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Optimization and performance validation for the fully automatic blood type analysis system

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ABSTRACT

To evaluate and validate the application of fully automatic blood type analysis system parameters under actual lab conditions. All key system parameters were optimized and validated accordingly. The optimized parameters were centrifugal speed at 550 rpm; centrifugal time 20 min; resuspension speed 1,200 rpm; resuspension time: 45 s; incubation temperature 25℃ ; incubation time 400 s; incubation rate: 0 rpm. The sampled red blood cell concentration was 3%. The ratio of plasma to red blood cells reagent was 60 μL:30 μL; the ratio of antibody (reagent) to sampled diluted red blood cell was 30 μL:30 μL. After applying our key parameters for optimization and validation, the automatic blood type detection system´s performance was found to meet the relevant requirements, effectively improving the accuracy and reliability of the detection system.

Keywords: blood type, performance validation, parameters

INTRODUCTION

In recent years, both the traditional manual method and semi-automatic methods of adding samples for blood group screening and identification have been phased out in blood-bank blood group detection, in favor of the automatic blood group detection system[1,2]. The optimization of these new systems is a prerequisite for their accurate operation in immunohematology laboratories. However, most automatic blood group analyzers are of the open detection type, which miss the relevant parameters for blood group detection reagents while only including the relevant hardware test parameters recommended by equipment manufacturers. At present, despite commercial blood group detection reagents being suitable for various detection methods, with reagent instructions providing reagent-to-blood cell or plasma reaction ratios, definite volumes suitable for automatic blood group systems are still lacking [3].

Orthogonal design is a popular experimental method that provides a convenient, fast and cost-saving resolution for optimization in many fields. We successfully applied orthogonal design to determine the optimal parameters combination for the Microlab STAR BG analytical system, which has potential to be a guide for future optimizing operations, with possible application to optimization related issues with other automated blood typing analyzers.

MATERIALS AND METHODS

Sample source

Vacuum blood sampling tubes containing anticoagulants were used for collecting of 4~5 mL venous blood from each volunteer donor at our center, which

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were centrifuged for 15 min at 1,500 g. Three tubes of A_2B blood type samples were obtained from Beijing Jinhao Pharmaceutical Co., Ltd., China.

Reagents

Anti-A and anti-B blood grouping reagents (lot number 20180801021) were provided by Shanghai Blood Biomedical Co., Ltd., China. Anti-D blood grouping reagent (lot number BMK1509D) was provided by Millipore Co., UK. Human blood type A, B, O cells reagents (lot number 20180801021) used for reverse typing, were provided by Beijing Jinhao Pharmaceutical Co., Ltd., China.

Instruments

The automatic blood group system (STAR-BG) was provided by Hamilton Co., Swiss.

Study flowchart

The study flowchart was seen in ***Fig.1***.

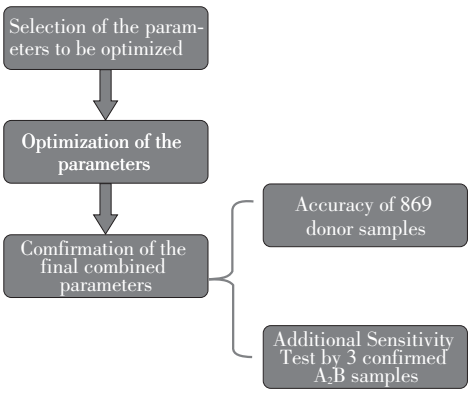

Fig.1 **The study flowchart**

Parameter identification

Test parameters were divided into fixed parameters and optimal parameters. Mixing conditions, centrifugal conditions and incubation temperature were set according to fixed parameters recommended by the manufacturers. Incubation rate, diluted erythrocyte sample loading volume, and monoclonal antibodies for forward typing, and the ratio of plasma to erythrocyte reagent in reverse typing were selected as parameters to be optimized, as shown in ***Table 1***.

Table 1 **Parameters to be optimized**

Parameter name	Equipment settable range	Equipment manufacturer recommended parameters	Reagent manufacturer recommended parameters	Final determined parameters
Mixing speed(rpm)	800–1,400	1,200	\	1,200
Mixing time(s)	80–160	120	\	120
Centrifugal speed(rpm)	100–2,500	550	\	550
Centrifugal time	0–30 min	20 min	Instructions 2.6: The reaction can be enhanced when placed at room temperature [(23±3) ℃] for a few minutes, but the time should not exceed 30 min (forward typing reagent); incubation at room temperature for 15–20 min(anti-D).	20 min
Incubation temperature( )	15–30	25	\	25
Scattered speed(rpm)	80–1,400	1,200	\	1,200
Scattered time(s)	30–60	45	\	45
Incubation speed(rpm)	0–500	0–500	\	to be optimized
Incubation time(s)	0–500	400	\	400
Erythrocyte sample concentration	\	\	\	3%
Erythrocyte reagent loading volume (reverse typing)	\	\	Instructions recommend the ratio of plasma to cells reagent be 1:1	The ratio of plasma to erythrocyte needs to be optimized
Plasma sample loading volume (reverse typing)	\	\	Instructions recommend the ratio of plasma to cells reagent be 1:1	The ratio of plasma to erythrocyte needs to be optimized
Anti-A, anti-B, anti-D reagent loading volume (forward typing and RH)	\	\	Instructions 2.1: add a drop of anti-A or anti-B reagent; Instruction 4.2: add a drop of anti-D reagent	The ratio of plasma to erythrocyte can be 1:1, but sampling quantity needs to be optimized.
Erythrocyte sample loading volume(forward typing and RH)	\	\	Instruction 2.2: one drop of cells suspension with 2%–5% concentration (ABO); Instruction 4.1: one drop of cells suspension with 3%–5% concentration (D)	The ratio of plasma to erythrocyte can be 1:1, but sampling quantity needs to be optimized.

Parameter optimization

For the three factors mentioned in ***Table 1*** to be optimized, the mixed-level orthogonal design was carried out. Based on the recommended range of 0–500 rpm given by the instrument manufacturer, the parameters of incubation speed were set at five levels: 0 rpm, 150 rpm, 300 rpm, 450 rpm and 500 rpm. The sample size of diluted red blood cells and monoclonal antibodies in forward typing was set at three levels: 30 μL:30 μL, 40 μL:40 μL, and 50 μL:50 μL, based on the recommendation of reagent manufacturer 1:1. The ratio of plasma to erythrocyte reagent was set at 30 μL:30 μL, 60 μL:30 μL, and 90 μL:30 μL. According to different parameters, 25-day experiments were carried out. Grouping accuracy was served to evaluate the performance of the optimized condition.

Further optimization of lipemic samples

According to our optimized parameters, the ratio of plasma to cell reagent in 16 lipemic samples was further fine-adjusted to reduce the influence of lipid blood on blood group detection.

Validation of optimized parameters

After setting the optimized parameters, the test results were validated, and a retrospective analysis was carried out three months after setting the parameters.

Performance of the detection system after optimization accuracy test

Totally 869 specimens (283 type A, 246 type B, 256 type O, 84 type AB) were tested for forward and reverse typing for 5 consecutive days, and the accuracy of grouping was evaluated. The acceptable accuracy was more than 95%.

Additional sensitivity test according to the *Chinese Pharmacopoeia*

Referring to the *Chinese Pharmacopoeia´*s requirement for sensitivity to anti-A reagent, three A2B samples were detected in parallel with routine samples, and the experimental results were recorded to validate the analyzer´s sensitivity to difficult blood groups.

Statistical analysis

SPSS 23.0 software was applied for the orthogonal modeling and data analysis, including direct–viewing analysis and variance analysis. P–values less than 0.05 were regarded as statistically significant.

RESULTS

Parametric optimization results

A total of 25 independent runs (128 samples in each run) of blood groups were carried out in 25 days (data shown in ***Table 2***). The variance analysis showed that the *P*-values of incubation speed and loading volumes of diluted red blood cells and monoclonal antibodies in forward typing were 0.364 and 0.153, respectively, indicating that these two factors did not significantly influence the ABO grouping, as shown in ***Table 3, Table 4*** and ***Table 5***. However, the *P*-value of the ratio of plasma to erythrocyte reagent was less than 0.001, indicating that the ratio of plasma to erythrocyte reagent was the major influencing factor, as shown in ***Table 6***. Finally, considering the results of direct viewing analysis and variance analysis, as shown in ***Table 5***, we chose 0 rpm as the optimal incubation speed. For the ratio of plasma to cell reagents, multiple comparisons showed no significant difference between proportion of 90 μL:30 μL and 60 μL:30 μL, and the parameters were then optimized in lipemic samples below. To minimize the cost of antibody reagents, 30 μL:30 μL was selected for the forward typing.

Further optimization results for lipemic samples

The ratio of plasma to cell reagents was tested at 90:30 and 60:30 in 16 lipemic samples. The results showed that two lipemic samples could not be correctly interpreted at 90:30, and the accuracy rate was 87.5%, while the accuracy was 100% at 60:30. There–

Table 2 **Schedule of an orthogonal design**

Test date (month/day)	Suspension (reaction) speed(rpm)	Erythrocyte sample volume (μL)	Ratio of plasma to erythrocyte (μL)	Rate of types A/B/O correctly interpretation (%)
6/14	450	50:50	60:30	100.0
6/15	300	40:40	30:30	100.0
6/16	300	30:30	60:30	100.0
6/19	500	40:40	90:30	91.7
6/20	150	40:40	60:30	100.0
6/21	0	40:40	60:30	100.0
6/26	500	30:30	30:30	100.0
6/29	150	50:50	30:30	100.0
7/03	450	40:40	90:30	82.4
7/04	450	40:40	30:30	96.9
7/05	150	30:30	60:30	100.0
7/06	300	50:50	90:30	55.8
7/07	450	40:40	60:30	100.0
7/10	0	30:30	90:30	60.5
7/12	500	50:50	60:30	99.2
7/13	300	30:30	60:30	99.2
7/14	0	50:50	30:30	100.0
7/17	300	40:40	30:30	100.0
7/18	500	30:30	30:30	100.0
7/19	500	40:40	60:30	100.0
7/20	450	30:30	30:30	100.0
7/21	150	40:40	30:30	98.9
7/24	0	30:30	30:30	100.0
7/25	150	30:30	90:30	56.9
7/26	0	40:40	60:30	100.0

Table 3 **Tests of between-subjects effects**

Source	Type Ⅲ sum of squares	Degree of freedom	Mean square	F value	*P* value
Modified model	4,052.003[a]	8	506.500	11.489	<0.001
Interception	162,688.642	1	162,688.642	3,690.356	<0.001
Suspension (reaction) Velocity	205.148	4	51.287	1.163	0.364
Loading volume of erythrocyte sample	186.267	2	93.133	2.113	0.153
Ratio of plasma to erythrocytes reagent	3,660.588	2	1,830.294	41.518	<0.001
Error	705.357	16	44.085		
Total	224,062.250	25			
Revised total	4,757.360	24			

Dependent variable: types A/B/O correctly interpretation rate.

a: R^2= 0.852 (adjusted R^2 = 0.778).

Table 4 **Multiple comparisons among different ratios of antibodies and diluted cells**

(I)Volume of antibodies and cell samples(μL)	(J)Volume of antibodies and cell samples(μL)	Difference of mean value(I-J)	Standard error	*P* value	95% confidence interval
30:30	40:40	−5.330	2.9693	0.092	−11.625~0.965
	50:50	0.660	3.6367	0.858	−7.049~8.369
40:40	30:30	5.330	2.9693	0.092	−0.965~11.625
	50:50	5.990	3.6367	0.119	−1.719~13.699
50:50	30:30	−0.660	3.6367	0.858	−8.369~7.049
	40:40	−5.990	3.6367	0.119	−13.699~1.719

Dependent variable: types A/B/O correctly interpretation rate.

Based on the actually measured average value.

The error term is mean square (error) = 44.085.

Table 5 **Multiple comparisons among different suspension speed**

(I) Suspension (reaction) speed	(J)Suspension (reaction) speed	Difference of mean value(I-J)	Standard error	*P* value	95% confidence interval
0 rpm	150 rpm	0.940	4.1993	0.826	−7.962~9.842
	300 rpm	1.100	4.1993	0.797	−7.802~10.002
	450 rpm	−3.760	4.1993	0.384	−12.662~5.142
	550 rpm	−6.080	4.1993	0.167	−14.982~2.8822
150 rpm	0 rpm	−0.940	4.1993	0.826	−9.842~7.962
	300 rpm	0.160	4.1993	0.970	−8.742~9.062
	450 rpm	−4.700	4.1993	0.280	−13.602~4.202
	550 rpm	−7.020	4.1993	0.114	−15.922~1.882
300 rpm	0 rpm	−1.100	4.1993	0.797	−10.002~7.802
	150 rpm	−0.160	4.1993	0.970	−9.062~8.742
	450 rpm	−4.860	4.1993	0.264	−13.762~4.042
	550 rpm	−7.180	4.1993	0.107	−16.082~1.722
450 rpm	0 rpm	3.760	4.1993	0.384	−5.142~12.662
	150 rpm	4.700	4.1993	0.280	−4.202~13.602
	300 rpm	4.860	4.1993	0.264	−4.042~13.762
	550 rpm	−2.320	4.1993	0.588	−11.222~6.582
550 rpm	0 rpm	6.080	4.1993	0.167	−2.822~14.982
	150 rpm	7.020	4.1993	0.114	−1.882~15.922
	300 rpm	7.180	4.1993	0.107	−1.722~16.082
	450 rpm	2.320	4.1993	0.588	−6.582~11.222

Dependent variable: types A/B/O correctly interpretation rate.

Based on the actually measured average value.

The error term is mean square (error) = 44.085.

fore the ratio of plasma to red blood cell reagents was finally determined as 60:30.

Final optimization of parameters

Taking both results from direct-viewing analy–sis and variance analysis into consideration, 0 rpm was selected as the optimal incubation speed and the proportion 60:30 was selected as the optimal ratio for plasma and reagent RBCs. To minimize the cost of antibody reagents, the ratio 30 μL:30 μL was selected for forward typing. The final optimization parameters are shown in ***Table 7***.

Validation results of optimization parameters

Under optimal conditions obtained from orthogonal design, a set of 322 random donor samples were car–ried out to confirm the optimized combination, and no erroneous results were found.

Table 6 **Multiple comparisons among different ratios of plasma and RBC reagents**

(I)Volume of plasma and erythrocyte reagent(μL)	(J)Volume of plasma and erythrocyte reagent(μL)	Difference of mean value(I-J)	Standard error	*P* value	95% confidence interval
90:30	60:30	−0.260	2.9693	0.931	−6.555~6.035
	30:30	30.120*	3.6367	<0.001	22.411~37.829
60:30	90:30	−0.260	2.9693	0.931	−6.035~6.555
	30:30	30.380*	3.6367	<0.001	22.671~38.089
30:30	90:30	−30.120*	3.6367	<0.001	−37.829~−22.411
	60:30	−30.380*	3.6367	<0.001	−38.089~−22.671

Dependent variable: types A/B/O correctly interpretation rate.

Based on the actually measured average value.

The error term is mean square (error) = 44.085.

* The significant level of difference between mean values is 0.05.

Table 7 **Final optimization of parameters**

Name of parameters	Final determined parameters
Mixing speed	1,200 rpm
Mixing time	120 s
Centrifugal speed	550 rpm
Centrifugal time	20 min
Incubation temperature	25
Scattered speed	1,200 rpm
Scattered time	45 s
Incubation speed	0 rpm
Incubation time	400 s
Erythrocyte sample concentration	3%
Loading volume and ratio of plasma and erythrocyte reagent (reverse typing)	60 μL:30 μL
Loading volume of antibody reagent and diluted erythrocyte sample	30 μL:30 μL

Accuracy of test results

Totally 869 samples (283 type A, 246 type B, 256 type O, 84 type AB) were tested with forward and reverse typing for 5 consecutive days. The accuracy of ABO interpretation was more than 99.60%, and the accuracy of Rh (D) grouping was 100% ***(Table 8)***.

Table 8 **Retrospective evaluation results**

Blood group	Total cases	Correctly grouped cases	Accuracy(%)
Type A	283	283	100.0
Type B	246	246	100.0
Type O	256	255	99.6
Type AB	84	84	100.0
Rh(D)	869	869	100.0

Additional sensitivity test results according to the *Chinese Pharmacopoeia*

Test results of three known A_2B samples were all correctly classified as AB type, which met the detection requirements of *Chinese Pharmacopoeia* for A_2B type [4].

Parameter reassessment

Up to Dec. 8, 2017, we did not receive any adverse feedback related to blood grouping, and no errors were found due to severe lipemic or hemolytic samples.

DISCUSSION

At present, the automatic blood group analysis system is an open system, which generally has no specified reagents. Although each equipment manufacturer provides the appropriate parameters for their equipment, these parameters need to be optimized when using different reagents. Also the sample status, centrifugal force, manufacturer of reagents, and sample loading volumes are different in each laboratory. Therefore, the setting of system parameters and reagent loading volume must be validated scientifically and systematically, instead of using empirical or estimated values[5,6]. Only by validating the parameters affecting the test results can the reliability of the results be ensured, especially for the correct detection of subtypes, weak antigens, weak antibodies and other difficult blood groups, so as to improve the safety of blood transfusion [7,8].

In the validation of the blood group detection system in this paper, the key parameters of the system firstly needed to be analyzed and identified. With regard to the mixing speed, mixing time, the centrifugal speed, and centrifugal time, they were classified as non-key parameters, as the reaction time, speed or time changes had little impact on the results. After scattering and suspending in the reaction process, strong agglutination changed little, but weak agglutination dispersed. The weak agglutination was then identified by the detection system after incubation. The incubation speed, the sample loading volume of diluted red blood cells and monoclonal antibodies in forward typing and the ratio of plasma to red blood cells reagent do have an impact on the blood group results, and were judged as the key parameters of the system. The orthogonal key parameters were analyzed by SPSS software to determine the appropriate parameters and the ratio of antigen to antibody. At the same time, lipemic samples were validated according to the appropriate parameters. So, by means of reducing plasma loading volume and increasing imaging

transmittance, the interference of lipemic samples on imaging can be avoided.

However, there were still some deficiencies in our work; for example, we did not compare two (or more) sets of blood–typing systems in our laboratory. Also, additional subtype blood samples, such as B subtype should be included to confirm that the system can meet the expected requirements after optimization.

In summary, by bearing in mind the manufacturer´s test parameter recommendations, assessing laboratory detection system related samples and reagents simultaneously, and selecting the key parameters for validation and further optimizing the parameters on the basis of validation, the processing ability and reliability of the detection system can be effectively improved. It also provides a method for other laboratories to evaluate the test parameters of their automatic blood group detection system and reagents.

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