody screen leads to inconclusive antibody identification. Exposure to new red cell antigens, as in past blood transfusions or past pregnancies, can lead to alloimmunization and the development of additional alloantibodies. The presence of circulating donor red cells for patients with recent transfusions may also affect testing. It is possible that lymphoproliferative syndromes, including malignancy, infection, or rheumatologic or autoimmune diseases, can lead to autoantibody formation, as seen in systemic lupus erythematosus, multiple myeloma, chronic lymphocytic leukemia, or lymphoma. However, one cannot dismiss the fact that patients with chronic illnesses tend to require frequent transfusions that increase the risk for alloimmunization. Lastly, treatment using Daratumumab, intravenous immunoglobulins (IVIg), or volume expanders may manifest pan-agglutination in kits. Daratumumab can bind to CD38 on the surface of reagents red cells, while IVIg can contain unexpected antibodies.¹⁶ To resolve this, autocontrol cells and direct Coombs testing (DAT) can facilitate the differentiation of autoantibodies.¹⁶ Kandasamy et.al. (2018) also suggested washing the reagent cells.¹⁷ Additional steps can also be done through phenotyping, using enzyme treatment such as papain and ficin which can inhibit MNS/Duffy antibodies, additive solutions like poly-ethylene glycol, or adsorption/elution methods.¹⁶

CONCLUSION

In comparing the methods, namely conventional tube-based technique versus the column agglutination or gel-based method, the resulting antibody screening and identification results showed statistical significance, with the latter being able to detect and identify more cases of Anti-Mi^a antibody. While the conventional tube method is considered the gold standard in the identification of antibodies, one cannot totally exclude the presence of Anti-Mi^a antibodies in patients who screened negative using this type of method. The high number of discrepant cases between the two methods highlights the advantage of using both in the screening and identification of RBC antibodies, done as a complement to the other.

ACKNOWLEDGMENTS

The authors thank Dr. Regine G. Bustonera, Ma. Rizalina F. Chua, Dr. Macario F. Reandelar Jr., and Mr. Jayson L. Guihama for their valuable contribution to the conduct of this study.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declare no conflict of interest.

FUNDING SOURCE

None.

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