Original Article

Application of magnetic bead method in detecting coagulation function

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Background: Blood samples indicative of jaundice, lipemia, hemolysis, and others are often encountered in the laboratory, and such features impact greatly on the detection of coagulation items. To understand the anti-interference ability of the magnetic bead method automatic coagulation instrument and the optical method automatic coagulation instrument against jaundice, lipemia, and hemolysis, anti-interference experiments of prolonged prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB) were conducted using ExC810 (magnetic bead method) and CS5100 (optical method).

Methods: Interference samples were prepared with bilirubin, hemoglobin, and lipids, while control samples were prepared with NaOH and distilled water. The samples contained different values of PT, APTT, TT, and FIB and were detected by magnetic bead method and optical method, respectively. The relative deviation was calculated according to the formula.

Results: In the anti-interference experiment of PT, APTT, TT, and FIB (jaundice, lipemia, hemolysis), the deviation between the test results and the control results with the addition of interfering substances tested by ExC810 was lower overall than that of CS5100. However, after the addition of interfering substances, most of the items were not detected by CS5100 in the anti-lipidemia experiment.

Conclusions: When testing coagulation function, the magnetic bead method has better anti-interference recognition of jaundice, lipemia, and hemolysis than the optical method, and its anti-lipemic interference has a particularly obvious advantage.

Keywords: Coagulation function; examination; magnetic bead method; optical method

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Introduction

In the laboratory, blood samples are often complicated by presentations such as jaundice, lipemia, hemolysis, and others, and the detection of coagulation items in such samples can be particularly challenging, which could affect the interpretation of the results and diagnosis of diseases by doctors (1-3).

In order to understand the anti-interference ability of the magnetic bead method automatic coagulation instrument and the optical method automatic coagulation instrument against jaundice, lipemia, and hemolysis, an anti-interference experiment of prolonged prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB) assessment were conducted by Shenzhen Mindray automatic coagulation instrument ExC810 (magnetic bead method; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen,

Guangdong, China) and Sysmex Automated Coagulation Analyzer CS5100 (optical method; Sysmex Corp., Kobe, Hyogo, Japan), respectively. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-2713).

Methods

Anti-bilirubin interference experiment

Preparation of bilirubin interference samples was conducted as follows: mixed plasma samples (more than 20 tubes of coagulated blood samples mixed for preparation) were prepared. One of each abnormal sample (PT, FIB, APTT, TT) was selected of the 4 routine examinations (beyond the normal reference range), respectively. A total of 0.02 g of bilirubin was weighed and dissolved in 2.5 mL of 0.1 M NaOH to prepare a bilirubin mother solution of 800 mg/dL. The ratios of bilirubin mother solution and mixed plasma samples were calculated to mix and prepare 40 and 80 mg/dL interference sample of PT; 40 and 80 mg/dL interference sample of APTT; 80 and 100 mg/dL interference sample of TT; 100 and 160 mg/dL interference sample of FIB; another mixed plasma sample with 0.1 M NaOH was taken to correspondingly prepare 40 and 80 mg/dL control sample of PT; 40 and 80 mg/dL control sample of APTT; 80 and 100 mg/dL Control sample of TT; and 100 and 160 mg/dL control sample of FIB. The ratio of bilirubin mother solution and abnormal plasma sample was calculated, and a PT 40 mg/dL interference sample was mixed and configured; 40 mg/dL interference sample of APTT; 80 mg/dL interference sample of TT; 100 mg/dL interference sample of FIB; another copy with 0.1 M NaOH abnormal plasma samples was taken to correspondingly prepare 40 mg/dL control samples of PT; 40 mg/dL control samples of APTT; 80 mg/dL control samples of TT; and 100 mg/dL control samples of FIB. Each coagulometer was calibrated before the experiment, and the quality control of all test items was passed with the supporting reagents. The test was repeated twice for the samples with 2 concentrations of mixed plasma and the corresponding control samples, mean value of the sample concentration was calculated, abnormal plasma sample and the control sample was tested once, data were recorded in *Table 1*, and the relative deviation was calculated according to the formula. The formula was as follows: deviation = [(y – x)/x] ×100%, where y is the detection value of interference sample, and x is the detection value of control sample (the same as below). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Foshan First People's Hospital [No. L(2020)23] and informed consent was taken from all the patients.

Anti-hemoglobin interference experiment

Preparation of hemoglobin interference samples: mixed plasma samples (preparation of coagulation samples with more than 20 tubes) were prepared. One sample was selected from each abnormal sample type of the 4 routine examinations (PT, FIB, APTT, TT; beyond the normal reference range). The ratio of hemoglobin interferent (28,400 mg/dL) and mixed plasma sample was calculated, then 3,155 and 3,550 mg/dL interference sample of PT, APTT, TT, and FIB was prepared with distilled water, respectively; another mixed plasma sample was taken, then 3,155 and 3,550 mg/dL control sample of PT, APTT, TT, and FIB was prepared with distilled water, respectively. The ratio of hemoglobin interferents and abnormal plasma samples was calculated, then 3,155 mg/dL interference sample of PT, APTT, TT, and FIB was prepared with distilled water, respectively; another abnormal plasma sample was taken, 3,155 mg/dL control sample of PT, APTT, TT, and FIB was prepared with distilled water, respectively. Each coagulometer was calibrated before the experiment, and quality control of all test items was conducted and passed with the supporting reagents. The test was repeated twice for the samples with 2 concentrations of mixed plasma and the corresponding control samples, mean value of the sample concentration was calculated, abnormal plasma sample and the control sample was tested once, data were recorded in *Table 2*, and the relative deviation was calculated according to the formula.

Anti-lipid interference experiment

Preparation of blood lipid interference sample was as follows: a mixed plasma sample (mix preparation of coagulation samples with more than 20 tubes) was prepared. A sample was selected of each abnormal sample of the 4 routine examinations (PT, FIB, APTT, TT; beyond the normal reference range). The ratios with blood lipid interference substances (50,000 mg/dL) and mixed plasma samples were calculated to mix and prepare as 2,500 and 2,941 mg/dL interference samples of PT; 2,941

Table 1 Experiment results of anti-bilirubin interference

Item and concentration	Bilirubin interference-data recording sheet						Deviation
	Control sample			Interference sample			
	Test 1 (s)	Test 2 (s)	Mean (s)	Test 1 (s)	Test 2 (s)	Mean (s)	
CS5100							
PT1 (mixed plasma) 40 mg/dL	12.7	13.0	12.85	12.6	12.7	12.65	–1.56%
PT2 (mixed plasma) 80 mg/dL	14.2	14.4	14.30	14.8	14.8	14.80	3.50%
PT3 (abnormal) 40 mg/dL	20.2	20.5	20.35	21.1	21.5	21.30	4.67%
APTT1 (mixed plasma) 40 mg/dL	33.9	34.0	33.95	33.6	34.1	33.85	–0.29%
APTT2 (mixed plasma) 80 mg/dL	53.6	54.2	53.90	53.5	53.7	53.60	–0.56%
APTT3 (abnormal) 40 mg/dL	57.0	57.6	57.30	58.2	58.5	58.35	1.83%
TT1 (mixed plasma) 80 mg/dL	18.7	20.1	19.40	19.3	20.4	19.85	2.32%
TT2 (mixed plasma) 160 mg/dL	22.7	23.6	23.15	23.7	25.5	24.60	6.26%
TT3 (abnormal) 80 mg/dL	29.5	28.7	29.10	45.4	38.2	41.80	43.64%
FIB1 (mixed plasma) 100 mg/dL	4.00	4.00	4.00	4.20	4.20	4.20	5.00%
FIB2 (mixed plasma) 160 mg/dL	3.47	3.55	3.51	3.81	4.00	3.91	11.25%
FIB3 (abnormal) 100 mg/dL	4.42	4.54	4.48	4.66	4.66	4.66	4.02%
ExC810							
PT1 (mixed plasma) 40 mg/dL	13.03	12.94	12.99	13.21	12.98	13.10	0.85%
PT2 (mixed plasma) 80 mg/dL	13.66	13.75	13.71	13.68	13.44	13.56	–1.06%
PT3 (abnormal) 40 mg/dL	19.87	20.33	20.10	19.92	20.04	19.98	–0.60%
APTT1 (mixed plasma) 40 mg/dL	35.15	34.96	35.06	35.13	35.73	35.43	1.07%
APTT2 (mixed plasma) 80 mg/dL	41.36	41.12	41.24	43.09	44.26	43.68	5.90%
APTT3 (abnormal) 40 mg/dL	44.53	45.11	44.82	43.58	44.65	44.12	–1.57%
TT1 (mixed plasma) 80 mg/dL	15.14	15.14	15.14	15.26	15.15	15.21	0.43%
TT2 (mixed plasma) 160 mg/dL	20.11	19.58	19.85	18.73	18.92	18.83	–5.14%
TT3 (abnormal) 80 mg/dL	21.63	22.13	21.88	22.1	22.51	22.31	1.94%
FIB1 (mixed plasma) 100 mg/dL	4.17	4.18	4.18	4.19	4.2	4.20	0.48%
FIB2 (mixed plasma) 160 mg/dL	3.55	3.55	3.55	3.39	3.49	3.44	–3.10%
FIB3 (abnormal) 100 mg/dL	3.68	3.97	3.83	4.91	4.8	4.86	26.93%

PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

and 3,333 mg/dL interference samples of APTT; 1,666 and 2,000 mg/dL interference samples of TT; 4,504 and 5,000 mg/dL interference sample of FIB; another mixed plasma sample was taken with distilled water to correspondingly prepare as 2,500 and 2,941 mg/dL control sample of PT; 2,941 and 3,333 mg/dL control sample of APTT; 1,666 and 2,000 mg/dL control sample of TT; 4,504 and 5,000 mg/dL control sample of FIB. The ratios of blood lipid interference substances and abnormal plasma samples were calculated to mix and prepare a 2,500 mg/dL interference sample of PT; 2,941 mg/dL interference sample of APTT; 1,666 mg/dL interference sample of TT; 4,504 mg/dL interference sample of FIB; another abnormal plasma sample was taken with the distilled water to

Table 2 Experiment results of anti-hemoglobin interference

Item and concentration	Hemoglobin interference-data recording sheet						Deviation
	Control sample			Interference sample			
	Test 1 (s)	Test 2 (s)	Mean (s)	Test 1 (s)	Test 2 (s)	Mean (s)	
CS5100							
PT1 (mixed plasma) 3,155 mg/dL	11.10	12.00	11.55	11.70	11.90	11.80	2.16%
PT2 (mixed plasma) 3,550 mg/dL	11.70	12.30	12.00	11.70	11.70	11.70	–2.50%
PT3 (abnormal) 3,155 mg/dL	20.00		20.00	20.30		20.30	1.50%
APTT1 (mixed plasma) 3,155 mg/dL	30.30	28.20	29.25	28.30	27.70	28.00	–4.27%
APTT2 (mixed plasma) 3,550 mg/dL	30.60	29.00	29.80	29.00	29.50	29.25	–1.85%
APTT3 (abnormal) 3,155 mg/dL	59.10		59.10	44.20		44.20	–25.21%
TT1 (mixed plasma) 3,155 mg/dL	16.10	17.00	16.55	15.90	16.50	16.20	–2.11%
TT2 (mixed plasma) 3,550 mg/dL	15.90	15.90	15.90		16.50	16.50	3.77%
TT3 (abnormal) 3,155 mg/dL	37.90		37.90	53.70		53.70	41.69%
FIB1 (mixed plasma) 3,155 mg/dL	2.99	3.18	3.09	3.05	2.87	2.96	–4.05%
FIB2 (mixed plasma) 3,550 mg/dL	2.99	2.93	2.96	2.99	2.71	2.85	–3.72%
FIB3 (abnormal) 3,155 mg/dL	4.79		4.79	4.10		4.10	–14.41%
ExC810							
PT1 (mixed plasma) 3,155 mg/dL	12.37	12.54	12.46	12.01	12.22	12.12	–2.73%
PT2 (mixed plasma) 3,550 mg/dL	12.51	12.58	12.55	11.95	11.87	11.91	–5.06%
PT3 (abnormal) 3,155 mg/dL	19.52		19.52	18.35		18.35	–5.99%
APTT1 (mixed plasma) 3,155 mg/dL	33.02	33.17	33.10	34.92	34.10	34.51	4.28%
APTT2 (mixed plasma) 3,550 mg/dL	33.95	32.80	33.38	32.53	33.40	32.97	–1.23%
APTT3 (abnormal) 3,155 mg/dL	37.29		37.29	40.14		40.14	7.64%
TT1 (mixed plasma) 3,155 mg/dL	15.39	15.11	15.25	13.80	13.57	13.69	–10.26%
TT2 (mixed plasma) 3,550 mg/dL	15.24	15.30	15.27	13.87	13.63	13.75	–9.95%
TT3 (abnormal) 3,155 mg/dL	23.89		23.89	23.67		23.67	–0.92%
FIB1 (mixed plasma) 3,155 mg/dL	3.35	3.22	3.29	3.15	3.27	3.21	–2.28%
FIB2 (mixed plasma) 3,550 mg/dL	3.22	3.25	3.24	3.31	3.17	3.24	0.15%
FIB3 (abnormal) 3,155 mg/dL	5.14		5.14	5.17		5.17	0.58%

PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

correspondingly prepare as 2,500 mg /dL control sample of PT; 2,941 mg/dL control sample of APTT; 1,666 mg/dL control sample of TT; 4,504 mg/dL control sample of FIB. Each coagulometer was calibrated before the experiment, and quality control of all test items was completed and passed with the supporting reagents. The test was repeated twice for the samples with 2 concentrations of mixed plasma and the corresponding control samples, the mean value of the sample concentration was calculated, abnormal plasma sample and the control sample was tested once, data were recorded in the following *Table 3*, and the formula was used to calculate relative deviation.

Table 3 Experiment results of anti-lipid interference

Item and concentration	Lipid interference-data recording sheet						
	Control sample			Interference sample			Deviation
	Test 1 (s)	Test 2 (s)	Mean (s)	Test 1 (s)	Test 2 (s)	Mean (s)	
CS5100							
PT1 (mixed plasma) 2,500 mg/dL	10.60	10.90	10.75	*	*	–	–
PT2 (mixed plasma) 2,941 mg/dL	10.80	10.90	10.85	*	*	–	–
PT3 (abnormal) 2,500 mg/dL	17.80		17.80	17.20		17.20	–3.37%
APTT1 (mixed plasma) 2,941 mg/dL	30.30	30.00	30.15	*	*	–	–
APTT2 (mixed plasma) 3,333 mg/dL	30.50	29.90	30.20	*	*	–	–
APTT3 (abnormal) 2,941 mg/dL	36.10		36.10	*		–	–
TT1 (mixed plasma) 1,666 mg/dL	18.00	18.00	18.00	52.70	50.10	51.40	185.56%
TT2 (mixed plasma) 2,000 mg/dL	19.70	19.70	19.70	80.20	78.10	79.15	301.78%
TT3 (abnormal) 1,666 mg/dL	22.00		22.00	*		–	–
FIB1 (mixed plasma) 4,504 mg/dL	3.18	2.87	3.03	*	2.81	2.81	–7.11%
FIB2 (mixed plasma) 5,000 mg/dL	2.87	2.87	2.87	*	*	–	–
FIB3 (abnormal) 4,504 mg/dL	4.54		4.54	4.79		4.79	5.51%
ExC810							
PT1 (mixed plasma) 2,500 mg/dL	11.12	11.37	11.25	11.36	11.90	11.63	3.42%
PT2 (mixed plasma) 2,941 mg/dL	11.37	10.93	11.15	11.63	11.49	11.56	3.68%
PT3 (abnormal) 2,500 mg/dL	17.75		17.75	17.64		17.64	–0.62%
APTT1 (mixed plasma) 2,941 mg/dL	31.89	32.12	32.01	31.20	31.51	31.36	–2.03%
APTT2 (mixed plasma) 3,333 mg/dL	31.75	32.22	31.99	31.75	31.76	31.76	–0.72%
APTT3 (abnormal) 2,941 mg/dL	50.48	51.39	50.94	43.38	43.16	43.27	–15.05%
TT1 (mixed plasma) 1,666 mg/dL	15.72	15.79	15.76	15.95	15.73	15.84	0.54%
TT2 (mixed plasma) 2,000 mg/dL	15.93	15.79	15.86	16.06	15.84	15.95	0.57%
TT3 (abnormal) 1,666 mg/dL	20.37		20.37	21.58		21.58	5.94%
FIB1 (mixed plasma) 4,504 mg/dL	3.13	2.92	3.03	2.92	2.94	2.93	–3.14%
FIB2 (mixed plasma) 5,000 mg/dL	2.97	2.99	2.98	3.05	3.13	3.09	3.69%
FIB3 (abnormal) 4,504 mg/dL	5.27		5.27	5.01		5.01	–4.93%

*, the undetectable. –, no data measured. PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

Statistical methods

Calculation of interference result: calculate the offset value according to EP7-A2 file, and the offset value = $\overline{X}$test -$\overline{X}$control. Observe the interference.

Results

Anti-bilirubin interference experiment

In the experiment, when bilirubin was added for interference, the deviation on the detection results of

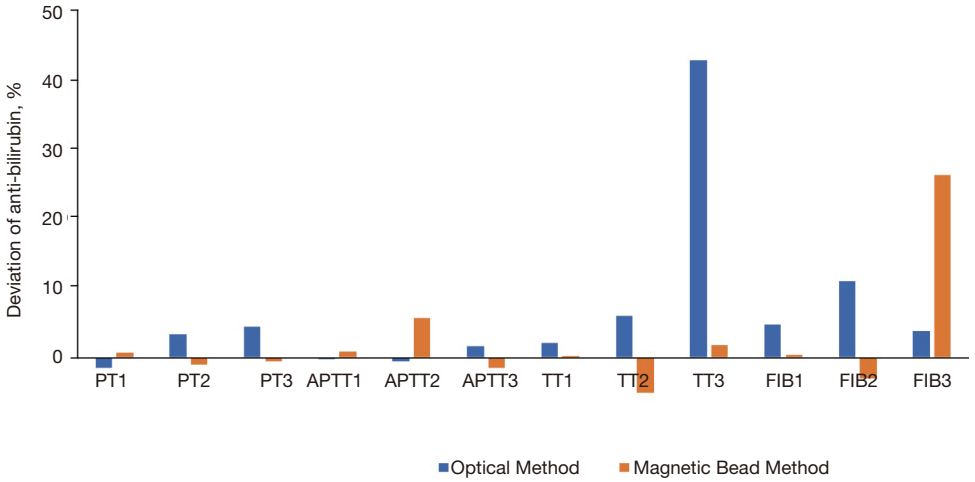

Figure 1 Deviation of anti-bilirubin. PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

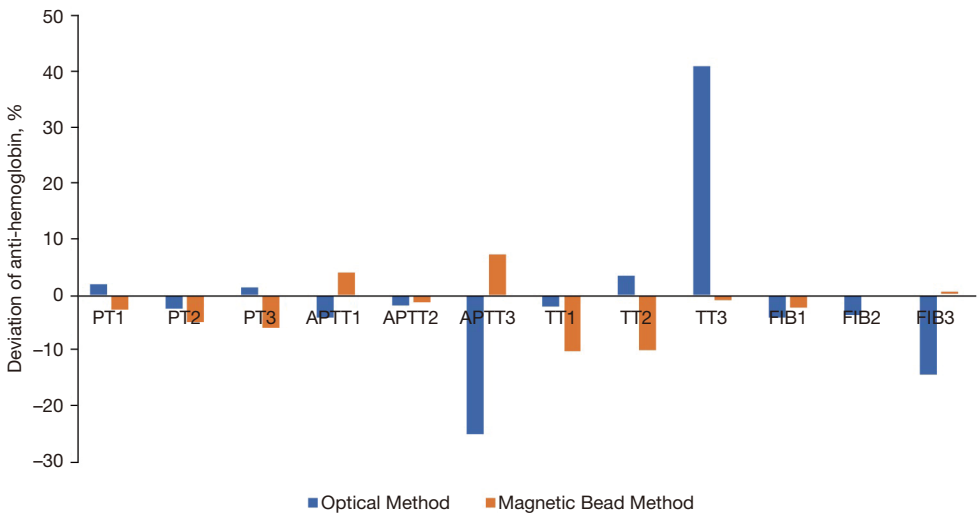

Figure 2 Deviation of anti-hemoglobin. PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

the optical method was generally greater than that of the magnetic bead method, especially the detection results of PT and TT, as shown in *Table 1* and *Figure 1*.

Anti-hemoglobin interference experiment

In the experiment, when hemoglobin was added for interference, the overall deviation of the optical method was greater than that of the magnetic bead method, especially the detection results of PT, APTT, and FIB, as shown in *Table 2* and *Figure 2*.

Anti-lipid interference experiment results

In the experiment, when lipids were added for interference, the overall deviation of the optical method detection result was greater than that of the magnetic bead method, especially the optical method often failed to detect the

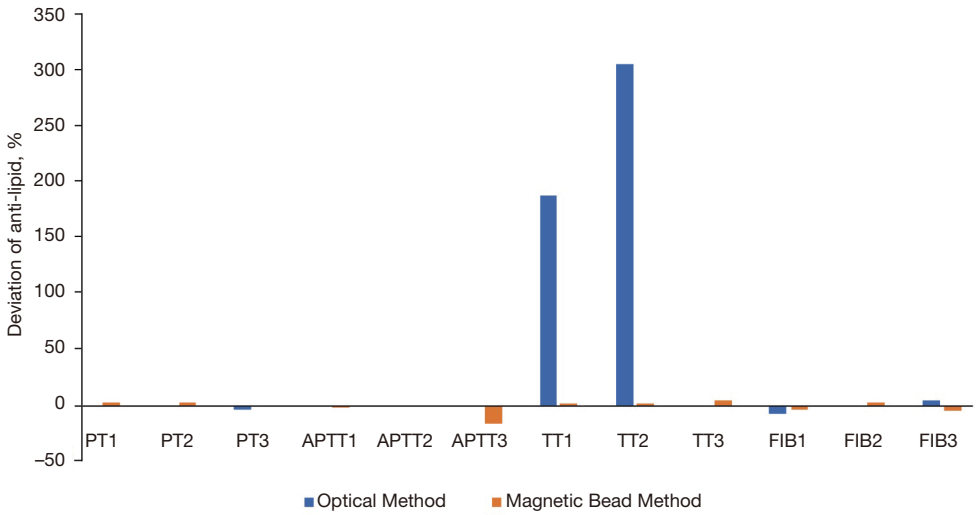

Figure 3 Deviation of anti-lipid. PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen.

specific values, as shown in *Table 3* and *Figure 3* (no tested results were presented as the missing part during drawing).

Discussion

In the anti-interference experiment of PT, APTT, TT, and FIB, after adding interfering substances (bilirubin, hemoglobin, and blood lipid), the overall deviation of the test results and the control results of ExC810 was lower than that of CS5100. However, most of the items failed detection in the results after adding interfering substances in the anti-lipidemia experiment of CS5100. Therefore, based on the current results, it appears that magnetic bead method instruments (such as ExC810) are better than optical instruments (such as CS5100) in anti-jaundice, lipemia, and hemolysis interference and the advantage of anti-lipemia interference is particularly significant.

Currently, there are 2 methods for detecting blood coagulation function—the optical method and magnetic bead method, among which the optical method is easily affected by blood components. In some disease states, blood pressure components often increase abnormally, and these abnormalities often combine with changes in coagulation function. It is more common in clinical practice that bilirubin is significantly increased with abnormal liver function, and coagulation function is decreased in severe cases, while the optical method is susceptible to the influence of bilirubin increase, resulting in inaccurate coagulation function test results. For the same reason, anticoagulation is often required for patients with acute coronary syndrome, whether it is in interventional surgery or the acute phase, and many of these patients have hyperlipidemia, which may also affect the test results of coagulation function. In recent years, the magnetic bead method has been widely used and subjected to in-depth research in sample detection (4,5). While the magnetic bead method mainly considers viscosity changes, the changes in mechanical motion are detected rather than optical changes. Therefore, it is not affected by jaundice, lipids, high concentrations of hemoglobin, and turbidity. The results of the study basically confirmed this viewpoint. In most of the test results, the deviation of the magnetic bead method was lower than that of the optical method, the latter was particularly susceptible to interferences in the detection of TT, it is often impossible to measure the result of TT with the lipid interference especially, and the magnetic bead method showed a better anti-interference ability. Therefore, the magnetic bead method can be preferred in the determination of coagulation function for patients who may have interferences such as the aforementioned jaundice, lipids, hemoglobin, and others.

However, there are some problems in the practical use of the magnetic bead method that should be taken into account. We know that temperature has a certain effect on coagulation function (6). It has been found that the accuracy of the magnetic bead method to detect coagulation function is significantly affected by temperature, and there are some differences in the coagulation function measured

at different temperatures (7). Therefore, the investigators suggested that when coagulation function is detected by the magnetic bead method, the indoor quality control should be established according to different temperatures (8). Secondly, the coagulation function analyzer used for the magnetic bead method has a complicated structure, for which the obtained single value of coagulation endpoint is not conducive to subsequent research. Integrating the results of numerous related studies, it appears that the optimum blood clotting time for magnetic bead detection is 8–25 s. As the clotting factors in platelets will affect the measurement results, the plasma must be platelet-poor; dilution with buffer plasma must be accurate, so that the optimum clotting time is 8–25 s, which is beneficial to FIB detection; reagents must be prepared and used immediately, otherwise the activity of thrombin is likely to be insufficient or disappear, and the clotting time will be prolonged; each laboratory must establish its own standard curve and monitor it closely. If there is any change of conditions, the standard should be re-established.

This study had some limitations. The simulated samples were adapted in the study without verification for performing the comparative tests of patients in clinical practices; moreover, the effects of temperature were not further verified. Future studies should include specific patients (such as patients with abnormally elevated bilirubin, abnormally elevated or decreased hemoglobin, and significantly elevated blood lipids) for comparative studies with the gold standard.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-2713

Data Sharing Statement: Available at https://dx.doi.org/10.21037/apm-21-2713

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-2713). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Foshan First People's Hospital (No. L(2020)23) and informed consent was taken from all the patients.

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