Original article

Effect of turbid plasma and biochemical levels on ABO blood group testing by an image-based automated analyzer

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Abstract:

Introduction: *Turbid plasma affects the analysis of several laboratory tests.* ***Objective:*** *To investigate the possible effects of plasma turbidity and the biochemical levels on the ABO blood group testing by QWALYS®3, an automated device that interprets the results by image-based analysis.* ***Materials and Methods:*** *A total of 205 known ABO blood group samples composed of non-error (n = 100), error "X" (n = 50), and error "?" (n = 55) results by QWALYS®3 were measured for biochemical levels and the turbidity of plasma, followed by their associations being tested. Then, the risk factors of the error results were calculated. To verify the risk factors of the biochemical levels, the plasma of blood group B with various concentrations of each biochemical substance was tested using QWALYS®3.* ***Results:*** *Plasma turbidity was positively correlated with the levels of globulin, total protein, and triglyceride. For ABO blood group testing by QWALYS®3, the turbid samples with OD > 1.000 were associated with errors "X" and "?". In multivariate analysis, the concentration of total protein ≤ 8.0 g/dL and the triglyceride > 500 mg/dL were the independent risk factors for both errors. The risk factors verified that a 1,700 mg/dL of triglyceride had a direct effect on ABO blood group testing with error "?" and, a 3,400 mg/dL of triglyceride affected error "X".* ***Conclusion:*** *The high triglyceride levels and total protein ≤ 8.0 g/dL could affect the ABO blood group testing using an image-based automated analyzer. Thus the rejection of specimens by turbidity assessment may reduce the error results of blood group testing, caused by high triglyceride levels.*

Keywords : ● *Turbid plasma* ● *Lipemia* ● *Interference* ● *ABO blood group testing*

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นิพนธ์ต้นฉบับ

ผลกระทบของความขุ่นและระดับสารชีวเคมีในพลาสมาต่อการตรวจหมู่เลือด ABO ด้วยเครื่องวิเคราะห์อัตโนมัติที่ใช้เทคนิคการอ่านผลจากภาพ

วชิราภรณ์ ยนต์วิเศษ[1] กรรณิการ์ กัวหา[2] วัชรินทร เทียนสันต์[1] และ วัชนันท์ วงศ์เสนา[1]

[1]ภาควิชาเทคนิคการแพทย์ คณะสหเวชศาสตร์ มหาวิทยาลัยนเรศวร [2]สาขาวิชาภูมิคุ้มกันวิทยาคลินิกและเวชศาสตร์การบริการโลหิต คณะเทคนิคการแพทย์ มหาวิทยาลัยขอนแก่น

บทคัดย่อ

บทนำ *พลาสมาขุ่นส่งผลกระทบต่อการตรวจวิเคราะห์ทางห้องปฏิบัติการได้หลายการทดสอบ* ***วัตถุประสงค์*** *เพื่อวิเคราะห์ผลของความขุ่นและระดับสารชีวเคมีในพลาสมาต่อการตรวจหมู่เลือด ABO ด้วยเครื่อง QWALYS®3 ซึ่งเป็นเครื่องอัตโนมัติที่มีการอ่านและแปลผลการตรวจจากภาพ* ***วัสดุและวิธีการ*** *นำตัวอย่างเลือด 205 ราย ที่มีผลตรวจหมู่เลือด ABO จากการตรวจด้วยเครื่อง QWALYS®3 ประกอบด้วย รายงานผลได้ 100 ราย รายงานผลไม่ได้ชนิด "X" 50 ราย และชนิด "?" 55 ราย นำมาตรวจระดับสารชีวเคมีและระดับความขุ่นของพลาสมา จากนั้นวิเคราะห์ความสัมพันธ์และปัจจัยเสี่ยงของตัวอย่างตรวจที่รายงานผลไม่ได้ รวมทั้งทวนสอบปัจจัยเสี่ยงของระดับสารชีวเคมีต่อผลการตรวจหมู่เลือดโดยใช้พลาสมาหมู่เลือด B ที่ถูกเติมสารชีวเคมีระดับต่างๆ แล้วนำมาทดสอบหมู่เลือด ABO ด้วยเครื่องอัตโนมัติ* ***ผลการศึกษา*** *ความขุ่นของพลาสมามีความสัมพันธ์เชิงบวกกับระดับโกลบูลิน โปรตีนรวม และไตรกลีเซอไรด์ การตรวจหมู่เลือด ABO ด้วยเครื่อง QWALYS®3 พบว่า ตัวอย่างขุ่นที่มีค่า OD > 1.000 มีความสัมพันธ์กับการรายงานผลไม่ได้ทั้งชนิด "X" และ "?" เมื่อวิเคราะห์หลายปัจจัยร่วมกัน พบว่า ความเข้มข้นของโปรตีนรวม ≤ 8.0 g/dL และไตรกลีเซอไรด์ > 500 mg/dL เป็นปัจจัยเสี่ยงอิสระต่อการรายงานผลไม่ได้ทั้งสองชนิด การทวนสอบปัจจัยเสี่ยงพบว่า ค่าไตรกลีเซอไรด์ 1,700 mg/dL และ 3,400 mg/dL มีผลต่อการตรวจหมู่เลือด ABO โดยทำให้อ่านผลไม่ได้ชนิด "?" และ "X" ตามลำดับ* ***สรุป*** *ไตรกลีเซอไรด์ระดับสูงและโปรตีน ≤ 8.0 g/dL มีผลต่อการตรวจหมู่เลือด ABO ด้วยการใช้เครื่องอัตโนมัติที่อาศัยการอ่านผลจากภาพซึ่งสนับสนุน การปฏิเสธสิ่งส่งตรวจจากการประเมินความขุ่นเพื่อลดการรายงานผลไม่ได้ของการตรวจหมู่เลือดด้วยเครื่องวิเคราะห์อัตโนมัติได้*

คำสำคัญ : ● *พลาสมาขุ่น* ● *เลือดที่มีไขมันสูง* ● *สารรบกวน* ● *การตรวจหมู่เลือดเอบีโอ*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2564;31:225-34.

Introduction

In clinical laboratory testing, lipemia is one of the interferences that can be a significant source of errors[1-4]. Lipemia is represented by turbid of plasma/serum due to increased light scatter and the adsorption of it by the large lipid particles, which are chylomicron and very low density lipoprotein (VLDL)[1]. Non-fasting specimen collection and in some diseases/conditions such as lipoprotein lipase deficiency, apolipoprotein C2 deficiency, diabetes mellitus, para-proteinemia, chronic kidney disease, which have glucose and protein metabolism disorders, cause blood samples to be turbid[5,6]. Turbid samples can interfere with laboratory analysis in several ways, such as physical and chemical interference, spectrophotometric method interference, non-homogeneity of the sample, and a volume displacement effect[1,2,5].

ABO blood group compatibility between donor and recipient is very important for blood transfusion and organ transplantation to prevent hemolytic transfusion reaction and organ rejection. In addition, ABO blood grouping was interesting to study for disease association and predictions such as several cancers[7-10], malignant lymphoma[11], cardiovascular diseases[12], liver diseases[13], and platelet transfusion[14,15]. There are many methods for ABO blood group testing, such as conventional tube test[16], column agglutination[17], solid-phase red cell adherence assay[18] and erythrocyte-magnetized technology (EMT)[19]. Conventional tube test is the standard method, but it is prone to human errors[20] because it has many limitations, such as being a labor-intensive process, and the inconsistency of observing the agglutination with the naked eye, therefore, automated devices have been developed to resolve these problems. QWALYS®3 (Diagast, France), is an image-based result processor analyzer, which is a fully automated system that uses EMT for ABO blood typing, antibody screening and identification, and cross-matching.

In the blood transfusion service, the donors are required to eat the main meal before giving blood, to maintain the blood glucose level, it can cause lipemia and abnormal levels of biochemical substances. Currently, turbid appearance blood units with various assessing protocols are discarded, but the effect of lipemia and abnormal level of biochemical substances has not yet been studied in detail. These may affect ABO blood group testing by using the image-based automated analyzer that leads to compromise the blood transfusion safety. The prevalence of turbid samples in the Regional Blood Centre, Nakhon Sawan, Thailand, is approximately 2%. We compared the methods for assessing the turbidity of each sample and analyzed its association with the biochemical substances, and also investigated the risk factors of plasma turbidity and biochemical levels that affect ABO blood group testing. These results were then verified by using the image-based automated analyzer compared to the conventional tube test method. As far as we know, this is the first study aimed to determine the interferences evidence of the lipemia and biochemical substances on the ABO blood group testing using an image-based automated analyzer.

Materials and Methods

This study was conducted in the Regional Blood Centre, Nakhon Sawan, Thai Red Cross Society, Thailand. Blood samples were collected from general blood donations. To enable ABO grouping, the samples are added to citrate phosphate dextrose (CPD). The protocol for this study was approved by the Ethics Review Committee of the Thai Red Cross Society, Bangkok. Thailand (NBC number 11/2018) and Naresuan University Institutional Review Board, Phitsanulok, Thailand (IRB number 0179/61).

Sample turbidity assessment

Blood samples collected in CPD tubes were centrifuged at 3,000 rpm for 10 minutes, then the plasma samples were assessed for the turbidity by visual grading and the absorbance measured at a wavelength of 660 nm by a spectrophotometer. Visual grading is carried out by comparing plasma samples to the plasma turbidity image chart which was prepared from non-turbid plasma

(grade 0) and turbid plasma with OD at λ 660 nm as 0.4, 0.8, 1.0, and 1.2, for grading as 1+, 2+, 3+, and 4+, respectively. This chart, which was developed by the Associated Medical Sciences Clinical Service Centre, Chiang Mai University, is commonly used in the routine laboratory. The comparison of the methods used to assess sample turbidity was performed by using a total of 398 blood samples that were randomly selected and divided into 2 groups, according to visual grading; 199 turbid- and 199 nonturbid- samples.

ABO blood group testing and biochemical measurement

ABO blood groups were determined by QWALYS®3. A total of 205 clotted blood samples comprised of non-error result (n = 100), error "X" result (the machine did not aspirate the samples) (n = 50), and error "?" result (weakly reactive) (n = 55).

Serum samples were recruited according to the ABO blood group testing results as above. Sera biochemical substances including albumin, total protein, triglyceride, and glucose were measured by an automated analyzer (Mindray Medical International Limited, Shenzhen, China). Sera globulin was calculated by sera total protein concentration diminished with sera albumin concentration. The plasma samples were assessed for turbidity by visual grading and optical density. Then, the association of sera biochemical levels with plasma turbidity were analyzed. Furthermore, the risk factors of the errors in ABO blood grouping testing was analyzed.

Evaluation of albumin, globulin, triglyceride and glucose levels that affect ABO blood group testing

Sixteen plasma samples from blood group B donors without unexpected antibodies were pooled and measured to determine the concentrations of albumin, total protein, glucose, and triglyceride. The pooled plasma was then aliquoted, followed by adding various amounts of albumin (Human albumin, Thai Red Cross Society, Thailand), globulin (Human normal immunoglobulin for intravenous administration, Thai Red Cross Society, Thailand), and glucose (Dextrose, Istanbul, Turkey) to determine the different concentrations in the plasma, as shown in Table 4. For triglyceride, a sample with hypertriglyceridemia plasma was measured for triglyceride concentration (3,400 mg/dL), then diluted as serial 2-fold dilution with pooled plasma to make plasma triglyceride concentrations of 1,700, 850, 524, 212, and 106 mg/dL, respectively.

Plasma with certain concentrations of each biochemical substance was duplicated tested for the ABO blood group by QWALYS®3 and conventional tube test.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistical for Windows, version 23.0 (Armonk, New York, USA). The nonparametric Kruskal Wallis test and Mann-Whitney U test were used to compare the methods for plasma turbidity assessments: visual grading and OD 660 nm. For the association study, the data of variables were expressed as median and range. Dichotomous values of each variable were analyzed by the Chi-square test and Fisher's exact test, and the p-values < 0.05 were considered statistically significant. The odds ratio (OR) and 95% confidence interval (CI) of OR were calculated for the risk factor analysis. The multivariate logistic regression analysis was used to identify the independent risk factors of the error results from the ABO blood group testing. A p-value < 0.10 in univariate analysis and < 0.05 in multivariate analysis were considered statistically significant.

Results

Assessment of plasma turbidity and its association with biochemical levels

The methods for plasma turbidity assessment were compared in 398 plasma samples. The plasma turbidity assessed by visual grading had a positive trend when compared to the OD at λ 660 nm (Figure 1), along with the boxplot showed the medians of OD were increased related to the degree of visual grading.

The association of sera biochemical levels with plasma turbidity was dichotomously analyzed (Table 1) in 205 samples, and the levels of albumin and glucose

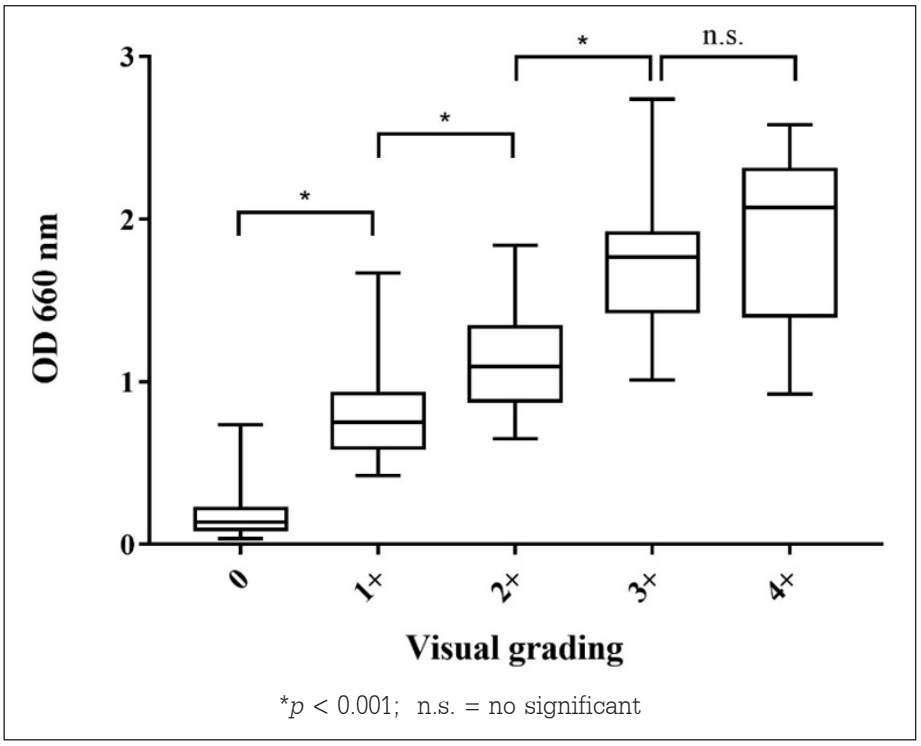

Figure 1 Comparison of plasma turbidity assessment methods: visual grading and OD 660 nm (n = 398 samples). Box and whiskers indicate median, 25th, and 75th percentiles, and range.

Table 1 Association of sera biochemical levels with plasma turbidity (n = 205 samples)

Biochemical levels	Levels of plasma turbidity					
	Visual grading			Optical density		
	0 to 2+ n (%)	3+ to 4+ n (%)	*p*-value	0.036 - 1.000 n (%)	1.001 - 2.980 n (%)	*p*-value
Albumin, g/dL (median: 4.9, range: 3.3-5.6)						
3.3-5.0	124 (62.9)	6 (75.0)	0.713	120 (62.2)	10 (83.3)	0.217
5.1-5.6	73 (37.1)	2 (25.0)		73 (37.8)	2 (16.7)	
Globulin, g/dL (median: 2.9, range: 2.1-6.1)						
2.1-3.5	175 (88.8)	1 (12.5)	< 0.001	172 (89.1)	4 (33.3)	< 0.001
3.6-6.1	22 (11.2)	7 (87.5)		21 (10.9)	8 (66.7)	
Total protein, g/dL (median: 8.1, range: 6.3-10.0)						
6.3-8.0	102 (51.8)	0 (0.0)	0.007	101 (52.3)	1 (8.3)	0.005
8.1-10.0	95 (48.2)	8 (100.0)		92 (47.7)	11 (91.7)	
Triglyceride, mg/dL (median: 155, range: 43-3,798)						
43-500	189 (95.9)	1 (12.5)	< 0.001	187 (96.9)	3 (25.0)	< 0.001
501-3,798	8 (4.1)	7 (87.5)		6 (3.1)	9 (75.0)	
Glucose, mg/dL (median: 78, range: 13-565)						
13-300	193 (98.1)	7 (87.5)	0.182	189 (97.9)	11 (91.7)	0.263
301-565	4 (2.0)	1 (12.5)		4 (2.1)	1 (8.3)	

were not associated with it ($p > 0.05$). In contrast, > 3.5 g/dL of globulin has a high proportion in the turbid samples; visual grading 3+ to 4+ and OD > 1.000 ($p < 0.001$). In addition, total protein concentrations of > 8.0 g/dL also have a high proportion in the turbid samples; visual grading 3+ to 4+ ($p = 0.007$) and OD > 1.000 ($p = 0.005$). Furthermore, triglyceride concentrations of > 500 mg/dL were also positively associated with plasma turbidity by both assessment methods ($p < 0.001$).

Risk factors of error results in ABO blood group testing

The analysis for the risk factors of the errors in ABO blood grouping by QWALYS®3 was performed in 205 samples which comprised of 50 with error "X", 55 with error "?", and 100 samples with non-error results. In univariate analysis, plasma OD > 1.000, globulin ≤ 3.5 g/dL, total protein ≤ 8.0 g/dL and triglyceride > 500 mg/dL were significantly different between error "X" and non-error results ($p < 0.10$) while, sex, age > 35 years, visual turbid > 2+, albumin ≤ 5.0 g/dL, and glucose > 300 mg/dL were not significantly different between error "X" and non-error results, as shown in Table 2. Multivariate logistic regression analysis revealed that total protein ≤ 8.0 g/dL and triglyceride > 500 mg/dL were the only two risk factors for error "X" results in ABO blood group testing ($p < 0.05$), as shown in Table 3.

For the error "?" result, univariate analysis, age > 35 years, plasma visual turbid > 2+, plasma OD > 1.000, total protein ≤ 8.0 g/dL, and triglyceride > 500 mg/dL were significantly different between error "?" and non-error results ($p < 0.10$) but, sex, albumin ≤ 5.0 g/dL, globulin ≤ 3.5 g/dL, and glucose > 300 mg/dL were not significantly different between error "?" and non-error results, as shown in Table 2. Multivariate logistic regression analysis revealed that total protein ≤ 8.0 g/dL and triglyceride > 500 mg/dL were the only two risk factors for error "?" results in ABO blood group testing ($p < 0.05$), as shown in Table 3.

Effects of biochemical levels on ABO blood group testing

For analysis, the levels of biochemical agents that affect ABO blood group testing were performed in blood group B samples using QWALYS®3 with duplication and the conventional tube test. The concentrations of albumin from 4.0-14.0 g/dL and globulin from 3.0-6.0 g/dL did not affect ABO blood group testing using both methods. Plasma with a glucose concentration of 1,000 mg/dL gave error "?" by QWALYS® 3, but a normal result (blood group B) from the conventional tube test. A triglyceride concentration of 1,700 mg/dL gave an error "?", while 3,400 mg/dL gave an error "X" from QWALYS®3, but a normal result from the conventional tube test.

Discussion

At present, automated chemistry analyzers can assess sample turbidity by measuring the OD at λ 660/700 nm, and report the results as lipemic (L)-index[21]. Although this method is more reliable than visual grading, it is time-consuming due to the increased workload of laboratory testing, especially in a blood transfusion service unit, which does not have a spectrophotometer. This study compared the assessment of plasma turbidity between OD 660 nm and visual grading measurements and found that they were related (Figure 1). This indicated that visual grading can be used for turbidity checking in routine blood bank samples. Therefore, we can reject the samples with plasma turbidity of ≥ 3+ that corresponds to OD > 1.000.

The high levels of triglyceride, globulin, and total protein were associated with the turbid samples, especially the ones with visual grades of 3+ to 4+ and OD > 1.000, which have high biochemical levels when compared to non-turbid samples (Table 1). Triglyceride is a non-soluble substance that needs to bind with lipoprotein before it can be transported to the tissue via circulation. Chylomicron and VLDL are lipoproteins with large particles at 70-1,000 nm and 27-200 nm[1] that affect light scattering, and both contain triglycerides at 90 and 65%, respectively[22]. Both lipoproteins may be the major cause of lipemia or turbid plasma, which corresponded with previous studies that reported triglyceride levels were associated with lipemia[23,24]. The results of this

Table 2 Univariate analysis of the risk factors for error results in ABO blood group testing (n = 205 samples)

Factors	Non error n=100 (%)	Error "X" n=50 (%)	*p*-value, OR (95% CI)	Error "?" n=55 (%)	*p*-value, OR (95% CI)
Sex: Female	30 (30.0)	21 (42.0)	0.149, 1.69 (0.83-3.42)	17 (30.9)	1.000, 1.04 (0.51-21.13)
Age: > 35 years	44 (44.0)	27 (54.0)	0.299, 1.49 (0.76-2.96)	36 (62.5)	0.012, 2.41 (1.22-4.77)
Visual turbid: > 2+	2 (2.0)	1 (2.0)	1.000, 1.00 (0.09-11.30)	5 (9.1)	0.098, 4.90 (0.92-26.16)
OD: > 1.000	1 (1.0)	4 (8.0)	0.043, 8.61 (0.94-79.19)	7 (12.7)	0.003, 14.44 (1.73-120.7)
Albumin: ≤ 5.0 g/dL	58 (58.0)	33 (66.0)	0.379, 1.41 (0.69-2.85)	39 (70.9)	0.122, 1.77 (0.87-3.57)
Globulin: ≤ 3.5 g/dL	17 (17.0)	2 (4.0)	0.035 4.92 (1.09-22.20)	10 (18.2)	0.829 0.92 (0.39-2.18)
Total protein: ≤ 8.0 g/dL	23 (23.0)	43 (86.0)	< 0.001, 20.57 (8.16-51.85)	36 (65.5)	< 0.001, 6.34 (3.07-13.10)
Triglyceride: > 500 mg/dL	2 (2.0)	4 (8.0)	0.096, 4.26 (0.75-24.11)	9 (16.4)	0.002, 9.59 (1.99-46.16)
Glucose: > 300 mg/dL	1 (1.0)	2 (4.0)	0.258, 4.13 (0.37-46.63)	2 (3.6)	0.287, 3.74 (0.33-42.16)

"X": the machine did not aspirate the samples; "?": weakly reactive; OR: Odds ratio

Table 3 Multivariate analysis of the risk factors for error results in ABO blood group testing

Risk factor	OR unadjusted (95%CI)	OR adjusted (95%CI)
Error "X"		
OD > 1.000	8.61 (0.94-79.19)	-
Globulin ≤ 3.5 g/dL	4.92 (1.09-22.20)	-
Total protein ≤ 8.0 g/dL	20.57 (8.16-51.85)	29.45 (9.22-94.10)
Triglyceride > 500 mg/dL	4.26 (0.75-24.11)	41.86 (3.60-486.54)
Error "?"		
Age > 35 years	2.41 (1.22-4.77)	-
Visual turbid: > 2+	4.90 (0.92-26.16)	-
OD > 1.000	14.44 (1.73-120.7)	-
Total protein ≤ 8.0 g/dL	6.34 (3.07-13.10)	8.95 (3.95-20.30)
Triglyceride > 500 mg/dL	9.59 (1.99-46.16)	10.35 (1.38-77.66)

"X": the machine did not aspirate the samples; "?": weakly reactive

study showed that the globulin level was associated with plasma turbidity, because of its high molecular weight. Corresponding to a previous report, a patient with a high concentration of IgM is indicative of the presence of monoclonal gammopathy, which caused a very high lipemic (turbidity) index[25]. Whereas, both albumin and glucose were not associated with plasma turbidity, because of their low molecular weight. These results supported that routinely discarded turbid donor plasma with visual grading ≥ 3+ in a blood transfusion service unit may reduce the transfused plasma, which has high levels of triglyceride, globulin, and total protein, more than 87.5 percent.

For the risk factor analysis of error "X" and "?" results in ABO blood group testing, the study was divided into two steps analysis. Firstly, we analysed univariate to find out for the risk factor of error "X" and "?" and then multivariate were analysed to exclude the confounding factors. The results showed that plasma OD > 1.000, total protein ≤ 8.0 g/dL and triglyceride > 500 mg/dL are risk factors of both errors in univariate analysis (Table 2), however, only total protein ≤ 8.0 g/dL and triglyceride ≥ 500 mg/dL are each independent risk factors in multivariate analysis (Table 3). As the previous study, age and sex are a risk factors of turbid plasma[23] that might be a source of error in using an image-based analyser, therefore, these two factors were included. However, the results showed that both of these factors were not the independent risk factors for the error results. The total protein is composed of albumin and globulin which both are important for antigen-antibody reaction. Albumin has decreased zeta potential around red cells, which brings them become closer to each other and cause better agglutination[26]. While the low level of albumin may cause weaker or no agglutination, especially for IgG antibodies, however, for the univariate analysis, albumin level was not a risk factor. For globulin, one part of globulin is called gamma globulin which is composed of antibodies or immunoglobulins. Thus, a low level of globulin may have a low level of antibodies in the plasma, which results in a weak reaction. Therefore, this may lead to ABO discrepancies between the results of ABO cell and serum grouping, and the error results. The sample with high triglyceride that was associated with turbidity may be involved in pipetting and nonhomogeneous samples, according to the low density of chylomicron < 0.95 g/mL and VLDL at 0.95-1.006 g/mL[22], causing the fat particles to float on top of the sample. In addition, the distance between red cell agglutination and the well width was measured and interpreted using image-based analysis. Due to the turbid samples showing unclear images, the software could not interpret them as error results, while only a high level of triglyceride was associated with sample turbidity, therefore, the rejection of the samples can only exclude the risk factors of errors from high triglyceride, but not for protein.

A triglyceride concentration of 3,400 mg/dL and 1,700 mg/dL in plasma, could interfere with the blood group testing, by giving the result "X" and "?", respectively (Table 4). The error "X" result, the machine did not aspirate the samples, of a triglyceride concentration of 3,400 mg/dL in plasma may be caused by its high viscosity and interfering of sample detector when pipetting. While the error "?", weakly reactive, of a triglyceride concentration of 1,700 mg/dL in plasma may be caused by unclear images and volume displacement effect because of non-homogeneous samples. This information supports the risk factor analysis results of errors "X" and "?" of high triglyceride level. However, triglyceride levels > 500 mg/dL can be visually graded as 3+ to 4+ (87.5%), and these samples may be rejected due to their high turbidity. A glucose concentration of 1,000 mg/dL gave an error "?", which may be caused by the high viscosity of the samples being interfered with during the aspiration process, and the antigen-antibody reaction.

Table 4 The effects of biochemical levels in ABO blood group testing

Biochemical levels	ABO blood group testing	
	QWALYS® 3	Conventional tube test
Albumin (g/dL)		
14.0	B	B
12.0	B	B
10.0	B	B
8.0	B	B
6.0	B	B
4.0	B	B
Globulin (g/dL)		
6.0	B	B
5.5	B	B
5.0	B	B
4.0	B	B
3.0	B	B
Glucose (mg/dL)		
1,000	?	B
800	B	B
600	B	B
400	B	B
200	B	B
100	B	B
Triglyceride (mg/dL)		
3,400	X	B
1,700	?	B
850	B	B
425	B	B
212	B	B
106	B	B

"X": the machine did not aspirate the samples; "?": weakly reactive

However, very high glucose concentrations were not found in healthy people, therefore, screening the donors by using a questionnaire, may help eliminate diabetic people from donating blood. Various concentrations of biochemical substances used in this study did not affect the results of ABO blood group testing by the conventional tube test method, which indicated its usefulness in verifying error results using an image-based automated analyzer.

Conclusions

This study found that both OD 660 nm and visual grading methods, were in agreement when assessing sample turbidity. This study also found that high levels of triglyceride and total protein ≤ 8.0 g/dL are the independent risk factors for error results in the automated ABO blood group testing. The high levels of triglyceride are associated with sample turbidity, therefore, the rejection of these turbid specimens may reduce the errors of these causes on the automated ABO blood group testing.

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Conflict of interest

None of the authors have any conflict to report.

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