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Interferences in coagulation tests – evaluation of the 570-nm method on the Dade Behring BCS analyser

Ralf Junker[1,2,*,], Margit Käse[1,*], Helmut Schulte[1], Ruth Bäumer[1], Claus Langer[1] and Ulrike Nowak-Göttl[3]**

[1] Institute of Arteriosclerosis Research,
[2] Institute of Clinical Chemistry and Laboratory Medicine,
[3] Paediatric Haematology and Oncology,
University of Münster, Münster, Germany

Abstract

The Dade Behring BCS is a coagulation analyser with optical reaction detection (standard 405 nm). The present study was conducted to evaluate measurement at 570 nm for analyses in interfering plasma samples. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-dimer in normal (n=50), lipaemic (n=60), icteric (n=113), and haemolytic (n=58) samples were measured at 405 and 570 nm. As they are unaffected by the optical properties of the sample, the mechanical STAcompact analyser (Roche Diagnostics) and an ELISA technique were defined as the ''comparison'' methods. The percentage of valid PT results using the 570-nm method varied from 54% (lipaemic samples) to 97% (haemolytic samples). Valid aPTT measurements were found in 67% (lipaemic samples) up to 93% (icteric samples) of samples. Fibrinogen measurement revealed valid results in 58% (lipaemic samples) to 100% (haemolytic samples) of samples. The number of valid D-dimer results varied from 28% (lipaemic material) up to 100% (haemolytic material). Significant inter-method differences were found: aPTT in lipaemic (BCS 405 vs. 570 nm) and icteric samples (STAcompact vs. BCS 405 and 570 nm); fibrinogen in lipaemic (BCS 405 vs. 570 nm), icteric (BCS 405 vs. 570 nm; STAcompact vs. BCS 570 nm) and haemolytic samples (STAcompact vs. BCS 405 and 570 nm). Differences between the BCS 570-nm and the STAcompact methods were in most cases low and less pronounced than between the BCS 570- and 405-nm methods, making the BCS 570-nm method an alternative to measurement at 405 nm. Limitations have to be taken into account regarding lipaemic plasma.

* Both authors contributed equally to this work.
**Corresponding author: Ralf Junker, MD, Abendrothsweg 31, 20251 Hamburg, Germany
Phone: +49-40-46090175, Fax: +49-40-46093045,
E-mail: junker@laboratory-medicine.de

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Introduction

Recent years have seen the development of coagulation analysers, on which clot detection is carried out by measuring changes in optical density or light scatter properties in the reaction chamber. Besides the opportunity to carry out chromogenic or turbidimetric assays simultaneously with clotting tests, advantages include cost containment by simplified reaction chambers and detection systems, and an enhanced sample rate. A major disadvantage of optical detection systems is the potential influence of analytical interferences. In particular, in undiluted samples, optical properties caused by hyperlipidaemia, hyperbilirubinaemia or haemolysis may interfere with optical clot detection (1, 2). Depending on the type of interference and the analysis required, various methods have been reported to minimise the influence of such interferences. These include ultracentrifugation, ultrafiltration, deproteinisation, molecular sieving, extraction of lipids by organic solvents, preincubation with bilirubin oxidase, etc. (3). However, each additional preanalytical step is time-consuming and a possible source of errors. Moreover, these methods themselves may cause a systematic or non-systematic shift in measurement results.

In the BCS analyser (Dade Behring, Marburg, Germany), a fully automated system for processing coagulometric, chromogenic and turbidimetric assays, the detection of fibrin clots or turbidity within the reaction chamber works photometrically or turbidimetrically, respectively (4). Aiming to reduce interferences by optical properties of plasma samples, the BCS enables light transmission to be measured not only at 405 nm, but also at 570 nm. Changing the wavelength should lead to more valid test results, because light transmission at 570 nm is less influenced by extinction values of triglycerides, bilirubin and haemoglobin (Figure 1) (5).

The present study was conducted to compare the BCS 570-nm with the 405-nm method for measurement of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-dimer in lipaemic, icteric and haemolytic plasma samples. As they are unaffected by the optical properties of plasma samples, the mechanical clot detection method of the STAcompact analyser (Roche Diagnostics, Mannheim, Germany) and an ELISA technique were defined as the comparison methods. D-Dimer testing at an alternative wavelength is not recommended by

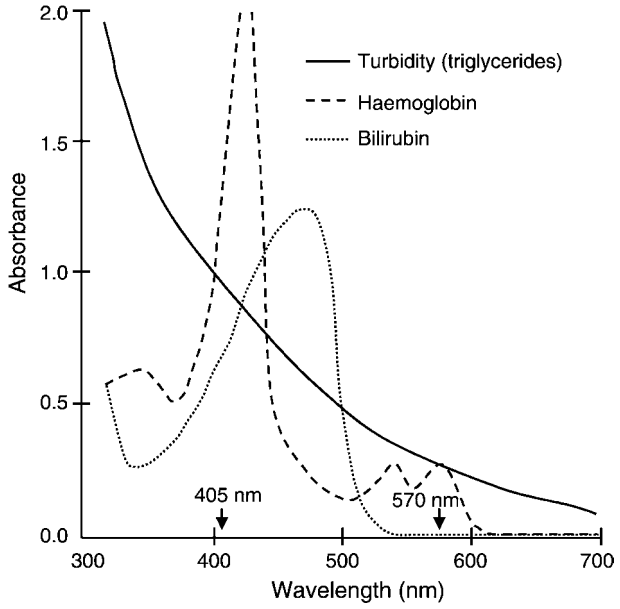

Figure 1 Absorbance spectra of turbidity (triglycerides), bilirubin and haemoglobin (modified according to ref. (5)).

the manufacturer of the test system (Dade Behring) and was therefore carried out on an explorative basis.

Methods

Blood samples

Method comparison was carried out using material remaining from routine laboratory testing. Samples were selected for study purposes if interference was visible (individual decision of the laboratory technician). Blood obtained by venepuncture was collected into plastic tubes containing 1/10 by volume trisodium citrate (0.106 mol/L; Sarstedt, Nümbrecht, Germany). Plasma was separated by centrifugation at room temperature for 15 min at 1400×*g*. On completion of routine laboratory tests, the remaining material was aliquoted into plastic tubes (Nunc, Wiesbaden, Germany) and kept in a −70°C freezer pending further laboratory analyses. Measurements for method comparison were made in one series on completion of blood sample collection.

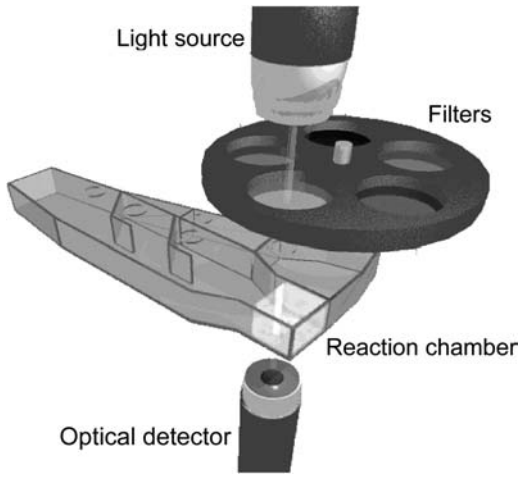

Figure 2 Principle of measurement using the optical detection of the BCS analyser (Figure by Dade Behring).

Coagulation analysers

Measurements were made on the Dade Behring BCS and the Roche STAcompact coagulation analysers. Instrument settings were used as recommended by the manufacturers.

The BCS coagulation analyser is a fully automated coagulation analyser for processing coagulometric, chromogenic and turbidimetric assays. Detection within the reaction chamber works photometrically. The light source of the BCS photometer is a xenon flasher lamp (frequency 40 Hz) with broadband emission. An interference filter with an appropriate main wavelength is swung into the beam of the light source to obtain light of the desired wavelength. The narrowband light generated in this manner is directed at the input of a two-channel light guide. The light is channelled in equal parts into a measurement channel and a reference channel. The lens system of the measurement channel adjusts the path of the light so that the light emitting from a light guide channel is practically parallel when passed through the volume to be measured in the cuvette. Behind the cuvette, stray light is removed by a system of diaphragms. The measurement beam illuminates the detector surface (measurement frequency 2 Hz). The detection wavelength is 405 nm (standard). The light of the second light guide channel goes directly to a second detector. This enables fluctuations in the brightness of the light source to be evened out. During the coagulation process (fibrin clot) as well as in turbidimetric testing (agglutination reaction) the preparation becomes increasingly turbid. In the case of chromogenic assays, a pigment is released during the reaction, and this also reduces the amount of light passing through the cuvette. Thus, on passing through the cuvette, the light beam is weakened due to absorption in the solution or to scattering by particles in the reaction chamber. Measurement results are calculated from changes in light transmission (Figure 2) (6).

To measure light transmission in interference plasma samples, changing the wavelength from 405 nm to 570 nm should lead to more valid test results, because light transmission at 570 nm is less influenced by extinction values of triglycerides, bilirubin and haemoglobin (Figure 1) (5).

In the fully automated STAcompact instrument, the measuring positions are capable of carrying out clotting (mechanical detection) as well as chromogenic (405 nm) and latex-enhanced immunological tests (546 nm). For coagulation methods (mechanical detection, applied in the present study), the change in the amplitude of an oscillating steel ball on the semicircular base of the cuvette is measured (Figure 3A). Clotting is detected by the change in amplitude (Figure 3B). Mechanical clot detection is unaffected by the optical properties of the sample (interference from bilirubin, free haemoglobin and lipids). Therefore, in the present study, the STAcompact system was defined as the reference method for measuring PT, aPTT and fibrinogen (7).

Coagulation assay procedures

Prothrombin time Thromborel S (Dade Behring) was applied for determination of PT. The coagulation process was started by incubation of a plasma sample with thromboplastin and calcium. The time to formation of a fibrin clot was measured. The results in percentage of normal were then derived from a standard curve obtained from measuring PT in diluted standard human plasma. In Thromborel S, thromboplastin was prepared from human placenta (international sensitivity index, 1.01).

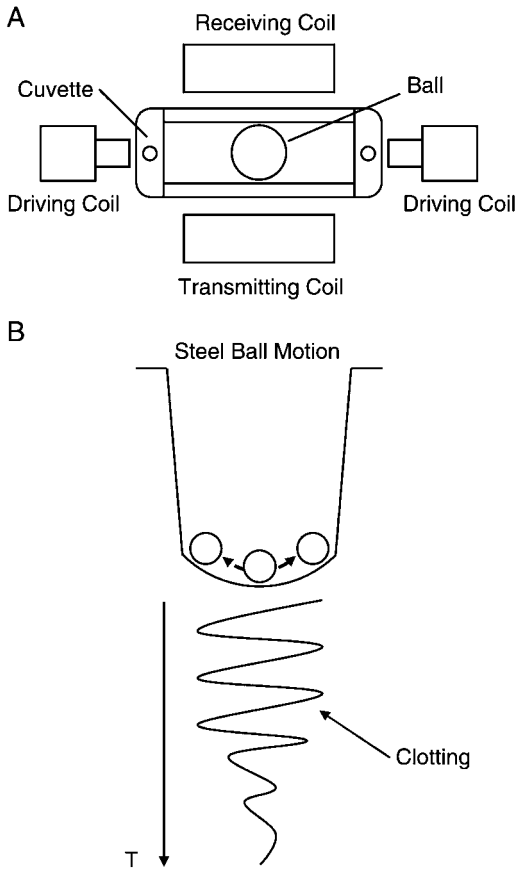

Figure 3 Principle of measurement using the mechanical detection of the STAcompact analyser (10). (A) View from above; (B) frontal view.

Partial thromboplastin time After incubation of a plasma sample with phospholipids and a surface activator (Pathromtin SL; Dade Behring) for activation of the coagulation cascade, calcium was added and the time to fibrin clot formation was measured.

Fibrinogen Fibrinogen was determined using Multifibren U (BCS analyser; Dade Behring), or STA Fibrinogen (STAcompact analyser; Roche Diagnostics). The coagulation process was initiated by adding a large excess of thrombin to the plasma sample. The time taken to form a fibrin clot was measured. The fibrinogen concentration was then calculated from a standard curve prepared by measuring fibrinogen calibrators of known concentrations.

D-Dimer A latex-enhanced turbidimetric test (D-dimer plus; Dade Behring) was used for quantitative determination of D-dimer on the BCS. The agglutination reaction was triggered by a monoclonal antibody against an epitope that occurs twice in one D-dimer molecule. The increase in turbidity was measured as an index of D-dimer agglutinates. The D-dimer concentration was derived from a standard curve prepared by diluting a standard material with known D-dimer concentration. D-dimer testing with the BCS 570-nm method is not recommended by the manufacturer of the test system. Therefore, measurement at an alternative wavelength was carried out on an explorative basis.

The Asserachrom D-dimer kit (Roche Diagnostics) was applied for measurement of D-dimer by a standard ELISA technique. Due to strong sample dilution, this method is virtually unaffected by the optical properties of plasma samples. Thus, in the present study, this ELISA technique was defined as the comparison method for measuring D-dimer.

All tests were carried out according to the manufacturers' instructions. For each parameter, test kits were based on the same reagent batch. A plasma pool and control plasma N and P (Dade Behring) were used as controls for PT, aPTT, and fibrinogen analyses (both on the BCS and on the STA-compact system). For D-dimer measurement on the BCS, D-dimer controls I and II (Dade Behring) were used, and for the D-dimer ELISA method, controls provided with the assay kit (Roche Diagnostics) were applied. Moreover, a plasma pool was used. All controls were measured in each measurement series.

Clinical chemistry

Measurements of triglycerides and total bilirubin were made on a Hitachi 747 autoanalyser, using an enzymatic or colorimetric test, respectively (TG, BIL-T; Roche Diagnostics). Free haemoglobin was measured on a BNII nephelometer (Dade Behring) (8).

Statistical analyses

Linear regression is a method used to describe the relationship between two variables and to predict one variable from another. In contrast to Passing and Bablok regression analysis, which is a procedure with no special assumptions regarding the distribution of samples and the measurement, this procedure should be applied if one of the comparison methods serves as a ''reference'' method. Concerning the main aspect of the present study (i.e., influence of optical interferences on measurement results) the STAcompact analyser with clot detection and the ELISA technique were chosen as ''gold standards'' because both are unaffected by the optical properties of the plasma sample. Moreover, the 405-nm BCS method is a well-evaluated ''standard'' method for coagulation analyses. Thus, linear regression and correlation analyses were used to compare methods instead of Passing and Bablok regression.

Because of the multiple test situation, significance levels were adjusted according to Bonferroni. For each parameter, 12 comparisons were made (BCS 405 nm vs. BCS 570 nm; BCS 405 nm vs. STAcompact/ELISA; BCS 570 nm vs. STA-compact/ELISA; normal, lipaemic, icteric and haemolytic samples). For this purpose, the level of significance was set to $p < 0.004$. For linear regression analyses, $p < 0.004$ indicates a significant deviation of the intercept from 0, whereas for the slope, a significant deviation from 1 is indicated by $p > 0.004$.

To determine any relationship between the level of inter-method differences and levels of interferences (triglycerides, bilirubin and haemoglobin) as well as levels of the measurement results (based on the comparison method), multiple regression analyses were carried out for each comparison showing a significant difference in bivariate analysis for slope, intercept or correlation, revealing partial β- and p-values for interference and level of measurement. Results with the flags ''result invalid'', ''result below/above measurement range'', and ''no clot'' were not considered for method comparison.

All statistical procedures were performed with SPSS (SPSS, Chicago, IL, USA) or MedCalc (MedCalc, Mariakerke, Belgium) software.

Results

The total number of measurements on the BCS and the ranges for measurement results are shown in

Table 1. The distribution of levels of interferences is displayed in Figure 4.

Results were considered not to be valid if one of the following measurement flags appeared on the BCS: ''result invalid''; ''result below/above measurement range''; or ''no clot''. Based on this definition, the overall increase in valid results with the 570-nm method instead of the 405-nm setting ranged from 499 (54.0%) to 771 (85.6%) of 924 measurements in total. For PT measurement, 89 of 231 samples (38.5%) led to a valid result with the 405-nm setting, in contrast to 197 (85.3%) valid results with the 570-nm method. Of 231 aPTT measurements, 123 (53.2%; 405 nm) and 198 (85.7%; 570 nm) produced a valid result. Fibrinogen measurements were valid in 197 cases (85.7%) with the 570-nm method, compared with 143 (62.2%) valid results using the 405-nm method. For D-dimer, valid results increased from 143 (61.9%; 405 nm) to 178 (77.1%; 570 nm) of 231 measurements in total (Table 2).

In most cases, method comparison revealed no significant inter-method difference. Results are shown in detail in Tables 3 and 4.

Results for controls and pooled plasma were within the range of expected values as given by the manufacturers. The inter-assay coefficient of variation calculated from the measurement results of control material and pooled plasma varied between 1.4 and 12.8%.

Discussion

The results presented here demonstrate that changing the detection wavelength from 405 to 570 nm on the BCS analyser leads to a marked increase in valid results and thus can further improve optical measurement of PT, aPTT, fibrinogen and D-dimer in interfering plasma samples. From a total of 231 measurements, the overall increase in valid results using the 570-nm method varied from 35 (D-dimer, from 143 to 178 results) to 108 (PT, from 89 to 197 results) (Table 2).

Significant inter-method differences were found in some cases. As inter-method differences varied for different parameters and different interferences, ''overall'' data interpretation is difficult. Thus, routine application of the 570-nm method in the laboratory requires detailed knowledge of the limitations of the method, even in the context of interfering plasma.

PT measurement did not lead to any significant inter-method difference (Table 3A). In contrast, aPTT measurement in interfering plasma samples in some cases produced significantly different results using the BCS 405-nm, BCS 570-nm and STAcompact methods. However, differences between measurement results of the BCS 570-nm method and the STAcompact were low and were less pronounced than differences between the BCS 570- and 405-nm methods.

Table 1 Measurement range. Data given apply to plasma samples measured on the BCS analyser at the 405-nm setting.

Parameter	Measurement range
Normal samples (n = 50)	
PT, %	18.8–105.9
aPTT, s	30.3–106.0
Fibrinogen, g/L	2.21–11.4
D-dimer, μg/L	0.073–0.629
Lipaemic samples (n = 60)	
Triglycerides, mmol/L	1.73–67.5
PT, %	20.2–122.3
aPTT, s	17.6–152.1
Fibrinogen, g/L	0.81–11.83
D-dimer, μg/L	0.050–1.02
Icteric samples (n = 113)	
Bilirubin, μmol/L	5.13–927.0
PT, %	11.8–90.8
aPTT, s	19.2–146.2
Fibrinogen, g/L	1.12–11.4
D-dimer, μg/L	0.05–5.11
Haemolytic samples (n = 58)	
Free haemoglobin, g/L	0.30–4.70
PT, %	9.3–107.6
aPTT, s	28.2–109.3
Fibrinogen, g/L	1.39–11.4
D-dimer, μg/L	0.12–1.65

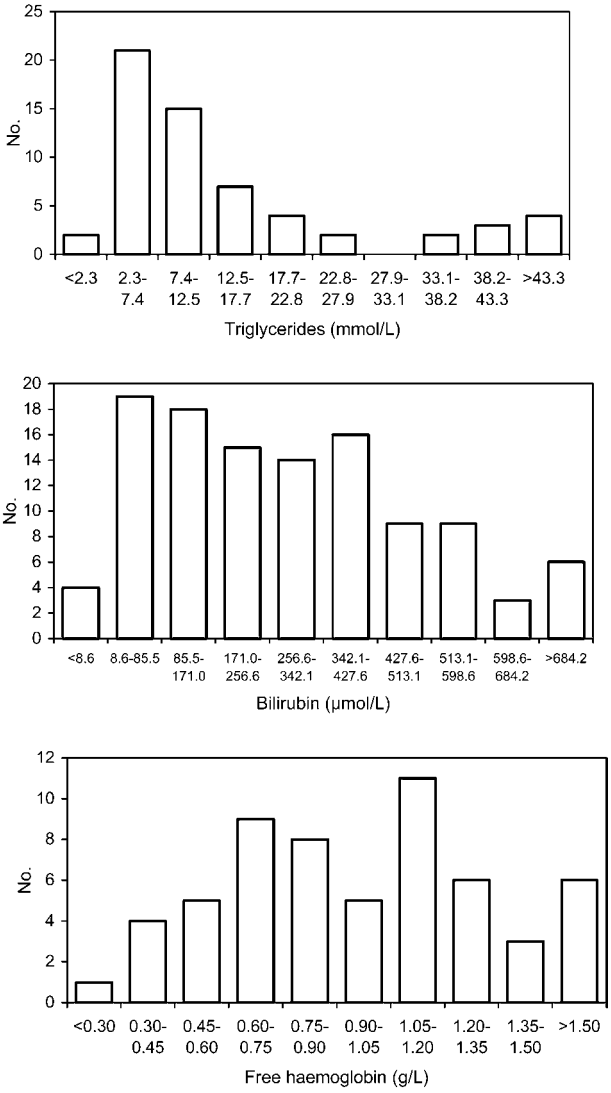

Figure 4 Distribution of levels of interferences investigated.

Table 2 Number and percentage of valid measurement results using the BCS 405- and 570-nm settings.

Parameter	Valid results		
	Lipaemic samples (n=60)	Icteric samples (n=113)	Haemolytic samples (n=58)
PT			
BCS 405 nm	13 (22%)	36 (32%)	40 (69%)
BCS 570 nm	38 (63%)	103 (91%)	56 (97%)
aPTT			
BCS 405 nm	15 (25%)	62 (55%)	46 (79%)
BCS 570 nm	40 (67%)	105 (93%)	53 (91%)
Fibrinogen			
BCS 405 nm	19 (32%)	72 (64%)	53 (91%)
BCS 570 nm	35 (58%)	105 (93%)	58 (100%)
D-dimer			
BCS 405 nm	10 (17%)	81 (72%)	52 (90%)
BCS 570 nm	17 (28%)	103 (91%)	58 (100%)

Table 3A Results of linear regression and correlation analysis between different methods: prothrombin time (unit of measurement, %).

		BCS 570 nm, Thromborel S		BCS 405 nm, Thromborel S	
		Result	p	Result	p
Normal samples					
BCS 405 nm,	n	50		–	
Thromborel S	Slope	0.97	<0.001	–	–
	Intercept	1.24	NS	–	–
	r	0.97	<0.001	–	–
STAcompact,	n	50		50	
Thromborel S	Slope	1.09	<0.001	1.13	<0.001
	Intercept	0.16	NS	−1.59	NS
	r	0.95	<0.001	0.98	<0.001
Lipaemic samples					
BCS 405 nm,	n	30		–	
Thromborel S	Slope	0.82	<0.001	–	–
	Intercept	14.2	NS	–	–
	r	0.84	<0.001	–	–
STAcompact,	n	58		31	
Thromborel S	Slope	0.85	<0.001	0.87	<0.001
	Intercept	8.05	NS	11.2	NS
	r	0.62	<0.001	0.83	<0.001
Icteric samples					
BCS 405 nm,	n	63		–	
Thromborel S	Slope	0.93	<0.001	–	–
	Intercept	0.46	NS	–	–
	r	0.93	<0.001	–	–
STAcompact,	n	108		62	
Thromborel S	Slope	1.10	<0.001	1.16	<0.001
	Intercept	0.07	NS	−0.32	NS
	r	0.90	<0.001	0.95	<0.001
Haemolytic samples					
BCS 405 nm,	n	49		–	
Thromborel S	Slope	0.98	<0.001	–	–
	Intercept	4.21	NS	–	–
	r	0.97	<0.001	–	–
STAcompact,	n	55		48	
Thromborel S	Slope	1.19	<0.001	1.15	<0.001
	Intercept	4.17	NS	3.19	NS
	r	0.96	<0.001	0.95	<0.001

r, correlation coefficient.

Table 3B Results of linear regression and correlation analysis between different methods: activated partial thromboplastin time (unit of measurement, s).

		BCS 570 nm, Pathromtin SL		BCS 405 nm, Pathromtin SL	
		Result	p	Result	p
Normal samples					
BCS 405 nm,	n	50		–	
Pathromtin SL	Slope	1.03	<0.001	–	–
	Intercept	−1.12	NS	–	–
	r	0.99	<0.001	–	–
STA compact,	n	50		50	
Pathromtin SL	Slope	1.00	<0.001	0.97	<0.001
	Intercept	−4.27	NS	−3.03	NS
	r	0.98	<0.001	0.99	<0.001
Lipaemic samples					
BCS 405 nm,	n	35		–	
Pathromtin SL	Slope	0.37	<0.001	–	–
	Intercept	34.9	0.001	–	–
	r	0.55	<0.001	–	–
STA compact,	n	54		36	
Pathromtin SL	Slope	0.78	<0.001	0.73	NS
	Intercept	12.3	NS	19.2	NS
	r	0.74	<0.001	0.42	NS
Icteric samples					
BCS 405 nm,	n	88		–	
Pathromtin SL	Slope	0.93	<0.001	–	–
	Intercept	10.4	NS	–	–
	r	0.81	<0.001	–	–
STA compact,	n	102		87	
Pathromtin SL	Slope	0.83	<0.001	0.56	<0.001
	Intercept	6.86	0.001	21.0	<0.001
	r	0.95	<0.001	0.78	<0.001
Haemolytic samples					
BCS 405 nm,	n	49		–	
Pathromtin SL	Slope	1.07	<0.001	–	–
	Intercept	−2.09	NS	–	–
	r	0.99	<0.001	–	–
STA compact,	n	52		48	
Pathromtin SL	Slope	0.98	<0.001	0.91	<0.001
	Intercept	−1.84	NS	0.72	NS
	r	0.95	<0.001	0.97	<0.001

r, correlation coefficient.

Thus, the BCS 570-nm method is best suited for measuring interfering plasma samples (Table 3B). In measuring fibrinogen in interfering plasma samples, absolute differences between the BCS 570-nm and the STAcompact methods appeared to be low or not significant, making the BCS 570-nm method a good alternative to measuring at 405 nm. Moreover, the finding of differences between the BCS 405- and 570-nm methods provides further evidence of inadequate measurement of fibrinogen in lipaemic plasma samples by the BCS 405-nm method. In the present study, different reagents were used for measuring fibrinogen on the BCS and STAcompact analysers. Because no significant inter-method difference was apparent for normal samples, any influence of the reagent can be excluded (Table 3C).

In general, for PT, aPTT and fibrinogen measurements, correlation coefficients were lowest in lipaemic plasma samples. When comparing the BCS 405-nm with the 570-nm and the STAcompact methods, this may reflect the strong influence of interferences, which were lower using the latter methods. However, especially in some cases of lipaemic samples, inter-method differences were apparently higher when comparing the BCS 570-nm method with the STAcompact, than when comparing the 405-nm method with the STAcompact as indicated not only by poor correlation, but also by the slope and intercept of the regression line. This was mainly due to some inter-method differences that could not be found applying the BCS 405-nm method, as at this wavelength the corresponding plasma samples did not reveal a valid result.

In some of the comparisons, the inter-method difference may have clinical relevance without reaching the level of significance. As a clear cut-off for clinical relevance cannot be given, the relevance of these differences remains a matter of debate.

In only one case (aPTT measurement in lipaemic samples, comparing BCS 405 nm and STAcompact),

Table 3C Results of linear regression and correlation analysis between different methods: fibrinogen (unit of measurement, g/L).

		BCS 570 nm, Multifibren U		BCS 405 nm, Multifibren U	
		Result	p	Result	p
Normal samples					
BCS 405 nm,	n	50		–	
Multifibren U	Slope	0.95	<0.001	–	–
	Intercept	0.225	NS	–	–
	r	0.99	<0.001	–	–
STA compact,	n	50		50	
STA Fibrinogen	Slope	1.24	<0.001	1.29	<0.001
	Intercept	−0.585	NS	−0.783	NS
	r	0.95	<0.001	0.94	<0.001
Lipaemic samples					
BCS 405 nm,	n	36		–	
Multifibren U	Slope	0.47	0.001	–	–
	Intercept	2.31	0.001	–	–
	r	0.55	<0.001	–	–
STA compact,	n	57		37	
STA Fibrinogen	Slope	1.10	<0.001	1.05	<0.001
	Intercept	−0.842	NS	−0.218	NS
	r	0.79	<0.001	0.62	<0.001
Icteric samples					
BCS 405 nm,	n	99		–	
Multifibren U	Slope	0.85	<0.001	–	–
	Intercept	0.569	0.026	–	–
	r	0.86	<0.001	–	–
STA compact,	n	102		95	
STA Fibrinogen	Slope	1.25	<0.001	1.10	<0.001
	Intercept	−0.628	<0.001	0.065	NS
	r	0.95	<0.001	0.85	<0.001
Haemolytic samples					
BCS 405 nm,	n	56		–	
Multifibren U	Slope	0.96	<0.001	–	–
	Intercept	0.121	NS	–	–
	r	0.98	<0.001	–	–
STA compact,	n	57		55	
STA Fibrinogen	Slope	1.38	<0.001	1.39	<0.001
	Intercept	−1.42	<0.001	−1.34	0.002
	r	0.93	<0.001	0.92	<0.001

r, correlation coefficient.

did inter-method differences correlate with the level of interference (triglycerides). Therefore, a clear cut-off for an interference level, necessitating a change of methodology, was not recorded.

We would like to emphasise that D-dimer testing with an alternative wavelength is not recommended by the manufacturer of the test system. Therefore, the BCS 570-nm method for measurement of D-dimer was carried out on an explorative basis. In measuring PT, aPTT and fibrinogen, the detection of fibrin clots is defined as the end point. In contrast, D-dimer measurement leads to an increase in turbidity on the formation of D-dimer agglutinates. One reason for the poor increase in valid results with the 570-nm method, compared to the 405-nm methods, may be because turbidity due to such agglutinates is less pronounced than turbidity due to fibrin clots. In normal plasma samples, significant differences between D-dimer results were recorded when comparing results from the ELISA method with both the BCS 405- and 570-nm methods. As the BCS methods did not differ from each other, this reflects a systematic shift between the BCS turbidimetric measurement and the ELISA method. For lipaemic samples, the low slope of the regression line for comparison of the BCS and ELISA methods indicates an underestimation of D-dimer when using the BCS methods. This may be explained by a reduction in interference from the washing steps used in the ELISA method, in which a limitation of the optical detection methods could be shown (Table 3D). Comparison studies of D-dimer measurement have normally led to the conclusion that assays differ strongly from each other (9, 10). Therefore, testing D-dimer using an alternative assay system is critical. Besides, sample dilution cannot generally be recommended, as this would cause a shift of the lower detection limit and would therefore lead to lower assay sensitivity.

Summarising these results, the number of valid results increased on using the alternative wavelength.

Table 3D Results of linear regression and correlation analysis between different methods: D-dimer (unit of measurement, μg/L).

		BCS 570 nm, D-dimer plus		BCS 405 nm, D-dimer plus	
		Result	p	Result	p
Normal samples					
BCS 405 nm,	n	50		–	
D-dimer plus	Slope	0.91	<0.001	–	–
	Intercept	0.006	NS	–	–
	r	1.00	<0.001	–	–
ELISA,	n	50		50	
Enzygnost D-dimer	Slope	0.10	<0.001	0.10	<0.001
	Intercept	0.113	<0.001	0.114	<0.001
	r	0.71	<0.001	0.73	<0.001
Lipaemic samples					
BCS 405 nm,	n	44		–	
D-dimer plus	Slope	0.60	<0.001	–	–
	Intercept	0.043	NS	–	–
	r	0.60	<0.001	–	–
ELISA,	n	56		44	
Enzygnost D-dimer	Slope	0.07	NS	0.05	NS
	Intercept	0.078	NS	0.142	NS
	r	0.29	NS	0.20	NS
Icteric samples					
BCS 405 nm,	n	100		–	
D-dimer plus	Slope	1.07	<0.001	–	–
	Intercept	−0.065	NS	–	–
	r	0.94	<0.001	–	–
ELISA,	n	75		71	
Enzygnost D-dimer	Slope	0.40	0.002	0.42	<0.001
	Intercept	−0.132	NS	−0.080	NS
	r	0.36	0.001	0.43	<0.001
Haemolytic samples					
BCS 405 nm,	n	56		–	
D-dimer plus	Slope	0.96	<0.001	–	–
	Intercept	−0.0148	NS	–	–
	r	0.99	<0.001	–	–
ELISA,	n	57		57	
Enzygnost D-dimer	Slope	0.34	<0.001	0.31	<0.001
	Intercept	−0.151	NS	−0.060	NS
	r	0.64	<0.001	0.59	<0.001

r, correlation coefficient.

The few other studies published on this issue give further support to the conclusion that optical detection with an appropriate wavelength is an adequate method for coagulation analysers (11–13). However, in a number of cases, flagging of the measurement result persisted. In these cases, a second sample without interference has to be tested if available (e.g., blood collection in a fasting state) or standard methods such as ultracentrifugation or measurement on a mechanical clot-detection system may be necessary. A suggested laboratory flow chart for optical clot detection in interfering plasma samples is displayed in Figure 5.

In the university hospital in which this study was carried out, the number of interfering plasma samples requiring a change of wavelength is 0.5% at most. Of these samples, approximately two-thirds reveal a valid result using the 570-nm method. For most of the remaining samples, another sample with optimised preanalytical conditions can be obtained. Thus, the necessity of ultracentrifugation or measurement with mechanical clot detection is low.

Another approach would be routine measurement with the BCS 570-nm method instead of at 405 nm. The main advantage of measuring light transmission at 405 nm is the higher sensitivity in detecting slight changes in light transmission. In contrast, the 570-nm setting is less influenced by interferences, such as lipaemia, bilirubinaemia and haemolysis. Therefore, in routine laboratory use the standard setting is 405 nm (high sensitivity) and only in specific cases should the wavelength be changed to 570 nm. In the present study, precision data for control material indicate the possibility of applying the 570-nm wavelength instead of 405 nm (data not shown). However, a complete system evaluation would be necessary before recommending this procedure.

Analyses carried out on lipaemic plasma samples led to the most pronounced inter-method differences. This reflects the strong influence of lipaemia on light

Table 4 Results of multiple regression analyses.

		BCS 570 nm		BCS 405 nm	
		Partial β	p	Partial β	p
aPTT					
BCS 405 nm (lipaemic samples)	Level of measurement	−0.837	<0.001		
	Triglycerides	0.159	NS		
STA compact (lipaemic samples)	Level of measurement			−0.290	NS
	Triglycerides			0.513	0.002
STA compact (icteric samples)	Level of measurement	−0.553	<0.001	−0.661	<0.001
	Bilirubin	0.065	NS	−0.122	NS
Fibrinogen					
BCS 405 nm (lipaemic samples)	Level of measurement	0.010	<0.001		
	Triglycerides	−0.228	NS		
STA compact (icteric samples)	Level of measurement	0.495	<0.001		
	Bilirubin	−0.139	NS		
STA compact (haemolytic samples)	Level of measurement	0.556	<0.001	0.541	<0.001
	Haemoglobin	−0.102	NS	−0.071	NS
D-dimer					
ELISA (icteric samples)	Level of measurement	−1.000	<0.001	−1.000	<0.001
	Haemoglobin	<0.001	NS	<0.001	NS

Partial β- and the corresponding p-values are given for influence of measurement level and interference level on inter-method differences. Multiple regression analysis was carried out only if a significant inter-method difference was apparent in bivariate regression and correlation analyses.

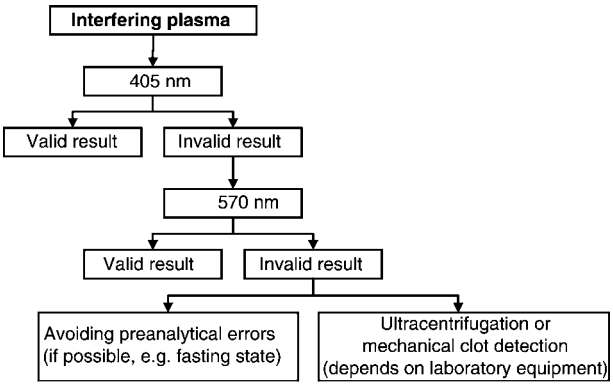

Figure 5 Suggested laboratory flow chart for optical clot detection in interference plasma samples.

absorption, not only at 405 nm, but also at 570 nm. Therefore, some limitations have to be taken into account, especially regarding lipaemic plasma samples. Routine application of the 570-nm method in the laboratory requires detailed knowledge of these limitations.

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