

Original article

Performance evaluation of in-house magnetized red cells prepared by Regional Blood Centre II Lopburi Province, Thai Red Cross Society for ABO serum grouping using the QWALYS® 3 EVO automated analyzer

Patcharakorn Kramkratok, Sirikorn Phengthaworn and Piyawan Thammakorn

Regional Blood Centre II Lopburi Province, Thai Red Cross Society

Abstract:

Introduction: ABO blood group testing using the QWALYS® 3 EVO analyzer employs erythrocyte-magnetized technology (EMT) with HEMALYS A1, B cells manufactured, imported from France. However, dependency on imported products poses risks, especially during crises, including the COVID-19 pandemic, which caused delivery delays and quality issues including hemolysis. **Objective:** This study aimed to evaluate the performance of ready-to-use in-house magnetized A1, B and O cells for ABO serum grouping. **Materials and Methods:** This prospective experimental study evaluated the performance of two formulations of in-house magnetized A1, B and O cells: standard formula (diluentlys 5 mL + magnelys reagent 75 µL + standard cells 80 µL each) and EDTA-supplemented formula (diluentlys 4 mL + magnelys reagent 75 µL + standard cells 80 µL each + EDTA 1.8 g/L, 1 mL). Performance was compared with HEMALYS A1, B using 1,123 known samples and 560 routine unknown samples. Cell stability was evaluated for 35 days. Testing was performed on the QWALYS® 3 EVO analyzer and confirmed with standard tube test (STT) for uninterpreted reactions. **Results:** Among 1,123 known samples, in-house magnetized cells with EDTA showed 98.04% accuracy (1,101 samples), comparable to HEMALYS A1, B cells at 98.13% (1,102 samples), with very high agreement (Kappa = 0.9462). Standard in-house magnetized cells without EDTA showed 90.29% accuracy (1,014 samples) with Kappa = 0.8412. Parallel testing with unknown samples showed 100% concordance. Both formulations demonstrated good stability for 35 days without contamination or hemolysis. **Conclusion:** In-house magnetized cells with EDTA demonstrated performance equivalent to imported products and can serve as a cost-effective alternative to reduce import dependency and enhance national blood service system security efficiently.

Keywords : ● ABO serum grouping ● QWALYS® 3 EVO ● Erythrocyte-magnetized technology
● In-house magnetized cells

J Hematol Transfus Med. 2025;35:169-78.

Received 18 April 2025 Corrected 29 May 2025 Accepted 17 June 2025

Correspondence should be addressed to Patcharakorn Kramkratok, Regional Blood Centre II Lopburi Province, Thai Red Cross Society, Amphur Muang Lopburi, Lopburi, 15000 E-mail: patcharakorn.k@redcross.or.th

นิพนธ์ต้นฉบับ

การประเมินประสิทธิภาพของเซลล์เม็ดเลือดแดงที่ถูกทำให้มีความเป็นแม่เหล็กที่เตรียมโดยภาคบริการโลหิตแห่งชาติที่ 2 จังหวัดลพบุรี สภากาชาดไทย สำหรับตรวจหมู่โลหิตเอบีโอ ด้วยเครื่องตรวจหมู่โลหิตอัตโนมัติ QWALYS® 3 EVO

พัชรากร กรำกระโทก สิริกร เพ็งถาวร และ ปิยะวรรณ ธรรมกรณ์

ภาคบริการโลหิตแห่งชาติที่ 2 จังหวัดลพบุรี สภากาชาดไทย

บทคัดย่อ

บทนำ การตรวจหมู่โลหิต ABO ด้วยเครื่อง QWALYS® 3 EVO ซึ่งใช้เทคโนโลยี Erythrocyte-magnetized technology (EMT) โดยใช้เซลล์ HEMALYS A1, B ซึ่งเป็นเซลล์ที่ผลิตและขนส่งจากประเทศฝรั่งเศส ใช้ร่วมกับเครื่องตรวจหมู่โลหิตอัตโนมัติ QWALYS® 3 EVO อย่างไรก็ตาม การพึ่งพาผลิตภัณฑ์นำเข้ามีความเสี่ยง โดยเฉพาะในช่วงวิกฤตการณ์ เช่น การแพร่ระบาดของโควิด-19 ทำให้เกิดความล่าช้าในการจัดส่ง และปัญหาคุณภาพของเซลล์ เช่น การเกิด hemolysis **วัตถุประสงค์** เพื่อประเมินประสิทธิภาพของเซลล์เม็ดเลือดแดงที่ถูกทำให้มีความเป็นแม่เหล็ก ซึ่งเป็นเซลล์ที่ผลิตขึ้นเองแบบพร้อมใช้ (ready-to-use) เรียกว่า in-house magnetized A1, B และ O cells สำหรับการตรวจหมู่โลหิต ABO serum grouping **วัสดุและวิธีการ** การศึกษานี้ออกแบบเป็นการวิจัยเชิงทดลองแบบไปข้างหน้า (Prospective Study) โดยมุ่งประเมินประสิทธิภาพของเซลล์ in-house magnetized A1, B และ O cells ที่พัฒนาขึ้นในสองสูตร ได้แก่ เซลล์แบบปกติ in-house magnetized cells : diluentlys 5 mL+ magnelys reagent 75 µL+ standard cells ชนิดละ 80 µL และ เซลล์แบบเติม EDTA: In-house magnetized cells with EDTA : diluentlys 4 mL+ magnelys reagent 75 µL+ standard cells ชนิดละ 80 µL+ EDTA 1.8 g/L ปริมาตร 1 mL เปรียบเทียบประสิทธิภาพกับ HEMALYS A1, B โดยทำการศึกษากับตัวอย่างสองกลุ่ม ได้แก่ ตัวอย่างโลหิตที่ทราบผลหมู่โลหิตเอบีโอแล้ว (known samples) จำนวน 1,123 ตัวอย่าง และตัวอย่างที่สุ่มจากงานประจำวัน (unknown samples) จำนวน 560 ตัวอย่าง รวมทั้งประเมินความคงทนของเซลล์เป็นระยะเวลา 35 วัน ทำการทดสอบด้วยเครื่อง QWALYS® 3 EVO โดยใช้เซลล์ทั้งสองสูตรเปรียบเทียบกับเซลล์ HEMALYS A1, B และในกรณีที่ไม่สามารถแปลผลการทดสอบได้ จะตรวจยืนยันด้วยวิธีมาตรฐานหลอดทดลอง **ผลการศึกษา** จากตัวอย่างโลหิตที่ทราบผลหมู่โลหิตแล้ว (known samples) จำนวน 1,123 ตัวอย่าง พบว่า in-house magnetized cells with EDTA มีความถูกต้องร้อยละ 98.04 (1,101 ตัวอย่าง) เกือบเคียงกับเซลล์ HEMALYS A1, B ที่ให้ผลถูกต้องร้อยละ 98.13 (1,102 ตัวอย่าง) และมีความสอดคล้องในระดับสูงมาก ($Kappa = 0.9462$) ในขณะที่ in-house magnetized cells แบบไม่เติม EDTA ให้ผลถูกต้องร้อยละ 90.29 (1,014 ตัวอย่าง) และมีค่า $Kappa = 0.8412$ การทดสอบคู่ขนานกับตัวอย่างในงานประจำวันพบผลการตรวจตรงกัน 100% โดยเซลล์ทั้งสองสูตรมีความคงทนดี สามารถเก็บรักษาและใช้งานได้ต่อเนื่องเป็นเวลา 35 วัน โดยไม่พบการปนเปื้อนหรือ hemolysis **สรุป** In-house magnetized cells with EDTA มีประสิทธิภาพเทียบเท่าผลิตภัณฑ์นำเข้า และสามารถใช้เป็นทางเลือกที่คุ้มค่าในการลดการพึ่งพาการนำเข้าและเสริมสร้างความมั่นคงในระบบบริการโลหิตของประเทศได้อย่างมีประสิทธิภาพ

คำสำคัญ : ● การตรวจหมู่โลหิต เอ บี โอ ในซีรัม ● เครื่องคลอลิส อีโว ● เทคโนโลยีการทำให้เม็ดเลือดแดงมีความเป็นแม่เหล็ก ● การผลิตเม็ดเลือดแดงที่มีความเป็นแม่เหล็กขึ้นมาเอง

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2568;35:169-78.

Introduction

Human blood groups are classified based on biochemical substances known as antigens, which are unique immune-stimulating substances present on the surface of red blood cells. Since Karl Landsteiner discovered the ABO blood group system and received the Nobel Prize in 1930.¹ There are currently 47 recognized blood group systems containing 366 red blood cell antigens (October 2024). The 47 systems are genetically determined by 52 genes.² Among all blood group systems, the ABO and Rh systems are considered the most clinically important, particularly in blood transfusion processes. It is critical to provide blood product of the compatible group to ensure patient safety. Therefore, laboratory testing to identify the donors and patients ABO blood groups is most importance.³

Blood group testing during the blood donation screening process is a crucial procedure for ensuring the quality and reliability of the national blood service system. Regional Blood Centre II Lopburi Province has implemented the QWALYS® 3 EVO automatic blood group analyzer⁴ (manufactured by Diagast, France) for ABO blood group determination. This analyzer utilizes erythrocyte magnetized technology (E.M.® Technology)⁵, which involves attaching magnetic particles to the surface of red blood cells. When red blood cells react with antibodies, cells they are attracted by a magnet and settle at the bottom of the well, effectively replacing traditional centrifugal precipitation. This process is conducted using HEMALYS A1 and B cells, which are produced and imported from France for exclusive use with the QWALYS® 3 EVO system. However, reliance on imported products poses significant risks, particularly during crises such as the COVID-19 pandemic. During crisis periods, delivery delays and issues such as cell hemolysis could compromise the accuracy and reliability of testing, thereby harmful for patients safety.

To address these challenges, the Regional Blood Centre II Lopburi Province, initiated the development of an innovative, ready-to-use cell replacement. This innovation includes in-house magnetized A1 and B cells, which have a one-month shelf life, and expanded the use of magnetized O cells for ABO serum grouping. The addition of magnetized O cells enhances the detection of IgM unexpected antibodies, such as anti-H, that may interfere with ABO indirect grouping results. This initiative builds upon findings from relevant prior studies.

Previous studies, in 2008, Olivier Bouix, et al. examined the erythrocyte-magnetized technology (EMT) for ABO-Rh-K phenotype blood group testing, comparing it with standard daily methods. Their study concluded that EMT was superior in detecting mixed-field or weak reactions and provided highly accurate results in routine practice.⁶

In 2009, Helge Schoenfeld, et al. evaluated the efficiency of the QWALYS 2 system, which employs EMT, for ABO/D blood group testing. They demonstrated that it could detect weak samples more effectively than traditional microtiter plate systems, highlighting the potential of EMT technology to enhance accuracy and efficiency in blood screening.⁷

Furthermore, in 2020, Wachiraporn Yonwisest, et al. investigated the preparation method for magnetized A1 and B red cells for ABO serum grouping using the QWALYS 3 system. Their findings the preparation of magnetized red cells by using 0.9% NSS + 1.8% EDTA is the most suitable approach in terms of ease of preparation, stability, and result accuracy. The method achieved an accuracy rate of 98.49% and remained effective for up to seven days.⁸

This study integrates existing research knowledge to develop a practical, locally applicable cells replacement innovation. The primary objectives are to enhance the stability of blood donation management, reduce dependency on imported products, and establish long-term security in Blood service operations.

Materials and methods

This prospective study aimed to evaluate the efficiency of ABO blood typing using in-house magnetized A1, B, O cells, compared to HEMALYS A1, B cells (Diagast, France).

Sample characteristics

Blood donation samples submitted for testing from Regional Blood Centre II Lopburi Province, were classified into two categories, known ABO samples and unknown samples. The known samples included 1,123 previously identified blood group samples, determined through routine daily blood typing at Regional Blood Centre II Lopburi Province. These were distributed as follows: group A1: 234 samples, group A3: 2 samples, group B: 360 samples, group B3: 4 samples, group O: 423 samples, group A1B: 91 samples, group A3B: 5 samples, group AB3: 4 samples.

The unknown samples were collected using a systematic sampling method from daily blood donations submitted to the Regional Blood Centre II Lopburi Province. Specifically, the first 28 samples arriving at the laboratory each day were selected over a five-days during working day for four consecutive weeks, resulting in a total of 560 samples. Samples exhibiting hemolysis, turbidity, or exceeding 48 hours post-collection were excluded from the study.

Methods

1. Preparation of in-house magnetized A1, B, and O cells for serum grouping using the QWALYS® 3 EVO automatic blood typing machine. Standard A1, B, and O red blood cells were produced from the National Blood Centre. Approximately 1 mL of each was placed in 13×100 mm test tubes. The cells were centrifuged and washed three times with 0.9% normal saline solution (NSS). After the final wash, the supernatant was completely removed to obtain packed A1, B, and O cells. Six sterilized 10 mL glass bottles were prepared and labelled with barcodes for in-house magnetized A1, B, and O cells, with two bottles allocated for each cell type. The bottles were divided into two sets as follows:

Set 1: For preparing in-house magnetized A1, B, and O cells without EDTA. Each sterilized glass bottle was filled with 5 mL of diluentlys, 75 µL of magnelys reagent, and 80 µL of concentrated washed red blood cells. The mixture was thoroughly gentle mixed to create a homogenous solution (standard formula 1).

Set 2: For preparing in-house magnetized A1, B, and O cells with EDTA. Each sterilized glass bottle was filled with 4 mL of diluentlys, 75 µL of magnelys reagent, 80 µL of concentrated washed red blood cells, and 1,000 µL of EDTA (1.8 g/L) to prevent hemolysis. The mixture was thoroughly gentle mixed to create a homogenous solution (EDTA-supplemented formula). Both preparations were left at room temperature (22-25°C) for at least 30 minutes prior to use, then stored at 2-8°C for subsequent testing.

2. Stability evaluation of in-house magnetized A1, B, and O cells were prepared in both formulations as described previously and divided into five sets for continuous testing over a period of five weeks (35 days).

Week 1: Set 1 was opened, and serum grouping was conducted daily using A, B, O, and AB plasma samples, with 28 samples tested per day for one working week.

Week 2: Set 2 was opened, and daily testing continued until the week was complete.

Subsequent weeks: Testing continued for five weeks in total, with a new reagent set used each week.

The test results of the in-house magnetized cells in both formulations were compared to those of HEMALYS A1, B cells throughout the testing period. Observations were recorded before each test to evaluate external characteristics, including evidence of hemolysis, clarity or turbidity of the solution and changes in red cell appearance.

3. Efficiency evaluation of in-house magnetized A1, B, and O cells compared to HEMALYS A1, B cells.

The efficiency of in-house magnetized A1, B, and O cells was evaluated by performing ABO serum grouping on systematically sampled blood donations from Regional Blood Centre II Lopburi Province. The study

design included: Collecting the first 28 samples received at the laboratory each day. Testing samples over 20 consecutive working days, resulting in a total of 560 samples. Conducting parallel testing using HEMALYS A1, B cells and both sets of in-house magnetized cells prepared by Regional Blood Centre II Lopburi Province.

Each sample was tested once using the QWALYS® 3 EVO automated blood typing machine. If the results were inconclusive or unreadable (ABO discrepancy)⁹, the standard tube test method¹⁰ was performed as a confirmatory procedure.

Data Analysis: Sensitivity and specificity were calculated using descriptive statistical methods. The standard tube test was used as the reference method for comparison. Cohen's Kappa coefficient^{11,12} was applied to determine the level of concordance between the results of the in-house and commercial cell types. A comparative analysis of the two cell formulations was conducted across individual blood groups by examining concordant interpretations, discrepant results, and accuracy percentages to evaluate whether each formulation demonstrates consistent reliability across all ABO blood group classifications.

Ethical Considerations: This study was approved by the Research Ethics Committee of the National Blood Service Centre, Thai Red Cross Society, on November 18, 2024 (reference number NBC 6/2024).

Results

The study compared the preparation methods of in-house magnetized A1 cells, B cells, and O cells for ABO blood grouping. Table 1 presents the number of serum samples for each ABO blood group tested using the QWALYS® 3 EVO analyzer, comparing the performance between in-house magnetized A1 cells, B cells and O cells against commercially available HEMALYS A1, B over a 35-day period. This study utilized a total of 1,123 samples, comprising group A: 234, group A3: 2 samples, group B: 360 samples, B3: 4 samples, group O: 423 samples, group AB: 91 samples, A3B: 5 samples, and AB3: 4 samples.

The testing procedure was conducted in two phases. Phase I involved testing 143 samples on Day 0, encompassing major blood groups and subgroups to establish baseline performance evaluation of the testing cells. Phase II consisted of continuous testing over 5 weeks (Days 1-35), utilizing 28 serum samples daily, totaling 980 samples during this period. This approach facilitated the assessment of performance consistency under real-world operational conditions.

Tables 2 and 3 present the performance results of the HEMALYS A1, B product, which demonstrate successful blood group determination in 1,102 out of 1,123 samples, yielding an overall success rate of 98.13%. There were 21 samples (1.87%) that could not be conclusively determined, predominantly consisting of group A

Table 1 Number of ABO serum grouping detected by QWALYS® 3 EVO compared between in-house magnetized A1, B, and O cells and HEMALYS A1, B

Group	Day 0	Week 1- Week 5							Sample 35 day	Total
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
A	24	6	6	6	6	6	6	6	210	234
A3	2	6	6	6	6	6	6	6	0	2
B	45	6	6	6	6	6	6	6	315	360
B3	4	6	6	6	6	6	6	6	0	4
O	38	6	6	6	6	6	6	6	385	423
AB	21	9	9	9	9	9	9	9	70	91
A3B	5	11	11	11	11	11	11	11	0	5
AB3	4	2	2	2	2	2	2	2	0	4
Total	143	28	28	28	28	28	28	28	980	1,123

Table 2 Results of ABO serum grouping detected by QWALYS® 3 EVO compared between in-house magnetized A1, B, and O cells and HEMALYS A1, B

Blood group	HEMALYS A1, B		In-house magnetized A1, B and O cells (standard formula)		In-house magnetized A1, B, and O cells with EDTA (EDTA-supplemented formula)		Total
	Interpreted	Discrepancy	Interpreted	Discrepancy	Interpreted	Discrepancy	
A	230	4	202	32	232	2	234
A3	2	0	2	0	2	0	2
B	345	15	308	52	342	18	360
B3	4	0	4	0	4	0	4
O	421	2	416	7	421	2	423
AB	91	0	73	18	91	0	91
A3B	5	0	5	0	5	0	5
AB3	4	0	4	0	4	0	4
Total	1,102	21	1,014	109	1,101	22	1,123
	98.13%	1.87%	90.29%	9.71%	98.04%	1.96%	

Table 3 Number of indeterminate samples detected by QWALYS® 3 EVO compared between in-house magnetized A1, B, and O cells and HEMALYS A1, B

Blood group	HEMALYS A1, B		Number of indeterminate samples	In-house magnetized A1, B, and O cells (standard formula)			Number of indeterminate samples	In-house magnetized A1, B and O cells with EDTA (EDTA-supplemented formula)			Number of indeterminate samples
	A1 Cell	B Cell		A1 Cell	B Cell	O Cell		A1 Cell	B Cell	O Cell	
A	0	(±)	4	(±)	(2+), (3+), (4+)	0	31	0	(±)	0	2
A				(4+)	(±)	0	1				
B	(±)	0	15	(2+), (3+), (4+)	(±)	0	47	(±)	0	0	18
B				(±)	0	0	5				
O	(±)	(±)	2	(4+)	(4+)	(±)	1	(±)	(3+), (4+)	0	2
O				(±)	(2+), (3+), (4+)	0	6				
AB				(±)	0	0	7				
AB				0	(±)	0	5				
AB				(±)	(±)	0	5				
AB				0	0	(±)	1				
Total	21			109				22			

(4 samples), B (15 samples), and O (2 samples), no false positive results were observed. The main cause was attributed to weak reactions with test cells. When tested with in-house magnetized A1, B, and O cells without EDTA, blood group results could be determined for 1,014 samples (90.29%) and could not be determined for 109 samples (9.7%). Among the indeterminate samples, the following patterns were observed: Group A showed

overreaction with A cells in 31 samples and weak reaction with B cells in 1 sample; Group B showed overreaction with B cells in 47 samples and weak reaction with A cells in 5 samples; Group O showed overreaction with O cells in 1 sample and weak reaction with A cells in 6 samples; Group AB showed overreaction with A cells in 7 samples, overreaction with B cells in 5 samples, overreaction with both A cells and B cells in 5 samples,

and overreaction with O cells in 1 sample. The high number of false positive results (97 cases) resulted in a specificity of 91.17%. Meanwhile, in-house magnetized A cells, B cells, O cells with EDTA showed improved blood group determination results at 1,101 samples (98.04%), with a decrease in indeterminate samples to 22 samples (1.96%). The main causes of indeterminate results were: group A: weak reaction with B cells in 2 samples; group B: weak reaction with A cells in 18 samples; and group O: weak reaction with A cells in 2 samples, no false positive results were observed.

Calculated performance metrics, as summarized in Table 4, showed the standard formula achieved 98.83% sensitivity and 91.17% specificity, while the EDTA-supplemented formula yielded 98.04% sensitivity and 100% specificity. When analyzing the Kappa coefficient to assess the agreement between HEMALYS A1, B cells, and in-house cells, the results showed that the in-house magnetized A cells, B cells, O cells without EDTA formula had a Kappa = 0.8412 (almost perfect agreement), and in-house magnetized A cells, B cells, O cells with the EDTA formula had a Kappa = 0.9462 (almost perfect agreement). Regarding stability testing results, the prepared in-house magnetized A cells, B cells, O cells

maintained good performance throughout the 35 days. Visual inspection showed no microbial contamination, no turbidity and hemolysis observed when stored at 2-8 °C.

As summarized in Table 5, stratified analysis by ABO blood group revealed that the commercial HEMALYS A1, B achieved the highest concordance in groups O (99.53%) and AB (100%), and the lowest in group B (95.88%). The standard formula had an overall concordance of 90.29%, with the lowest in group AB (82.00%) and the highest in group O (98.34%). When EDTA was added (EDTA-supplemented formula), overall concordance improved to 98.04%, approaching commercial standards. Notably, group B concordance rose from 85.71% to 95.05%, and group AB reached 100%.

Table 6 shows parallel testing results of routine ABO blood grouping using systematic random sampling, comparing in-house magnetized cells (both normal and EDTA added) with HEMALYS A1, B method using the QWALYS® 3 EVO analyzer. The study included 560 blood samples over 20 days, consisting of 94 group A samples, 199 group B samples, 220 group O samples, and 47 group AB samples. Test results showed 100% concordance among all three methods.

Table 4 Performance of in-house magnetized A1, B, and O cells for ABO serum grouping: comparison between standard formula and EDTA-supplemented formula

In-house magnetized cells	True positive (TP)	False negative (FN)	True negative (TN)	False positive (FP)	Sensitivity (%)	Specificity (%)
In-house magnetized A1, B, and O cells (standard formula)	1,014	12	1,002	97	98.83	91.17
In-house magnetized A1, B, and O cells with EDTA (EDTA-supplemented formula)	1,101	22	1,079	0	98.04	100

Table 5 Comparison of blood group typing concordance between in-house magnetized A1, B, and O cells and HEMALYS A1, B

Blood Group	HEMALYS A1, B			In-house magnetized A1, B, and O cells			In-house magnetized A1, B, and O cells with EDTA		
	Interpreted	Discrepancy	Concordance	Interpreted	Discrepancy	Concordance	Interpreted	Discrepancy	Concordance
A	232	4	98.31%	204	32	86.44%	234	2	99.15%
B	349	15	95.88%	312	52	85.71%	346	18	95.05%
O	421	2	99.53%	416	7	98.34%	421	2	99.53%
AB	100	0	100.00%	82	18	82.00%	100	0	100.00%
Total	1,102	21	98.13%	1,014	109	90.29%	1,101	22	98.04%

Table 6 Results of ABO serum grouping detected by QWALYS® 3 EVO A parallel testing with routine work using systematic random sampling between in-house magnetized A1, B, and O cells and HEMALYS A1, B

Blood group	HEMALYS A1, B		In-house magnetized A1, B, and O cells		In-house magnetized A1, B, and O cells with EDTA	
	N	Percent	N	Percent	N	Percent
A	94	100	94	100	94	100
B	199	100	199	100	199	100
O	220	100	220	100	220	100
AB	47	100	47	100	47	100
Discrepancy	0	0	0	0	0	0
Total	560	100	560	100	560	100

Importantly, the cell preparations remained stable over the 35-day testing period, with no observed degradation in agglutination intensity, hemolysis, or microbial contamination. when stored at 2-8 °C. This confirms their suitability for routine use in blood bank.

Discussion

This study demonstrates that both in-house magnetized A1, B, and O cells without EDTA and in-house magnetized A1, B, and O cells with EDTA preparation methods showed very high agreement (Kappa coefficient) with HEMALYS A1, B achieving almost perfect agreement with Kappa values of 0.8412 and 0.9462, respectively.

Notably, the in-house magnetized A1, B, and O cells with EDTA preparation method demonstrated efficiency comparable to HEMALYS A1, B product, with successful blood group determination in 98.04% of cases and 1.96% indeterminate results. The majority of issues were due to weak reactions. These findings align with previous research on erythrocyte magnetized technology (EMT), particularly the work of Olivier Bouix⁶ (2008) which found that EMT reported a 98.04% interpretability rate across 11,022 samples, with 1.96% uninterpretable results, primarily due to weak or mixed-field reactions mirroring the indeterminate rate observed in our study. Similarly, Helge Schoenfeld⁷ (2009) observed a 99.4% concordance rate in ABO/D grouping using the QWALYS 2 system. However, evaluating the QWALYS 2 system demonstrated better detection of weak samples compared to the traditional microtiter plate system

An interesting observation in this study was that blood group B showed the greatest difference across both testing methods, particularly in cells prepared without EDTA, which showed differences in 52 samples. This highlights the importance of EDTA addition in the blood group cell preparation process. These findings align with the study by Wachiraporn Yonwiset⁸ (2020) on the preparation of magnetized red cells for ABO serum grouping, specifically regarding the EDTA addition method, which helped reduce hemolysis and adverse reactions, particularly in blood groups A and B that showed weak reactions or overreactions, thus improving result accuracy.

Stratified analysis by blood group found that the standard formula showed the lowest consistency in group AB (82.00%) and group B (85.71%), mainly due to overreactions and weak reactions. In contrast, the EDTA-supplemented formulation demonstrated improved performance across all groups, achieving 100% consistency in group AB and 99.15% in group A. These findings confirm that EDTA enhances both the stability and consistency of in-house magnetized cells in ABO serum grouping.

In this testing, two samples were found positive for antibody screening using indirect antiglobulin test (IAT) with column agglutination technique¹³. Identification testing revealed anti-Le^a+ anti-Le^b and anti-Mi^a antibodies. Since serum grouping was performed at room temperature, unlike IAT which uses 37°C, no reactions were observed with the added in-house magnetized O

cells. Interestingly, in cases where cold-reactive antibodies such as anti-M¹⁴, anti-N¹⁵, anti-P1¹⁶ or anti-H¹⁷ are present with sufficient potency, may interfere with ABO serum grouping when testing is conducted at room temperature. These antibodies can cause agglutination with O cells, potentially leading to positive reactions patterns. Although no ABO discrepancies related to these antibodies were observed in this study, laboratories adopting this method should exercise caution. It is recommended to perform confirmatory testing using methods such as the standard tube technique in cases of uncertainty to ensure accurate ABO interpretation.

In terms of practical efficiency, parallel testing with routine samples at the Regional Blood Centre II Lopburi Province, showed that both in-house magnetized A1, B, and O cells and in-house magnetized A1, B, and O cells with EDTA preparations achieved 100% concordance with no discrepancies. This demonstrates that all three methods have equivalent accuracy and reliability in blood group determination. Furthermore, the developed cells demonstrated good stability, remaining usable for 35 days without microbial contamination or hemolysis when stored at 2-8°C, indicating cost-effectiveness in production and usage compared to imported commercial products

This development of in-house blood grouping cell preparation represents a significant innovation for the country, reducing reliance on imported products, which are often expensive and may face challenges related to transportation and procurement at certain times. Additionally, this approach enables control over production and allows adjustments to meet specific demands, contributing to the long-term stability of the national blood service system. More importantly, the development of this innovation enhances domestic knowledge and increases the capacity of local personnel. Notably, laboratories both domestically and internationally using the same blood grouping system can adopt and adapt this method. However, the successful implementation of in-house magnetized A1, B, and O cells in laboratories

depends on several key factors: proper temperature control (2-8°C for up to 35 days), sterile preparation conditions, accurate volume and concentration using calibrated tools, clear labeling, SOP-based staff training, daily internal quality control, and expert approval. Adhering to these standards supports efficient and accurate testing while strengthening the security of blood service.¹⁸ This highlights Thailand's capability to develop innovations with potential applications on a global scale.

Conclusion

This study demonstrates that the in-house magnetized A1, B, and O cells preparation method with EDTA exhibits efficiency comparable to the HEMALYS A1, B product. It achieved a blood grouping success rate of 98.04%, with 1.96% of samples yielding inconclusive results, primarily due to weak reactions which needs standard tube test to confirm. The method also showed good stability, with a usability duration of 35 days without any observed microbial contamination or hemolysis when stored at 2-8°C.

Acknowledgment

We would like to express our gratitude to the National Blood Centre, Thai Red Cross Society, and the staff of the Blood Screening Unit, Regional Blood Centre II Lopburi Province, for their kind assistance in preparing samples and helping with sample analysis in this study.

References

1. Reid M, Shine I. *The discovery and significance of the blood groups*. Massachusetts: SBB Books; 2012.
2. International Society of Blood Transfusion. *Red cell immunogenetics and blood group terminology* [Internet]. Amsterdam: ISBT; [cited 2025 Apr 3]. Available from: <https://www.isbtweb.org/isbt-working-parties/rcibgt.html>.
3. Pechchantorn, S. *Blood groups*. Bangkok: The Thai Society of Hematology. Available from: <https://tsh.or.th/Knowledge/Details/69> 1 Jan, 2024.
4. E for L International Co. *Standard operation procedure for QWALYS® 3 EVO analyzer*. France: Diagnost; 2014.

5. QWALYS® 3 EVO EM technology [Internet]. France: Diagast; [cited 2024 Jan 1]. Available from: <https://www.diagast.com/en/automatons-reagents/automate-methods/QWALYS-3-evo-en/em-technology/>
6. Bouix O, Ferrera V, Delamaire M, Redersdorff JC, Roubinet F. Erythrocyte-magnetized technology: an original and innovative method for blood group serology. *Transfusion*. 2008;48:1878-85.
7. Schoenfeld H, Bulling K, Von Heymann C, Neuner B, Kalus U, Kieseewetter H, et al. Evaluation of immunohematologic routine methods using the new Erythrocyte-magnetized technology on the QWALYS 2 system. *Transfusion*. 2009;49:1347-52.
8. Vashiraporn Y, Kasama E, Sathit T. Preparation of magnetized red cells for ABO serum grouping by Qwalys 3 blood grouping automation. *J Hematol Transfus Med*. 2020;30:119-27.
9. Roback JD, Combs MR, Grossman BJ, Harris T, Hillyer CD, eds. Technical manual. 16th ed. Bethesda, MD: AABB; 2008. p. 368-72.
10. Roback JD, Combs MR, Grossman BJ, Harris T, Hillyer CD, eds. Technical manual. 16th ed. Bethesda, MD: AABB; 2008. p. 899-900.
11. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas*. 1960;20:37-46.
12. Banerjee C, Capozzoli M, McSweeney L, Sinha D. Beyond kappa: a review of interrater agreement measures. *Can J Stat*. 1999;27:3-23.
13. Reis KJ, Chachowski R, Cupido A, Davies D, Jakway J, Setcavage TM. Column agglutination technology: the antiglobulin test. *Transfusion*. 1993;33:639-43.
14. Ranjan V, Khillan K. Naturally occurring anti-M with high thermal amplitude complicating ABO blood grouping. *Curr Med Res Pract*. 2018;8:71-2.
15. Samarah F, Srour M. An unusual report of anti-N antibody presenting as ABO discrepancy in an old female patient in Palestine. *Asian J Transfus Sci*. 2019;13:140-1.
16. Ferdowsi S, Mohammadi S, Ahmadnezhad M, Herfat F, Rezvani A, Eshghi P, et al. Naturally occurring anti-P1 with high thermal amplitude complicating ABO blood grouping. *Asian J Transfus Sci*. 2024;18:135-7.
17. Sathima L, Kulvara A, Pasuporn P. AB para-Bombay phenotype with anti-H a case report. *J Hematol Transfus Med*. 2019;29:131-7.
18. National Blood Centre, Thai Red Cross Society. National blood service operation plan of Thailand 2022-2027. 1st ed. Bangkok: Udomsuksa; 2021. p. 26-40.