International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

Effects of haemolysis, icterus and lipaemia on coagulation tests as performed on Stago STA-Compact-Max analyser

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doi:10.1111/ijlh.12498

Received 6 January 2016;
accepted for publication 18 March 2016

Keywords
Coagulation, HIL, mechanical endpoint, APTT, PT

SUMMARY

Introduction: Haemolysis, icterus and lipaemia (HIL) may affect haemostasis test results. This may be influenced by the level of interfering substance and the reagents and end-point detection system used.

Methods: We assessed the influence of HIL on prothrombin time, activated partial thromboplastin time and fibrinogen assay using a viscosity-based detection analyser.

Results: Spontaneous haemolysis that occurred during sample collection and processing had no effect on PT with either a rabbit tissue factor extract or recombinant human tissue factor reagents. In contrast, addition of mechanically haemolysed cells impacted on PT for the highest haemoglobin concentration.

For APTTs determined with STA®-Cephascreen® reagent, there was no significant difference between results in haemolysed and nonhaemolysed samples. For the other two reagents studied, APTTs were statistically significantly shorter in haemolysed samples compared with nonhaemolysed samples. This bias was clinically significant only for STA®-PTT Automate. For all three APTT reagents, the impact of haemolysis was sufficient to impact patient management decisions, and in some samples, the effects of lipaemia and icterus were not clinically significant.

Conclusion: Overall, our results confirm that PT and fibrinogen were not clinically significantly affected by HIL. The APTTs of some haemolysed samples were falsely normal. Haemolysed samples for APTT determination should be rejected.

INTRODUCTION

Laboratory diagnosis is more and more prominent in modern medicine: it is commonly accepted that approximately 70% of all medical decisions are based on the laboratory results. Accurate results are therefore key for appropriate diagnosis. Limiting errors is hence one of the major objectives of clinical laboratories. Sources of errors can occur in the different steps of a test, that is pre-analytics, analytics and post-analytics. The importance of pre-analytics has been emphasized in past years with between one-third and

three quarters of laboratory errors being attributable to this phase [1]. Haemolysis, icterus and lipaemia (HIL) in patients' specimen may interfere in the measurement of many analytes, including coagulation parameters. *In vitro* haemolysis which occurs during sample collection transport or processing is a particular problem as it is the most common source of interference in plasma samples [2].

This possible interference can be influenced by several factors such as (i) the level of interfering substance in plasma, (ii) the assay principle and (iii) the end-point detection system, that is optical versus viscosity-based detection system (mechanical detection).

One of the requirements for a clinical laboratory is that common interferences related to sample integrity such as haemolysis, icterus and lipaemia be evaluated with each reagent system. Because of limited resources and budgetary constraints, the clinical laboratory relies on the manufacturer to document HIL estimates. The clinical relevance of the observed bias should be reviewed [3].

In this study, the authors describe a study conducted to assess the influence of haemolysis, icterus and lipaemia on the results of three routine coagulation assays, namely prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen assay performed with different reagents on a viscosity-based detection analyser. The effects of spontaneous *in vitro* haemolysis on clotting tests has been studied by analysis of rejected patient samples where haemolysis is judged to have occurred in the samples but is not present *in vivo* (4) or more commonly following addition of cell lysate to plasma *in vitro* [5].

As the cause of haemolysis in rejected patient samples may be multifactorial and it is possible that addition of lysate to plasma may not replicate all the effects of haemolysis in rejected patient samples, both methods were studied here.

MATERIALS AND METHODS

Sample collection

Blood samples were collected in 0.109M/3.2% trisodium citrate anticoagulant (BD VacutainerTM Plus, Becton Dickinson, Franklin Lakes, NJ, USA) in the proportion 1 volume of citrate to 9 volumes of blood and processed in accordance with the CLSI Guideline H03-A6 'Procedures for the collection of diagnostic blood specimens by venipuncture' and with the CLSI guideline H21-A5 'Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular haemostasis assays'[6] by centrifugation at room temperature for 15 min at 2000 g.

All experiments were carried out on residual material remaining after the completion of any diagnostic tests in accordance with local ethical committee requirements at the site where samples were collected.

Haemolysis interference

Interference of haemolysis was studied in two different ways: spontaneous *in vitro* haemolysis judged to have occurred during sample collection transport or processing and spurious haemolysis.

Spontaneous haemolysis

This part of the haemolysis study is based on the comparison of assay results in rejected patient samples, received haemolysed samples by the investigating laboratory and a replacement nonhaemolysed sample obtained from the same patient within 4 h of the first (i.e. haemolysed) sample. The absence of haemolysis in the replacement samples was taken as an indication that the haemolysis had occurred *in vitro*.

The level of haemolysis was semiquantitatively estimated based on the measurement of haemoglobin concentration using a XN-10™ (Sysmex, Kobe, Japan). The levels of plasma haemoglobin were at levels substantially below those present in whole blood samples, and due to the lack of precision of haematology counters for such low haemoglobin concentrations, numbers were converted into semiquantitative units defined as follows:

- < 0.3 g/L: no significant haemolysis
- 0.3 – 1.0 g/L: (+)
- 1.1 – 3 g/L: (++)
- > 3.0 g/L: (+++)

This semiquantitative scale is in agreement with equivalent semiquantitative scales proposed by others [2, 3].

Spurious/Mechanical haemolysis

Nonhaemolysed plasma samples referred to the laboratory covering a wide range of PT (12 to 50 s), APTT (30 to 80 s) and fibrinogen concentrations (1 to 8 g/L) were selected to prepare plasma pools of a minimal volume of 8 mL. All samples had to be pooled within 4 h of sample collection.

Each pool was divided into four 2 mL-aliquots: equal volumes of packed red cells were added to each aliquot after which one sample (H0) was kept nonhaemolysed (reference), whereas incremental haemolysis was artificially created in the three other aliquots (H1, H3 and H5) by aspiration of anticoagulated blood (1 time, 3 times and 5 times, respectively) through a fine needle connected with an insulin syringe. Aliquots were then centrifuged before immediate testing for plasma haemoglobin, PT, APTT and fibrinogen.

Icterus interference

Leftover plasma samples without icterus covering a wide range of PT (12 to 50 s), APTT (30 to 50 s) and fibrinogen (1 to 8 g/L) were selected to constitute plasma pools of a minimal volume of 8 mL. All samples had to be pooled within 4 h of sample collection.

From each pool, four aliquots of 2 mL were prepared, B0, B5, B20 and B40 spiked with exogenous bilirubin (Sigma-Aldrich, Saint Louis, MO, USA) to achieve a final bilirubin concentration of 0, 5, 20 and 40 mg/dL, respectively, and then tested for PT, APTT and fibrinogen. Spiking was performed in such a manner that spiking-induced pool dilution was identical for the four bilirubin concentrations.

Lipaemia interference

Leftover plasma samples without lipaemia covering a wide range of PT (12 to 50 s), APTT (30 to 50 s) and fibrinogen (1 to 8 g/L) were selected to constitute plasma pools of a minimal volume of 8 mL. All samples had to be pooled within 4 h of sample collection.

From each pool, four aliquots of 2 mL were prepared, L0, L200, L500 and L1000 spiked with an exogenous triglyceride standard solution (Lipofundin S 20%, B. Braun SpA, Milan, Italy) to achieve a final concentration of 0, 200, 500 and 1000 md/dL, respectively, and then tested for PT, APTT and fibrinogen. Spiking was performed in such a manner that spiking-induced pool dilution was identical for the four triglyceride concentrations.

Coagulation assays

All the assays were performed using an STA-Compact-Max® analyser (Stago, Asnières sur Seine, France).

All reagents were from Stago (Asnières sur Seine, France).

All assays were performed according to the manufacturer's instructions.

All tests were run in duplicate, except for the paired haemolysed samples that were run in singlicate as volume of some samples was insufficient to perform tests in duplicates.

PT

Two different PT reagents were used in parallel:

- STA®-Neoplastine CI Plus 10, rabbit brain thromboplastin,
- STA®-Neoplastine R, human recombinant thromboplastin.

APTT

Three different APTT reagents were used in parallel:

- STA®-PTT Automate® 5, cephalin prepared from rabbit cerebral tissue + silica activator
- STA®-Cephascreen® 10, cephalin prepared from rabbit cerebral tissue + polyphenolic activator,
- STA®-C.K. Prest® 5, cephalin prepared from rabbit cerebral tissue + kaolin activator

Fibrinogen

One reagent, STA®-Liquid Fib, titrated human calcium thrombin, was used for the measurement of fibrinogen.

Statistics

The main objective of the study was to check for possible clinically significant interferences. The main statistical analyses were therefore based on equivalence

tests. Difference tests were also performed, as secondary analyses.

Equivalence test was performed to evaluate the clinical relevance of potential statistical difference. This relies on clinical acceptance criteria determined according to the CLSI C56-A guideline [3]. Accordingly, a clinically significant interference is evidenced when the test result in the presence of the interfering substance differs more than $1.96 \times (CVa2 + CVw2)1/4$ from the result without the interfering substance (CVa is the analytical coefficient of variation, and CVw is the within-subject biological variation) [7]. Briefly, CVa was given by the manufacturer; it corresponds to the intraassay variability of each reagent. CVw has been determined as the mean of observed biological variation published in three different papers [8–10].

In these conditions, the maximum acceptable bias (MAB) was determined as follows:

- PT: 8.6%,
- APTT: 9.2%,
- Fibrinogen: 16.7%.

Equivalence analyses were based on the confidence interval method; that is, the equivalence was significant if the 95% confidence interval of the CV was included in the equivalence interval, computed as [− MAB; + MAB]. CV and their 95% confidence intervals were computed from means and standard errors estimated by ANOVAs followed by Dunnett's tests for all the analyses except the one of spontaneous *in vitro* haemolysis. Spontaneous haemolysis was coded as a binary variable (i.e. presence/absence) and it was analysed by paired Student's t-tests.

RESULTS

Haemolysis interference

Haemolysed paired samples

Forty pairs of samples (spontaneous *in vitro* haemolysis versus nonhaemolysed) were available and tested with all the reagents for PT, APTT and fibrinogen.

Haemolysis distribution across samples was as follows:

- (+): 15 samples,
- (++): 19 samples,
- (+++): 6 samples.

Table 1 and Figures 1–6 depict the results obtained for the different assays.

No significant difference was observed between the results obtained in haemolysed versus nonhaemolysed sample groups with both PT reagents, namely STA®-Neoplastine CI Plus and STA®-Neoplastine R, and for fibrinogen measured using the STA®-Liquid Fib reagent.

Table 1. Summary of results obtained for nonhaemolysed versus haemolysed paired samples. Rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection and processing were analysed and results compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other

		PT (s)		APTT (s)			Fibrinogen (g/L)
		STA®-Neoplastine® CI Plus	STA®-Neoplastine® R	STA®-PTT Automate®	STA®-Cephascreen®	STA®-C.K. Prest®	STA®-Liquid Fib
Nonhaemolysed	Min	11.9	12.9	29.5	25.0	25.1	1.94
	Max	32.8	63.2	99.7	60.6	67.1	9.45
	Mean	16.6	20.3	44.4	36.8	32.5	5.22
	Median	14.3	16.6	40.2	35.2	30.2	5.38
Haemolysed	Min	12.0	12.3	23.1	21.8	20.8	1.84
	Max	36.5	68.8	79.3	62.9	65.9	9.91
	Mean	16.6	20.7	41.6	36.2	31.5	5.38
	Median	14.2	16.6	38.6	34.1	29.3	5.02

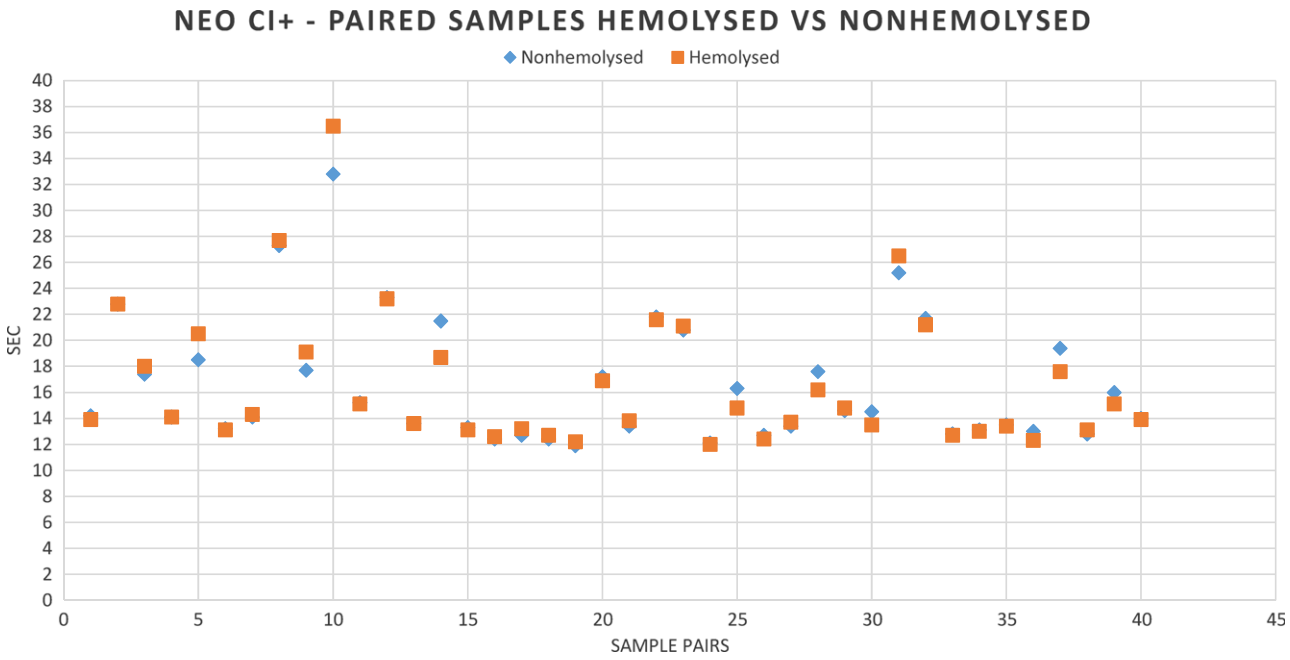

Figure 1. Results obtained for nonhaemolysed versus haemolysed paired samples for PT (STA®-Neoplastine® CI Plus). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

In contrast, APTT was statistically significantly shorter in haemolysed versus nonhaemolysed samples with two test reagents, STA®-PTT Automate® and STA®-C.K. Prest® ($P = 0.0289$ and 0.0382 respectively), but not with STA®-Cephascreen®. However, this difference was demonstrated to be not clinically significant for STA®-C.K.Prest®.

Results of clinical relevance analysis in paired samples for haemolysis are displayed in Table 2.

No correlation was observed between the level of haemolysis and the test result variation for all assays whatever the reagent used (data not shown).

Spurious/mechanical haemolysed samples

No statistical difference was observed between H1 and H3 versus H0 pools for PT measured using either STA®-Neoplastine® CI Plus or STA®-Neoplastine® R. In contrast, PT was statistically different between H5 and H0 ($P = 0.0117$ and $P = 0.0001$ for STA®-Neoplastine® CI Plus and STA®-Neoplastine® R, respectively). However, these differences were demonstrated to be not clinically significant.

APTT was not significantly impacted by haemolysis regarding H1 versus H0 whatever the reagent used. APTT was significantly shorter for H3 and H5 with STA®-PTT Automate® ($P = 0.0024$ for H3 and 0.0014 for H5). However, these differences were demonstrated to be not clinically significant. In contrast, no statistically significant difference was observed for the different levels of mechanical haemolysis when APTT was measured using either STA®-Cephascreen®, or STA®-C.K.Prest®. Provoked haemolysis had no significant impact on the fibrinogen assay result using the STA®-Liquid Fib reagent.

Clinical relevance analysis for mechanical haemolysis is depicted in Table 3.

Icterus interference

Icterus had no statistically significant impact on PT measured using STA®-Neoplastine CI Plus, or STA®-Neoplastine R.

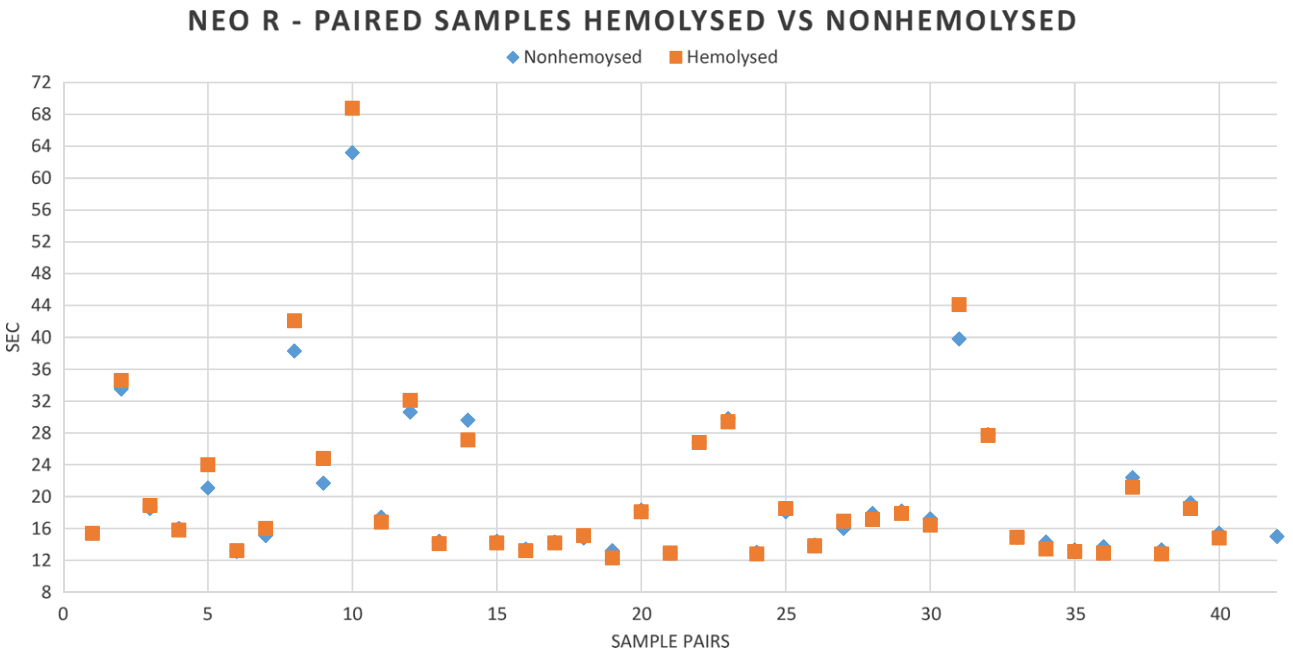

Figure 2. Results obtained for nonhaemolysed versus haemolysed paired samples for PT (STA®-Neoplastine® R). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement non-haemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

Regarding APTT, a statistically significant difference was observed between the B5 pools and the B0 reference pools ($P = 0.0050$), but not between the B20 and B40 versus B0 pools when APTT was measured using STA®-PTT Automate reagent. No significant impact of icterus was observed for APTT measured using STA®-Cephascreen® reagent, whatever the level of icterus was (B5, B20 or B40 versus B0). Finally, only the lowest level of icterus (B5) had no impact on APTT measured using STA®-C.K.Prest® reagent, while B20 and B40 spiked pools significantly differed from B0 with this reagent ($P = 0.0001$ for B20 and $P < 0.0001$ for B40). However, these differences were demonstrated to be not clinically significant.

Icterus did not significantly impact the fibrinogen assay result at the two lowest bilirubin concentrations (B5 and B20). However, fibrinogen assay result was impacted for the highest tested bilirubin concentration (for B40 $P = 0.0015$). Nevertheless, these differences were demonstrated to be not clinically significant.

Clinical relevance analysis for icterus interference is depicted in Table 4.

Lipaemia interference

Only the lowest triglyceride concentration (L200) had no impact on PT measured with STA®-Neoplastine CI Plus, whereas the two highest concentrations (L500 and L1000) significantly affected the PT result ($P = 0.0403$ and 0.0028, respectively). In contrast, lipaemia had a significant effect only on PT measured using the STA®-Neoplastine R reagent for the highest level L1000 ($P = 0.0238$). However, these differences were demonstrated to be not clinically significant.

Lipaemia had no impact on APTT measured with either STA®-PTT Automate® or STA®-C.K.Prest®. In contrast, lipaemia at the two highest concentrations (L500 and L1000) significantly affected APTT results generated using STA®-Cephascreen® reagent ($P = 0.0054$ and 0.0005, respectively). However, these

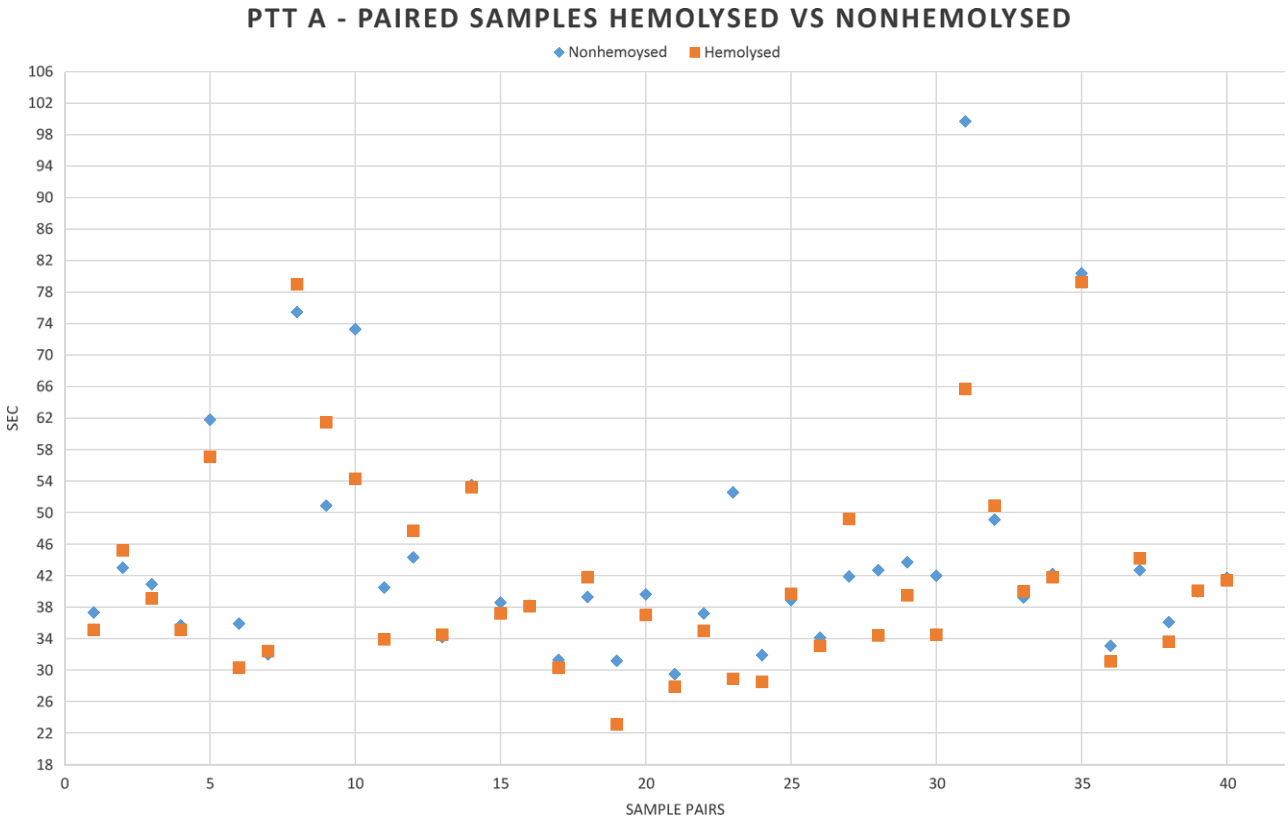

Figure 3. Results obtained for nonhaemolysed versus haemolysed paired samples for APTT (STA®-PTT Automate®). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

differences were demonstrated to be not clinically significant.

Finally, lipaemia had no impact on fibrinogen assay results when measured with STA®-Liquid Fib.

Clinical relevance analysis for lipaemia is depicted in Table 5.

DISCUSSION

Important diagnostic and therapeutic decisions are based on laboratory test results. However, many variables may affect plasma-based coagulation test results. Haemolysis, lipaemia and icterus are common conditions in clinical laboratories: indeed, they have been reported to affect up to 19.5%. 0.3%. and 0.3%, respectively, of samples referred to coagulation laboratories [11]. To date, most laboratories use automated analysers to analyse screening tests of haemostasis including PT, APTT and fibrinogen. In general, the principle of detection system can be divided into two categories: electromechanical and photo-optical methods. It is known that the photo-optical method can be limited by a high degree of coloured or particulate interferences in plasma samples. Coagulation test results from these samples could lead to erroneous or undetectable errors [12].

There are multiple ways an interfering substance may impair the reliability of a test result: this depends on the nature of the interfering substance, the nature of the analyte and the principle of the assay including the detection method. Indeed, interference can derive from absorbance of the interfering substance at

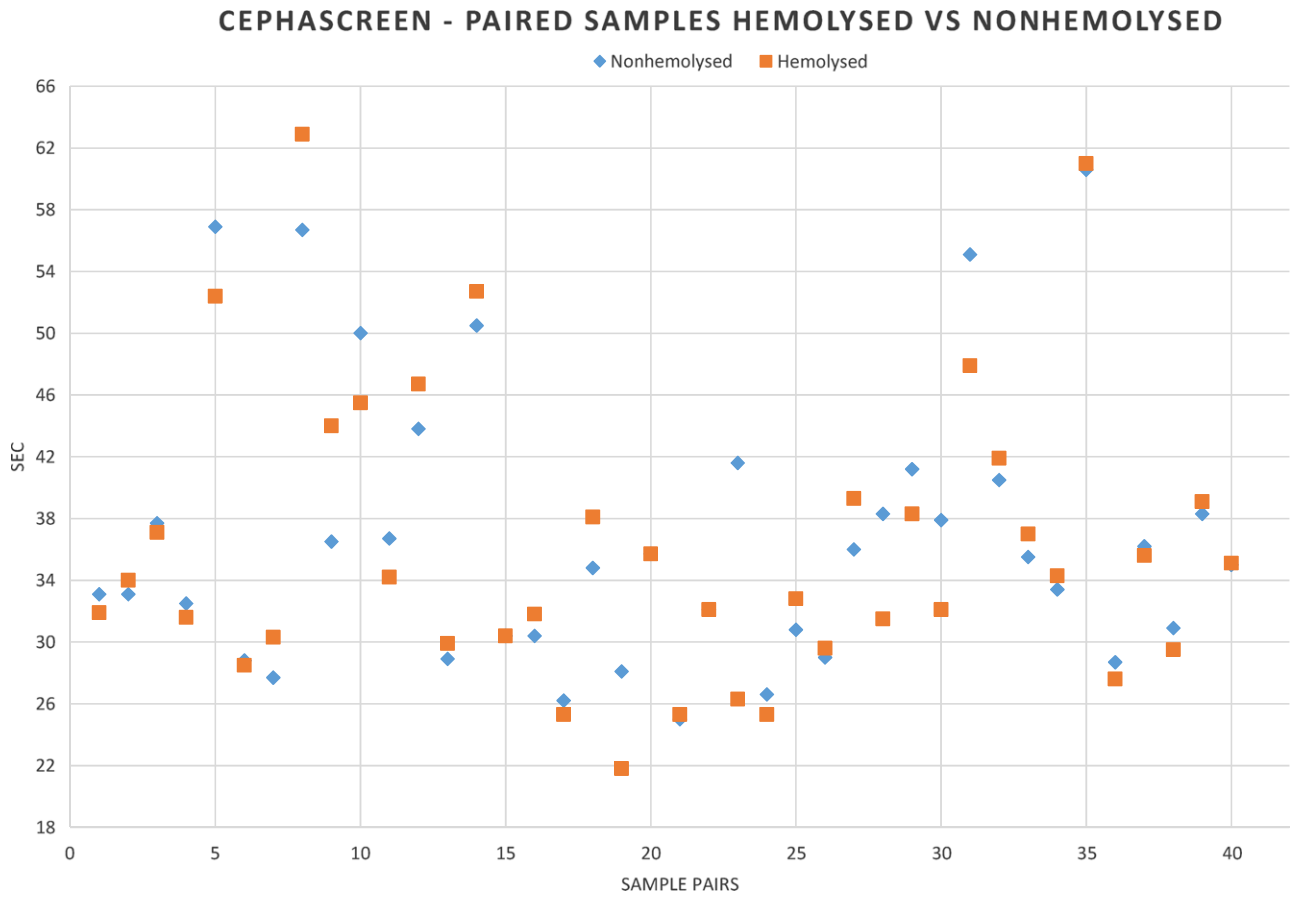

Figure 4. Results obtained for nonhaemolysed versus haemolysed paired samples for APTT (STA®-Cephascreen®). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

wavelengths that are commonly used for the optical detection in coagulation analysers [2]. Besides this analytical interference, the interfering substance or interfering substance-related components can induce a direct interference with haemostasis. For instance, phospholipid membranes from haemolytic red cells may interfere with the coagulation reaction by providing a phospholipid-rich surface that accelerates coagulation reactions [4]. Platelet activation by haemolysed red cells can impact on coagulation test results, indicating that effects will not be restricted to those induced by change in the colour of the plasma samples under analysis [13].

In contrast to photo-optical detection systems, viscosity-based detection systems (VBDS) also referred to as mechanical or electromechanical detection systems may overcome at least some of the analytical interference limitations. The aim of this study was to assess the sensitivity of various PT and APTT reagent and of one fibrinogen assay run on a VBDS to haemolysis, icterus, and lipaemia.

The results demonstrate that interfering substances may impact assays differently depending on (i) the parameter, (ii) the specific reagent used to run an assay, (iii) the nature of the interfering substance, and (iv) the level of the interfering substance.

The impact of sequential changes in patient samples that might occur in the interval between the occurrence of *in vitro* haemolysis, for example during sample collection, and receipt/analysis of that sample

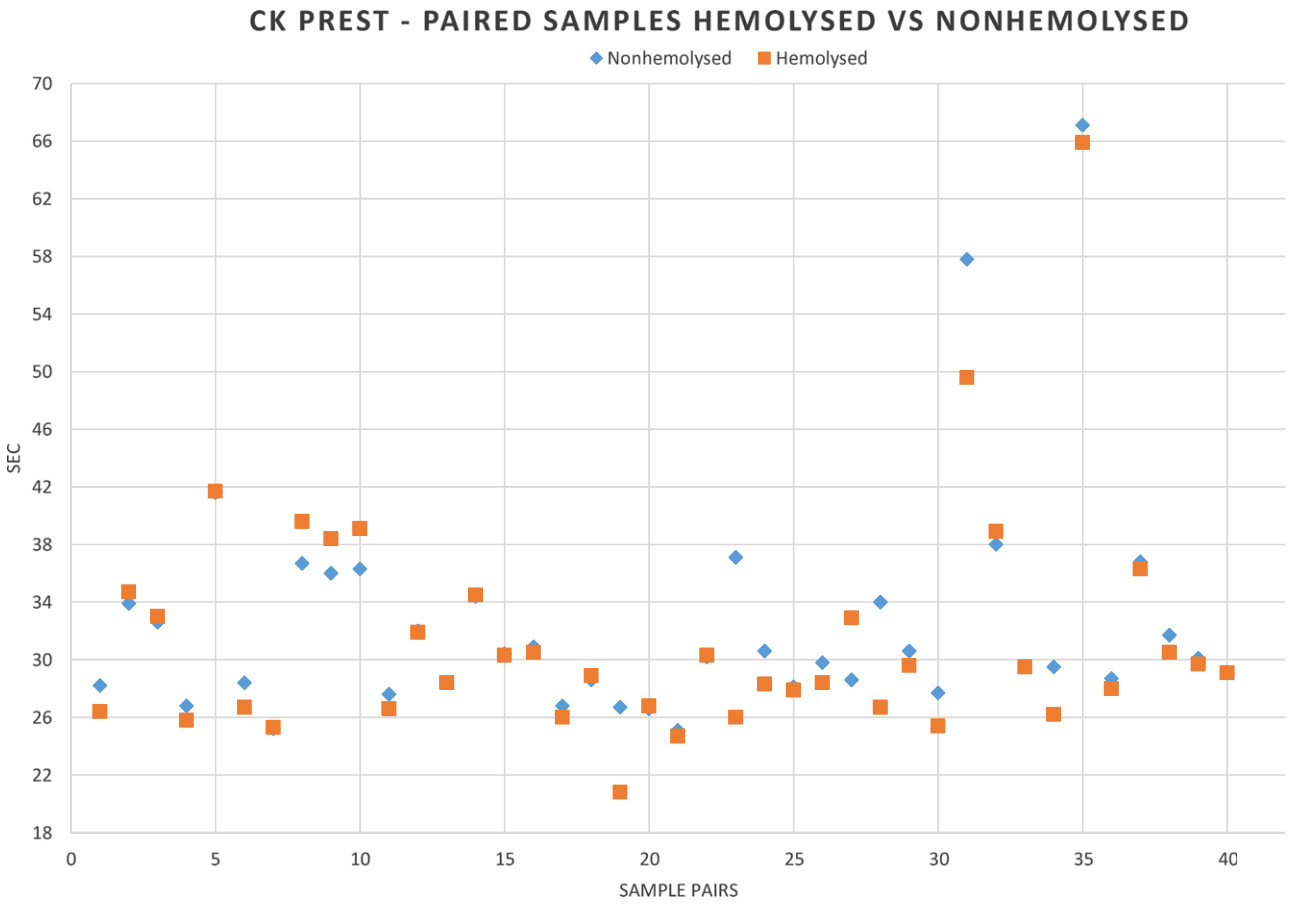

Figure 5. Results obtained for nonhaemolysed versus haemolysed paired samples for APTT (STA®-C.K. Prest®). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

in the laboratory, is unknown but could involve both activation of and consumption of clotting factors. Some of these effects could be missing from experiments involving addition of lysates to plasma samples so one of the important strengths of this study is that pairs of rejected spontaneously haemolysed samples and matched nonhaemolysed samples from the same subjects were analysed. It is usually considered that haemolysis is visible for plasma haemoglobin concentrations of 0.2 – 0.3 g/L [4, 14] although analytical systems typically used for such plasma haemoglobin measurements are designed for use with whole blood samples and a much higher measuring range. In addition, the influence of spurious haemolysis was studied. There are number of techniques in use to create spurious haemolysis, including spiking plasma with haemolysate preparations, freezing and thawing whole anticoagulated blood, lysis of whole anticoagulated samples by means of deionized water with or without detergents, mechanical lysis of whole anticoagulated blood by sonication, stirring with a metallic bar, application of the blade of a tissue homogenizer and aspiration through a fine blood collection needle (< 25 gauges). The most reliable techniques seem to be those that more closely reproduce mechanical haemolysis of blood during collection: serial aspiration of whole anticoagulated blood by a 0.5-mL insulin syringe equipped with a very thin needle most closely reproduces the breakdown of cells due to traumatic blood collection. Furthermore, the number of

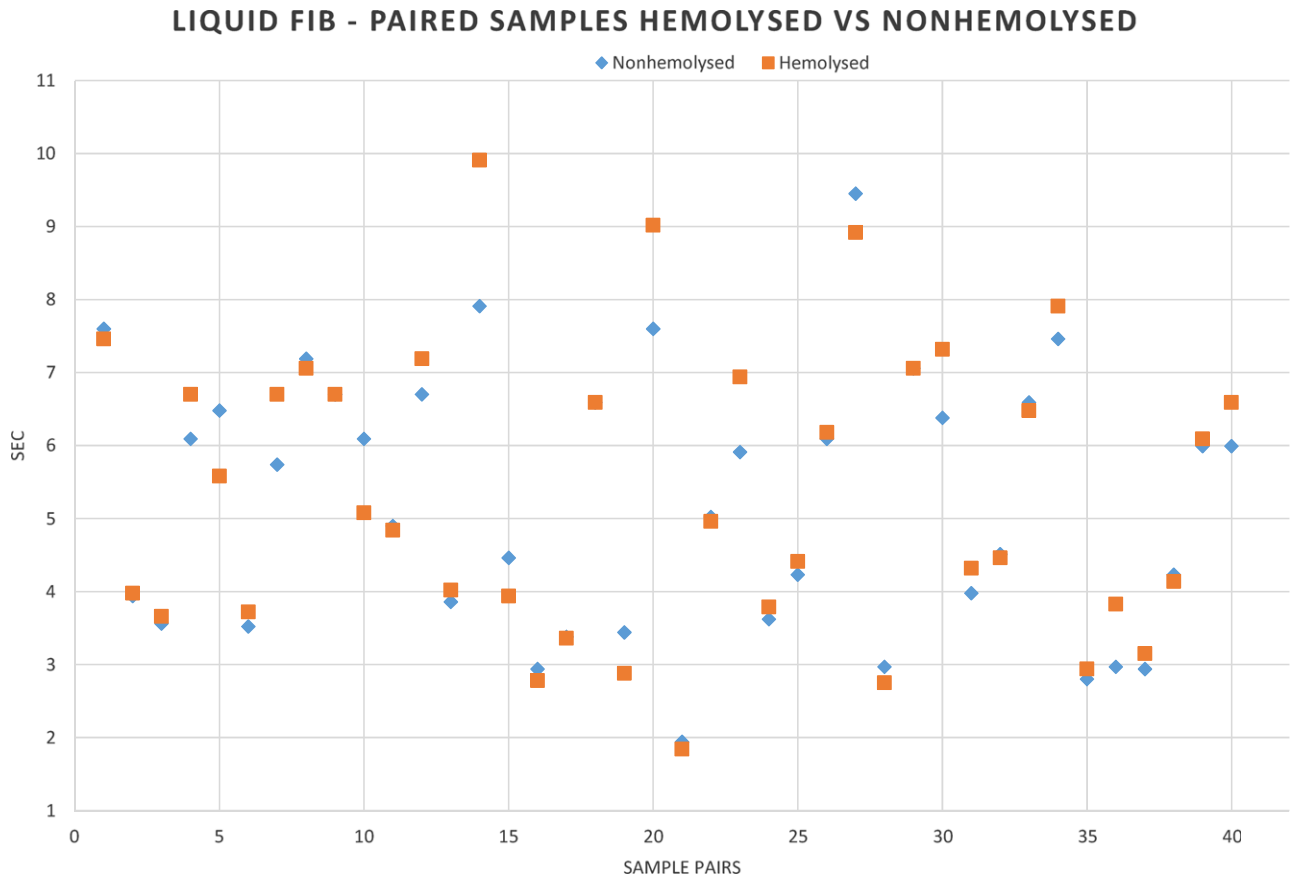

Figure 6. Results obtained for nonhaemolysed versus haemolysed paired samples for fibrinogen (STA®-Liquid Fib). Individual results on rejected patient samples in which haemolysis had occurred spontaneously *in vitro* during blood sample collection/processing compared with replacement nonhaemolysed samples from the same subjects. The two samples were collected within 4 h of each other.

aspirations is directly linked to the degree of damaged blood cells and cell-free haemoglobin, so that an arbitrary scale of haemolysis can be easily and suitably reproduced [2]. *In vitro* haemolysis in rejected patient samples is likely to be derived from a number of mechanisms including this type of mechanical haemolysis.

Spontaneous haemolysis that occurred during sample collection and processing had no effect on PT in our study for either the rabbit tissue factor extract or recombinant human tissue factor reagents, namely STA®-Neoplastine® CI Plus and STA®-Neoplastine® R, respectively. In contrast, addition of mechanically haemolysed cells impacted statistically significantly PT for the highest haemoglobin concentration. Any test result reported by the laboratory as a single number actually represents a range of numbers that has a definable dispersion. Therefore, statistically significance of the difference between two different conditions is easily reached, but may not be of clinical significance. As a consequence, the clinical relevance of the observed bias should be reviewed instead [7]. This approach allows a more relevant comparison of results obtained in samples containing a potentially interfering substance. When looking at the clinical relevance of the observed difference between the highest haemolysis level studied and the nonhaemolysed reference pools for PT, it was demonstrated that the bias would have no clinical impact.

The results obtained in this study for haemolysis indicate that not only the analyte has to be taken into account, but also the particular test used for the

Table 2. Clinical relevance analysis for haemolysis interference in paired samples

Test	Reagent	Mean Nonhaemolysed	Mean haemolysed	Diff. min	Diff. max	Mean diff	SD diff	CI95% min	CI95% max	Range min%	Range max%
PT (maximum acceptable bias: 8.64%)	STA-Neoplastine CI+	16.61	16.60	−2.8	3.7	−0.01	1.03	−0.33	0.33	−2.02	1.94
	STA-Neoplastine R	20.35	20.67	−2.5	5.6	0.31	1.58	−0.19	0.82	−0.93	3.96
APTT (maximum acceptable bias: 9.22%)	STA-PTT Automate	44.39	41.62	−34	10.6	−2.77	7.73	−5.24	−0.31	−12.61	−0.73
	STA-Cephascreen	36.78	36.16	−15.3	7.5	−0.62	3.94	−1.88	0.64	−5.19	1.77
	STA-C.K. Prest	32.45	31.48	−11.1	4.3	−0.97	2.87	−1.88	−0.06	−6.00	−0.18
Fibrinogen (maximum acceptable bias: 16.71%)	STA-Liquid Fib	5.22	5.38	−1.01	2	0.16	2.87	−0.02	0.35	−0.43	6.42

measurement of the analyte: three APTT reagents have been evaluated in this study. For APTTs determined with STA®-Cephascreen® reagent, there was no significant difference between results in haemolysed and nonhaemolysed samples. For the other two reagents studied, there were statistically significantly shorter APTTs in haemolysed samples compared with matched nonhaemolysed samples from the same subjects. However, this bias was clinically significant only for STA®-PTT Automate.

For all three APTT reagents, there were some matched samples amongst the 40 pairs in which the difference between the result obtained on the two samples (haemolysed and nonhaemolysed) was sufficient to have a potential impact on patient management decisions. False normal results associated with more than 10% shortening of APTT in the presence of haemolysis occurred in four sample pairs with STA®-PTT automate, three sample pairs with STA®-Cephascreen® and two sample pairs with STA®-CK Prest®. Even though all APTT assays are based on the same principle (clotting time of the recalcified plasma sample measured in the presence of phospholipids + an activator), the nature/concentration of the phospholipids and activator may make the test variably sensitive to the potentially interfering substances. This finding is in agreement with previous findings in clinical chemistry: the interference may depend on the method used, but even in some cases, it may not be dependent on the methods [15]. Rejected haemolysed samples may not always be replaced in time to benefit patient management decisions. This means there are risks arising from sample rejection which must be balanced against the risks of issuing potentially erroneous results on haemolysed samples. It would be an advantage to make use of a reagent test system that is insensitive to the impact of haemolysis as there are potential benefits for assessing samples that might otherwise be rejected.

Furthermore, no correlation was observed between the level of free haemoglobin in haemolysed samples and the level of bias. This is in agreement with previous observations [4].

It has been widely held that mechanical clot detection is unaffected by turbid samples and, hence, is superior to photo-optical detection, which, in contrast, may be affected by turbid samples [16]. A study comparing mechanical and photo-optical systems using samples containing haemolysis generated

Table 3. Clinical relevance analysis for spurious haemolysis

Test	Reagent	Haemolysis Level	Mean	Mean diff	SD diff	Range min	Range max	Range min%	Range max%
PT (maximum acceptable bias: 8.64%)	STA-Neoplastine CI+ (mean H0: 21.02)	H1	21.03	0.01	0.18	−0.34	0.37	−1.62	1.72
		H3	21.03	0.01	0.18	−0.34	0.37	−1.62	1.72
		H5	21.57	0.55	0.18	0.20	0.91	0.94	4.29
	STA-Neoplastine R (mean H0: 28.41)	H1	28.42	0.01	0.29	−0.56	0.58	−1.96	2.04
		H3	27.8	−0.61	0.29	−1.18	−0.05	−4.14	−0.15
		H5	27.05	−1.36	0.29	−1.93	−0.80	−6.78	−2.79
APTT (maximum acceptable bias: 9.22%)	STA-PTT Automate (mean H0: 43.76)	H1	42.61	−1.15	0.65	−2.43	0.13	−5.55	0.30
		H3	41.36	−2.4	0.65	−3.68	−1.13	−8.40	−2.57
		H5	41.23	−2.53	0.65	−3.81	−1.26	−8.70	−2.86
	STA-Cephascreen (mean H0: 34.72)	H1	34.32	−0.4	0.39	−1.16	0.37	−3.36	1.06
		H3	34.5	−0.22	0.39	−0.98	0.55	−2.84	1.58
		H5	35.41	0.69	0.39	−0.07	1.46	−0.22	4.20
	STA-C.K. Prest (mean H0: 31.32)	H1	31.06	−0.26	0.58	−1.39	0.88	−4.45	2.80
		H3	30.95	−0.37	0.58	−1.50	0.77	−4.80	2.45
		H5	32.62	1.3	0.58	0.16	2.44	0.52	7.78
Fibrinogen (maximum acceptable bias: 16.71%)	STA-Liquid Fib (mean H0: 4.285)	H1	4.33	0.05	0.06	−0.06	0.17	−1.52	3.77
		H3	4.31	0.02	0.06	−0.09	0.14	−2.15	3.14
		H5	4.21	−0.07	0.06	−0.18	0.04	−4.36	0.92

Table 4. Clinical relevance analysis for icterus interference

Test	Reagent	Icterus level	Mean	Mean diff	SD diff	Range min	Range max	Range min%	Range max%
PT (maximum acceptable bias: 8.64%)	STA-Neoplastine CI+ (mean B0: 13.62)	B5	13.53	−0.09	0.07	−0.22	0.06	−1.65	0.40
		B20	13.54	−0.07	0.07	−0.21	0.07	−1.58	0.47
		B40	13.52	−0.09	0.07	−0.23	0.05	−1.72	0.33
	STA-Neoplastine R (mean B0: 14.63)	B5	14.6	−0.03	0.12	−0.26	0.21	−1.78	1.38
		B20	14.56	−0.07	0.12	−0.30	0.17	−2.05	1.11
		B40	14.35	−0.28	0.12	−0.51	−0.05	−3.49	−0.34
APTT (maximum acceptable bias: 9.22%)	STA-PTT Automate (mean B0: 37.47)	B5	36.5	−0.97	0.28	−1.52	−0.42	−4.07	−1.11
		B20	37.14	−0.33	0.28	−0.88	0.23	−2.36	0.61
		B40	38.11	0.64	0.28	0.08	1.20	0.21	3.20
	STA-Cephascreen (mean B0: 31.27)	B5	31.07	−0.2	0.14	−0.47	0.08	−1.53	0.26
		B20	31.13	−0.14	0.14	−0.41	0.14	−1.34	0.45
		B40	31.53	0.26	0.14	−0.01	0.54	−0.06	1.73
	STA-C.K. Prest (mean B0: 29.72)	B5	29.78	0.06	0.14	−0.21	0.34	−0.72	1.14
		B20	30.38	0.66	0.14	0.38	0.94	1.29	3.15
		B40	30.73	1.01	0.14	0.73	1.29	2.46	4.33
Fibrinogen (maximum acceptable bias: 16.71%)	STA-Liquid Fib (mean B0: 4.34)	B5	4.32	−0.02	0.05	−0.10	0.08	−2.48	1.66
		B20	4.23	−0.11	0.05	−0.19	−0.02	−4.51	−0.38
		B40	4.16	−0.18	0.05	−0.26	−0.09	−6.15	−2.01

mechanically or by freeze thaw [17] reported much more marked prolongation of PT and APTT for the optical system, although reading optical end-points at 570 nm reduced the impact of haemolysis when compared to optical readings at 405 nm. This study did not include any testing of samples in which haemolysis had occurred in patient samples during their collection and processing.

Although not being a head-to-head comparison between these two types of detection systems, this

Table 5. Clinical relevance analysis for lipaemia interference

Test	Reagent	Lipaemia level	Mean	Mean diff	SD diff	Range min	Range max	Range min%	Range max%
PT (maximum acceptable bias: 8.64%)	STA-Neoplastine CI+ (mean L0: 16.95)	L200	16.67	−0.28	0.18	−0.63	0.08	−3.72	0.43
		L500	16.49	−0.46	0.18	−0.81	−0.11	−4.79	−0.64
		L1000	16.3	−0.65	0.18	−1.00	−0.30	−5.91	−1.76
	STA-Neoplastine R (mean L0: 20.00)	L200	19.8	−0.2	0.32	−0.83	0.44	−4.16	2.17
		L500	19.5	−0.5	0.32	−1.13	0.14	−5.66	0.67
		L1000	19.1	−0.9	0.32	−1.53	−0.27	−7.66	−1.34
APTT (maximum acceptable bias: 9.22%)	STA-PTT Automate (mean L0: 38.66)	L200	38.51	−0.15	0.29	−0.71	0.42	−1.86	1.09
		L500	38.23	−0.43	0.29	−0.99	0.14	−2.58	0.37
		L1000	38.69	0.03	0.29	−0.53	0.60	−1.39	1.55
	STA-Cephascreen (mean L0: 31.79)	L200	31.49	−0.3	0.15	−0.59	−0.01	−1.85	−0.03
		L500	31.29	−0.5	0.15	−0.79	−0.21	−2.48	−0.66
		L1000	31.16	−0.63	0.15	−0.92	−0.34	−2.89	−1.07
	STA-C.K. Prest (mean L0: 28.87)	L200	28.75	−0.12	0.13	−0.37	0.14	−1.29	0.46
		L500	28.75	−0.12	0.13	−0.37	0.14	−1.29	0.46
		L1000	28.76	−0.11	0.13	−0.36	0.15	−1.25	0.50
Fibrinogen (maximum acceptable bias: 16. 7%)	STA-Liquid Fib (mean L0: 4.71)	L200	4.27	−0.00	0.06	−0.11	0.11	−2.75	2.58
		L500	4.34	0.07	0.06	−0.04	0.19	−1.00	4.33
		L1000	4.35	0.07	0.06	−0.03	0.19	−0.90	4.43

study illustrates that VDBS is not influenced by icterus and lipaemia interferences to an extent which would create clinically relevant bias in the result of PT, APTT and Fibrinogen. This absence of interference to haemolysis for PT and kaolin-cephalin APTT is in agreement with previous findings [4]. Indeed, only one APTT reagent generated a clinically relevant bias for the highest haemolysis level tested. For the three reagents studied, there were between two and four individual samples amongst 40 pairs (5 -10%) in which haemolysis (in the range <0.5 – 2.1 g/L free plasma haemoglobin) led to a 10% or greater reduction in APTT and which normalized what should be a prolonged result. Individual centres need to take these effects into account when establishing policy on sample acceptance and rejection. The vast majority of haemolysed specimens detected in clinical laboratories are only mildly haemolytic (i.e. with cell-free haemoglobin < 0.6 g/L) [18]. In contrast to what has been described for fibrinogen measured in a grossly haemolysed plasma sample using a photo-optical detector, the fibrinogen level was not clinically significantly affected by haemolysis in our study [12]. Initial visual observation of samples upon receipt in the laboratory for processing should be retained as detection of incorrect sample tubes and grossly haemolysed or lipaemic specimens by initial observation can initiate the replacement of unacceptable specimens and reduce overall turnaround time (TAT) [3].

CONCLUSION

Reporting accurate results is a critical concern for clinical laboratories. It is therefore important that the laboratory staff be aware of potentially clinically relevant bias due to interferences. HIL is a group of the most commonly observed source of interferences. Over the past years, visual inspection of plasma samples has been the system for detection and reporting of HIL interference. However, there are some limitations to this inspection, including the difficulty to adequately identify HIL. The aim of this study was to evaluate the absence of clinically relevant bias induced by HIL interference for PT, APTT and fibrinogen measured with different reagents using a VBDS analyser. These results confirm that PT and fibrinogen are not clinically significantly affected by HIL. The APTTs of some haemolysed samples were falsely normal with one reagent more affected than two others. Haemolysed samples should be continuously rejected. Conversely,

from a clinical standpoint, lipaemia and icterus did not significantly affect APTT measured with the different reagents tested in combination with a VBDS analyser.

ACKNOWLEDGEMENTS

The authors are grateful to Diagnostica Stago for providing reagents and funding to conduct this study.

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