

Ann Helen Kristoffersen*, Anne V. Stavelin, Eva Ajzner, Gunn B.B. Kristensen, Sverre Sandberg, Marjan Van Blerk, Steve Kitchen, Dagmar Kessler, Timothy A.L. Woods and Piet Meijer

Pre-analytical practices for routine coagulation tests in European laboratories. A collaborative study from the European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM)

<https://doi.org/10.1515/cclm-2019-0214>

Received February 23, 2019; accepted April 1, 2019; previously published online May 14, 2019

Abstract

Background: Correct handling and storage of blood samples for coagulation tests are important to assure correct diagnosis and monitoring. The aim of this study was to assess the pre-analytical practices for routine coagulation testing in European laboratories.

Methods: In 2013–2014, European laboratories were invited to fill in a questionnaire addressing pre-analytical requirements regarding tube fill volume, citrate concentration, sample stability, centrifugation and storage conditions for routine coagulation testing (activated partial thromboplastin time [APTT], prothrombin time in seconds [PT-sec] and as international normalised ratio [PT-INR] and fibrinogen).

*Corresponding author: Ann Helen Kristoffersen, MD, PhD, Department of Medical Biochemistry and Pharmacology, Helse Bergen HF, Haukeland University Hospital, Postboks 1400, NO-5021 Bergen, Norway; and Norwegian Quality Improvement of Laboratory Examinations (Noklus), Haralds plass Deaconess Hospital, Bergen, Norway, Phone: +47 55973113, E-mail: ann.kristoffersen@helse-bergen.no

Anne V. Stavelin and Gunn B.B. Kristensen: Norwegian Quality Improvement of Laboratory Examinations (Noklus), Haralds plass Deaconess Hospital, Bergen, Norway

Eva Ajzner: Central Laboratory, Jóna University Hospital, Nyíregyháza, Hungary

Sverre Sandberg: Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway; Norwegian Quality Improvement of Laboratory Examinations (Noklus), Haralds plass Deaconess Hospital, Bergen, Norway; and Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

Marjan Van Blerk: Quality of laboratories, Sciensano, Brussels, Belgium

Steve Kitchen: UK NEQAS for Blood Coagulation, Sheffield, UK; and Sheffield Haemophilia and Thrombosis Centre, Sheffield, UK

Dagmar Kessler: Quality Control Center Switzerland (CSCQ), Chêne-Bourg, Switzerland

Timothy A.L. Woods: UK NEQAS for Blood Coagulation, Sheffield, UK

Piet Meijer: ECAT foundation, Voorschoten, The Netherlands

Results: A total of 662 laboratories from 28 different countries responded. The recommended 3.2% (105–109 mmol/L) citrate tubes are used by 74% of the laboratories. Tube fill volumes $\geq 90\%$ were required by 73%–76% of the laboratories, depending upon the coagulation test and tube size. The variation in centrifugation force and duration was large (median 2500 g [10- and 90-percentiles 1500 and 4000] and 10 min [5 and 15], respectively). Large variations were also seen in the accepted storage time for different tests and sample materials, for example, for citrated blood at room temperature the accepted storage time ranged from 0.5–72 h and 0.5–189 h for PT-INR and fibrinogen, respectively. If the storage time or the tube fill requirements are not fulfilled, 72% and 84% of the respondents, respectively, would reject the samples.

Conclusions: There was a large variation in pre-analytical practices for routine coagulation testing in European laboratories, especially for centrifugation conditions and storage time requirements.

Keywords: activated partial thromboplastin time; coagulation tests; fibrinogen; haemostasis; pre-analytical phase; prothrombin time.

Introduction

Correct handling and storage of blood samples for coagulation tests are important to assure a correct diagnosis of bleeding and thrombotic disorders as well as anticoagulant monitoring [1, 2]. Incorrect handling of samples may lead to erroneous results which could jeopardise patient safety [1, 3]. In the case of traumatic blood sampling or vigorous or insufficient mixing of the sample, coagulation factors could be pre-activated. In the case of insufficient mixing a clot could be formed in the sample [3]. Under-filled tubes or prolonged storage before testing may lead to a decrease of coagulation factor activities or altered impression of the anticoagulant effect in a monitoring situation [4–7]. In these circumstances, results would represent the situation *in vitro* and not *in vivo*. Because of

the risk of erroneous results of coagulation tests caused by handling in the pre-analytical phase, a harmonisation of the different pre-analytical practices is important [3, 8].

The most extensive international guideline for pre-analytical handling of coagulation tests is the Clinical and Laboratory Standards Institute (CLSI) H21-A5 [7]. However, there are several other less extensive guidelines and reviews [1–3, 8–14] dealing with pre-analytical procedures in the coagulation field. It is uncertain to what extent laboratories use published guidelines, studies or reviews, or if they use their own studies or expert opinions for pre-analytical requirements. The CLSI guideline takes a conservative approach regarding pre-analytical handling of samples for coagulation testing and states that, if less strict requirements are considered, laboratories must perform their own studies to show that their requirements are acceptable [7].

To the best of our knowledge, there is no published information on pre-analytical practices of routine coagulation tests in laboratories from several European countries. The aim of this study was therefore to assess the actual pre-analytical practices for routine coagulation testing in European laboratories.

Materials and methods

This study was performed by the Haemostasis Working Group within the European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM). A questionnaire on pre-analytical practices for routine coagulation testing was offered to European medical laboratories from their national external quality assurance (EQA) organiser through EQALM [15]. Each EQA organiser distributed the questionnaire either by email or via a web-link at the website of the EQA provider. The link to the study questionnaire was open from the autumn of 2013 to the spring of 2014. As the questionnaire was offered through the web in some countries, it was not possible to calculate the response rate in each country, as the total number of invited laboratories is unknown.

The questionnaire dealt with the most frequently used routine coagulation tests; i.e. activated partial thromboplastin time (APTT), prothrombin time in seconds (PT-sec) and as the international normalised ratio (PT-INR) and fibrinogen. The following topics were included in the questionnaire with a total of 29 questions: type of laboratory; type of coagulation tests performed; if external samples are received in the laboratory and how laboratories control that received samples are citrated plasma; citrate concentration of the tubes used; requirements for tube fill volume; centrifugation conditions; acceptable storage time (stability) for citrated blood in the primary tube before centrifugation, and after centrifugation (plasma on cells) and for aliquoted citrated plasma (fresh plasma transferred to a secondary tube), both at room temperature and refrigerated; and if a system for temperature control is applied during storage and transport. Laboratories were also asked to mention

the action they take if their requirements are not fulfilled, which source of guidance they use for tube fill and stability requirements and if they have national guidelines available (see questionnaire in the Supplementary material). Ethical approval was not necessary for this study.

Statistics

Descriptive statistics with median, 10- and 90-percentiles were used to describe the requirements and practices in the laboratories. Percentages were calculated based upon the number of laboratories responding to each question (missing responses excluded). The number of missing responses is only reported for questions where the response rate was less than 85%. SPSS version 22.0 (SPSS Inc.) was used for statistics and figures.

Results

In total, 662 laboratories from 28 different countries completed the questionnaire (Figure 1). The response rate to each question was generally above 85%. Sixty-seven percent of responding laboratories were public hospital laboratories, 19% were private outpatient laboratories, 7% private hospital laboratories, 3% public outpatient laboratories and 4% other types. Half of the responding laboratories (55%) perform only routine coagulation testing (one or more of the following tests: APTT, PT-sec, PT-INR and/or fibrinogen), while the remaining laboratories also perform more specialised coagulation tests (e.g. coagulation factors, thrombophilia- and/or platelet-function testing).

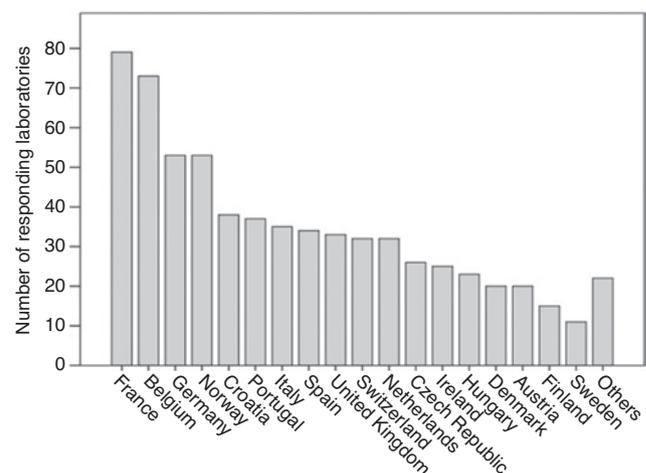


Figure 1: The number of responding laboratories from each of the participating countries.

Samples received from outside the hospital and verification of citrated plasma

Sixty-six percent of the responding laboratories receive samples from outside the hospital. Of them, 62% receive *only* citrated whole blood, whereas the others receive whole blood *and* aliquoted plasma or *only* aliquoted plasma. Of the laboratories receiving aliquoted plasma, 36% do not routinely check if the received sample material is citrated plasma (and not, e.g. serum, EDTA or heparin plasma), 29% only check in the case of unexpected results, and 34% always check. Of the laboratories performing a check, 50% check that “citrated plasma” is written on the tube label and 50% perform one or more laboratory tests. Of the latter, only 50% (n = 26) specified in this survey which laboratory test(s) they perform. Fibrinogen measurement is performed by 20 laboratories, while some perform either an electrophoresis or measure one or more coagulation screening tests (APTT, PT-sec, thrombin time) or electrolytes (calcium, sodium, potassium and/or magnesium). Two laboratories require the primary citrate tube to be attached to the aliquoted sample (e.g. with an elastic band).

Citrate concentration and tube fill requirements

The majority of respondents (74%) use tubes with a citrate concentration of 3.2% (105 or 109 mmol/L), 12% use tubes with a citrate concentration of 3.8% (129 mmol/L) and 14% use both.

Depending upon the routine coagulation test in question, 73%–76% only accept tube fill volumes of $\geq 90\%$ for both regular and paediatric (low volume) tubes, while 15%–17% accept fill volume of $\geq 80\%$ and 5%–8% fill volumes of $\geq 70\%$. Only 1%–4% answered specifically that they do not have tube filling requirements or that they accept tube fill volumes of less than 70% (Figure 2). The question regarding tube fill volume was not answered by 4%–15% for the different routine coagulation tests for regular tubes, and by 41%–46% for paediatric tubes. When underfilled tubes are received by laboratories, 84% would reject the sample and ask for a replacement (Figure 3). Forty-four percent stated own experience, while 56% stated at least one guideline as their source of requirements for tube fill volume. Of those using guidelines, only 28% stated a guideline also mentioned by others (Table 1) and 67% did not state any name.

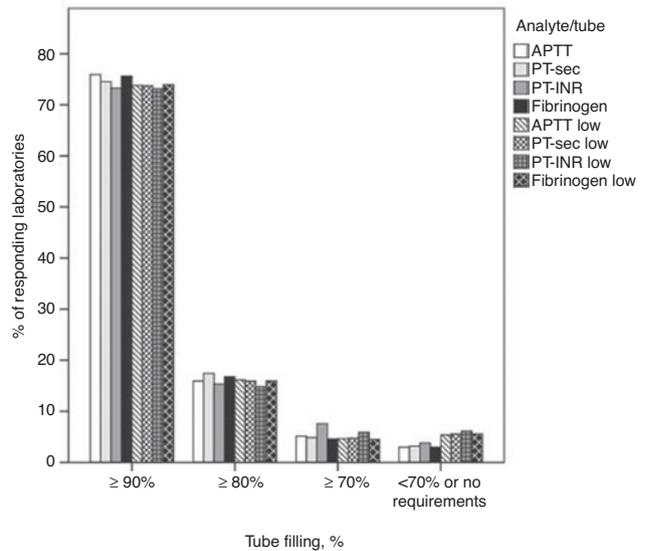


Figure 2: Percentage of laboratories (y-axis) accepting the different tube fill volumes (x-axis) in regular and low volume/paediatric tubes for different routine coagulation tests. APTT, activated partial thromboplastin time; PT-sec, prothrombin time in seconds; PT-INR, prothrombin time in the international normalised ratio.

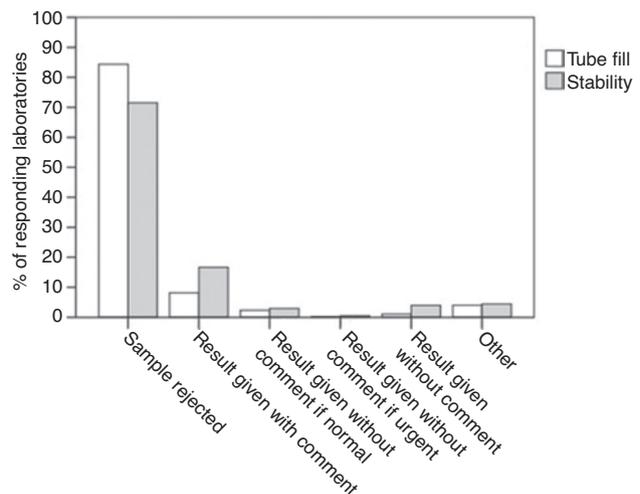


Figure 3: The percentage of responding laboratories (y-axis) performing different actions (x-axis) if a sample does not fulfill tube fill volume requirements (white bars) or stability requirements (grey bars).

Centrifugation conditions

The median (10- and 90-percentiles) centrifugation force was 2500 g (1500 and 4000), the centrifugation duration was 10 min (5 and 15) and the median centrifugation temperature was 20 °C (18 and 24). The variation of combinations of centrifugation force and duration was large

Table 1: Recommendations for citrate concentration, centrifugation condition, tube fill volume and acceptable storage times for APTT, PT-sec, PT-INR and fibrinogen given by guidelines used by the responding laboratories, and the number (%) of laboratories using these guidelines for tube fill and sample stability requirements.

Guideline	Citrate concentration		Centrifugation conditions		Tube fill volume		Sample stability		Laboratories n (%)
	Recommendation		Recommendation		Recommendation		Recommendation		
CLSI H21-A5 [7]	3.2% recommended 3.8% may be used		At least 1500 g 15 min Higher force and duration can be used		90% or perform own studies		APTT, PT, fibrinogen 4 h, citrated blood/plasma, room temp PT-INR 24 h, citrated blood/plasma, room temp		31 (8.5)
French (GEHT) [9]	3.2% recommended 3.8% abandoned		Good 2 × 2000 g 15 min Fair 1 × 2000 g 15 min Poor <1000 g 10 min Refers to CLSI		100% good 90% fair		APTT-UH 1 h, citrated blood/plasma, room temp <2 h good, citrated blood/plasma, room temp <4 h fair, citrated blood/plasma, room temp		48 (13.1)
Dutch (SKML) [10]	3.2% recommended		Refers to CLSI		Not given		APTT-UH <1 h, citrated blood/plasma, room temp APTT, PT, fibrinogen 4 h, citrated blood/plasma, room temp/refrig		10 (2.7)
British (BSH) [11]	3.2% recommended		2000 g at least 10 min		Ensure correct filling of the tube		PT-INR 24 h, citrated blood/plasma, room temp Ideal <1 h, room temp		5 (1.3)
WHO (Guder) [12]	3.2% recommended	NA	NA		NA		Acceptable <4 h, room temp APTT: 2–8 h, citrate plasma, room temp PT: 4–24 h, citrated blood/plasma, room temp PT: 8–24 h, citrate plasma, refrig		8 (2.2)
Others	NA	NA	NA		NA		Fibrinogen: 8 h, citrated blood, room temp Fibrinogen: 24–168 h, citrate plasma, room temp/refrig		31 (8.5)

CLSI, Clinical and Laboratory Standards Institute; GEHT, Groupe d'Etude sur l'Hémostase et la Thrombose; SKML; Stichting Kwaliteitsbevordering Stollingsonderzoek (the Dutch EQA organisation); BSH, the British Society for Haematology; WHO, World Health Organisation; NA, not available; temp, temperature; refrig, refrigerated; 3.2%, 105 or 109 mmol/L and 3.8%, 129 mmol/L.

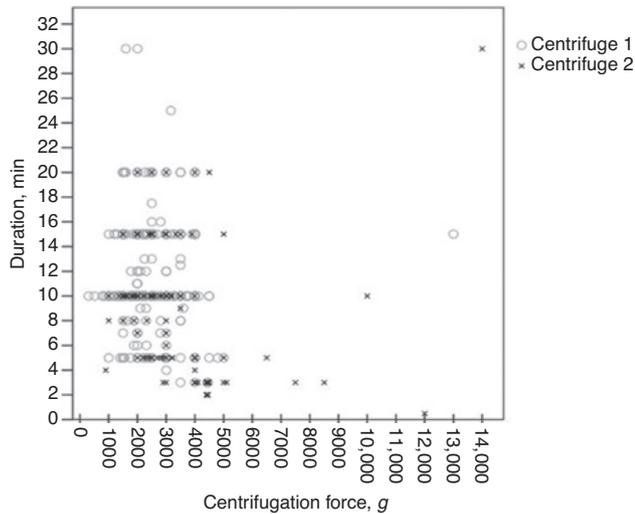


Figure 4: The combination of centrifugation force (g) (x-axis) and duration (minutes) (y-axis) for all the centrifuge conditions given by the responding laboratories (several laboratories reported two different conditions).

(Figure 4). When only laboratories using two centrifuges were included (n = 110 [17%]), the median centrifugation force was 2200 g (1500 and 3000) and the duration was 10 min (7 and 15) for the centrifuge with the lowest centrifugation force while it was 3200 g (2000 and 4500) and 5 min (3 and 15) for the centrifuge with the highest centrifugation force. More than 95% of the laboratories centrifuged their samples at room temperature (18–24 °C).

Sample stability and storage

Large variation in the accepted storage time of the samples was found between laboratories for all coagulation tests studied, for all sample materials and for both room temperature and refrigerator storage (Figure 5). Median stabilities given for the different routine coagulation tests in the different sample materials and temperatures were rather similar, but a wider range was seen for PT-INR and fibrinogen compared to APTT (Figure 5 and Table 2).

Fewer laboratories have storage time requirements for refrigerated samples compared to samples stored at room temperature, and 32%, 35%, 30% state not to accept refrigerated citrated whole blood for APTT, PT-INR and fibrinogen, respectively (Table 2). Fewer laboratories have specific requirements for APTT in patients treated with unfractionated heparin (APTT UH) and for PT-INR in patients treated with vitamin K antagonists (INR VKA) than for APTT and PT-INR in general (Table 2).

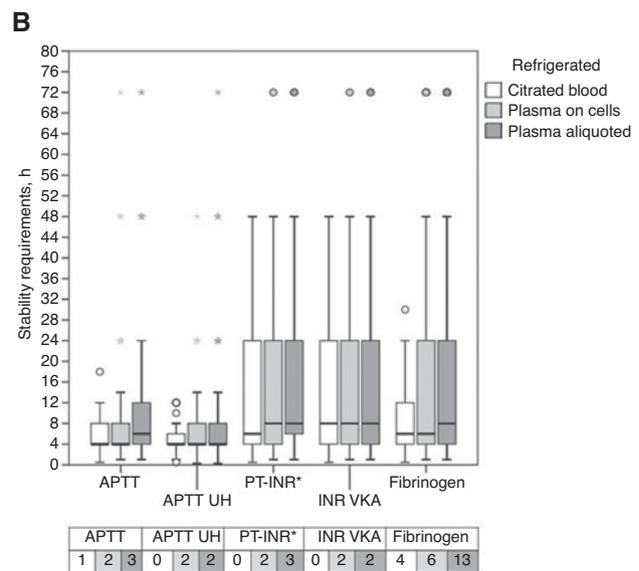
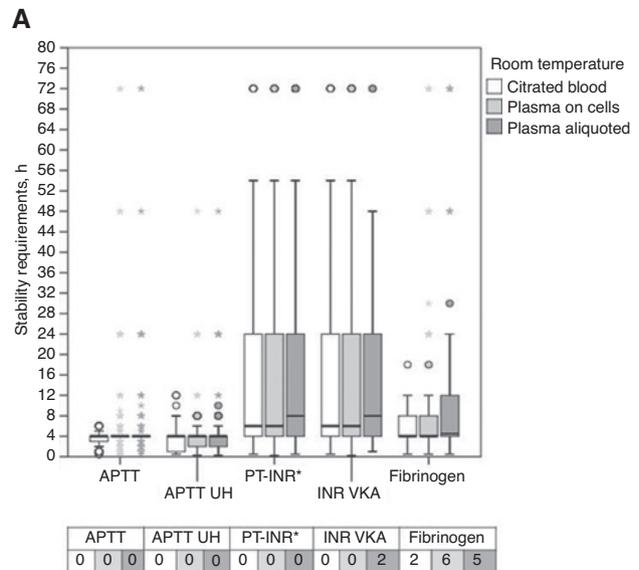


Figure 5: Stability requirements (y-axis) at room temperature (A) and in refrigerator (B) given by the responding laboratories for the different routine coagulation tests (x-axis) in different sample materials (citrated whole blood, citrated plasma on cells and citrated aliquoted plasma).

The tables below the Figure panels show the number of laboratories stating stability requirements of more than 72 h for the given condition and analyte. The boxes in the figure represent the median (horizontal black line) and the 25- and 75-percentiles (lower- and upper border, respectively). The range is depicted by the vertical lines from the boxes, the circles are outliers, while the asterix* are extreme outliers (SPSS version 22.0 [SPSS Inc.]). APTT, activated partial thromboplastin time; UH, unfractionated heparin; PT-INR, prothrombin time international normalised ratio; VKA, vitamin K antagonists. *Stability requirements for PT-INR and PT-sec were merged in the same question.

Table 2: Sample stability requirements at room temperature and refrigerator for routine coagulation testing reported by the laboratories.

	APTT			APTT UH			PT-INR ^a			PT-INR VKA			Fibrinogen		
	Citrate whole blood ^b	Citrate plasma on cells ^c	Citrate aliquot plasma ^d	Citrate whole blood	Citrate plasma on cells	Citrate aliquot plasma	Citrate whole blood	Citrate plasma on cells	Citrate aliquot plasma	Citrate whole blood	Citrate plasma on cells	Citrate aliquot plasma	Citrate whole blood	Citrate plasma on cells	Citrate aliquot plasma
Room temperature															
Requirements median (10–90 perc) in hours	4 (1–8)	4 (2–8)	4 (2–9)	4 (1–6)	4 (1–8)	4 (1–8)	6 (2–24)	6 (2–40)	6 (2–48)	6 (2–24)	6 (2–48)	8 (2–35)	8 (2–48)	8 (2–24)	4 (2–24)
Number (% of total) of laboratories stating their requirements	543 (82%)	435 (66%)	276 (42%)	446 (67%)	354 (54%)	235 (36%)	567 (86%)	506 (76%)	394 (60%)	499 (75%)	105 (16%)	398 (60%)	260 (39%)		
Number (% of total) of laboratories stating not to accept the condition ^e	13 (2%)	33 (5%)	101 (15%)	23 (4%)	40 (6%)	100 (15%)	12 (2%)	32 (5%)	104 (16%)	15 (2%)	34 (5%)	218 (33%)	20 (3%)	36 (5%)	103 (16%)
Refrigerator															
Requirements median (10–90 perc) in hours	4 (2–24)	4 (2–24)	6 (4–24)	4 (1–17)	4 (1–24)	4 (2–24)	6 (2–24)	8 (2–24)	8 (4–48)	8 (2–24)	8 (2–24)	8 (2–48)	8 (2–24)	6 (2–24)	8 (4–72)
Number (% of total) of laboratories stating their requirements	164 (25%)	154 (23%)	140 (21%)	131 (20%)	126 (19%)	111 (17%)	157 (24%)	144 (22%)	125 (19%)	139 (21%)	124 (19%)	105 (16%)	160 (24%)	149 (23%)	144 (22%)
Number (% of total) of laboratories stating not to accept the condition ^e	210 (32%)	210 (32%)	202 (31%)	192 (29%)	192 (29%)	194 (29%)	230 (35%)	233 (35%)	226 (34%)	220 (33%)	223 (34%)	218 (33%)	195 (30%)	198 (30%)	191 (29%)

^aStability requirements for PT-INR and PT-sec were merged in the same question, ^buncentrifuged whole blood stored in the primary citrate tube (blue stopper), ^ccentrifuged citrate tube stored in the primary tube with the plasma on top of the cells, ^dcentrifuged citrated plasma stored in a secondary tube (transferred aliquot), ^econdition; combination of the coagulation test, sample type and storage temperature. APTT, activated partial thromboplastin time; UH, unfractionated heparin; PT-INR, prothrombin time international normalised ratio; VKA, vitamin K antagonists; perc, percentiles.

If samples are received after the maximum storage time, 72% would reject the sample and ask for a replacement (Figure 3). More than half of the laboratories (61%) use at least one guideline for acceptable storage time, while 39% stated that the requirements were based on own experience or other studies. Of those stating to use at least one guideline, only 28% stated a guideline also mentioned by others (Table 1), and 64% did not state any name.

In more than half of the laboratories (56%), the samples are routinely stored in primary tubes (plasma on cells after centrifugation) for potential extra requests. Of these laboratories, 79% store the samples capped, 12% covered (e.g. with a plastic film or cardboard) and 9% uncapped and uncovered. Half of the laboratories (51%) usually store the samples at room temperature, 25% in a refrigerator, while 9% and 3% store the samples at -20 and -80 °C, respectively (12% did not answer this question).

In general, 72%, 96% and 95% of the laboratories have a system for temperature control at room temperature, in refrigerators and freezers, respectively. Only 42% have a system for temperature control during transport (24% did not answer).

Availability of national guidelines on pre-analytical issues in coagulation testing

Among the laboratories, 63% answered “yes” we have a national guideline. Of these, 58% stated to have a guideline from a national society, 8% from a government or national health care service, 26% from experts in the field and 8% from “others”. Only 45% specified the name of the guideline or the source of their recommendations. Of the French laboratories, 73% specified a guideline from Groupe d’Etude sur l’Hémostase et la Thrombose (GEHT) [9], also stated by 3% of the Belgian laboratories. Of the Dutch laboratories, 53% stated a guideline from Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek (SKML) (Dutch EQA organisation) [10], also stated by 1.5% of the Belgian laboratories. Of the laboratories in the UK and Ireland, 15% and 8%, respectively, stated the name of a guideline from the British Society for Haematology (BSH) [11]. Overall, 19 and three laboratories stated the name of the *international* guidelines from CLSI (H21-A5) [7] and the World Health Organization (WHO) [12], respectively.

Discussion

The main findings of this study were that the pre-analytical practices for routine coagulation testing in European

laboratories varied widely, especially regarding centrifugation conditions and sample storage time requirements. In addition, few countries have national guidelines for pre-analytical issues in haemostasis testing acknowledged by a considerable number of the responding laboratories in the country.

Samples received from outside the hospital and citrate plasma verification

Performing coagulation testing in the wrong sample type (EDTA or heparin plasma or serum) could lead to erroneous results that could jeopardise patient safety [2]. However, it is challenging for laboratories who receive aliquoted plasma (secondary tubes) to verify that the correct sample material is received. Most laboratories in this study check that citrated plasma is written on the secondary tube and a few state that the primary tube should be sent together with the secondary tube (recommended in a very recent guidance [14]), but this is no guarantee. A few laboratories measure electrolytes, which may be helpful to distinguish EDTA plasma from citrated plasma and serum/lithium heparin plasma if used according to a specific algorithm [16]. However, this is resource demanding and is probably only performed if the wrong sample material is suspected. Fibrinogen measurement, mentioned by some laboratories, may indicate that the sample material is serum (no fibrinogen). Electrophoresis, mentioned by others, is a more resource-demanding fibrinogen method (no fibrinogen band in serum). Extremely prolonged or unmeasurable APTT, PT-sec and PT-INR are measured in sodium heparin plasma or serum [3] but could also be caused by supra-therapeutic concentrations of heparin/heparin contamination from a catheter. Measuring APTT, PT-sec, PT-INR and fibrinogen in EDTA plasma may apparently give reasonable results (normal or slightly pathological) and it is therefore more difficult to suspect wrong sample material [3]. Consequences may be erroneous dosing with unfractionated heparin (APTT) or vitamin K antagonists (PT-INR), or if further testing is performed, e.g. false diagnosis of factor VIII deficiency [3]. Unfortunately, an easily accessible and effective way to guarantee citrated plasma is to the best of our knowledge not available. It is preferable for the laboratories to receive primary tubes because this would ensure correct sample material and the opportunity to check the tube fill volume [5]. However, this requires that the samples are stable during transport.

Citrate concentration and tube fill requirements

The majority of the European laboratories use 105 or 109 mmol/L (3.2%) sodium citrate tubes, as recommended by most guidelines (Table 1). It was found that the international sensitivity index (ISI) was approximately 10% lower when determined in 129 mmol/L (3.8%) compared to 3.2% tubes, resulting in higher PT-INR results in 3.8% tubes, especially for the most sensitive thromboplastin reagents [17] in warfarin users [18]. In addition, the results from 3.8% tubes are more prone to be affected by underfilling, also potentially resulting in prolonged clotting times (e.g. APTT, PT-sec, PT-INR) [4].

In general, clotting times increase with decreasing tube fill volumes, which is caused by increased plasma dilution (liquid citrate in the tube) and increased calcium binding citrate per volume blood [4, 19, 20]. Most responding laboratories require the sample tubes to be almost completely filled ($\geq 90\%$), which is also recommended by the CLSI guideline [7]. It is, however, shown that the results were not statistically significantly different when obtained in 3.2% 4.5–5.0 mL citrate tubes with 60%, 70% and 60% tube fill volume, for PT-sec, APTT and fibrinogen, respectively [4, 19, 20]. In 3.6 mL tubes (3.2%), the acceptable fill volume was found to be 67%, 89% and 78% for PT-sec, APTT and fibrinogen, respectively [8], and in 2.7 mL (3.2%) tubes, it was 70%, 90% and 80% [21]. It should be noted that the acceptance criteria in these studies are different or not given. Paediatric tubes (small volume tubes [≤ 2.5 mL]) seem to be more prone to underfilling than regular tubes [22], still fewer responding laboratories had tube fill requirements for these tubes. In general, the tubes should be completely filled, however, the consequences of underfilling should be studied to be able to give advice to clinicians in emergency situations where underfilled tubes are received. Laboratories using different tubes (manufacturer and/or volume) than tested in published studies should validate their own system for acceptable tube fill volume.

Centrifugation conditions

Centrifugation forces and durations stated by laboratories varied widely and may be reflected by the fact that different guidelines give different recommendations (Table 1). Short centrifugation times may give increased coagulation times in routine coagulation tests if low centrifugation forces are used, i.e. 1500 g for less than

2 min prolong APTT and less than 5 min reduce fibrinogen concentration (Clauss method) [23]. Other studies have shown that high speed centrifuges (>4000 g) can be combined with short centrifugation times (2–5 min) for PT-sec, PT-INR, APTT and fibrinogen [24–26], and these centrifuges are used to some extent by the laboratories (Figure 4). Criteria for acceptable changes differ in the different studies; most of the studies only evaluate the results when the samples were analyzed right after sampling and centrifugation and some only have samples with results within the reference interval. Studies comparing different centrifugation conditions and their effect on the routine coagulation results are highly warranted. The CLSI guideline states that plasma should be platelet poor (thrombocytes $<10 \times 10^9/L$) and that centrifugation procedure should be validated every 6 months or after modification of the centrifuge to ensure the thrombocyte count does not exceed this limit [7]. However, the CLSI guideline does not state any acceptance criteria regarding the size of acceptable changes in coagulation test results. The need for harmonising centrifugation conditions in coagulation is increasing due to time efficiency problems of full automation laboratory systems when coagulometers are included.

Sample stability and storage

The acceptable storage time varies widely amongst European laboratories, especially for PT-INR and fibrinogen (in all sample materials). The CLSI guideline is very strict, demanding all coagulation tests to be centrifuged and analyzed within 4 h, except PT-INR within 24 h and APTT with unfractionated heparin treatment within 2 h, but also states that longer storage time could be used if this is validated in one's own laboratory [7]. There are studies indicating that both PT-INR and fibrinogen are stable for 48 h and APTT for 24 h, both in citrated blood and plasma [5, 6]. PT-INR and fibrinogen have even been shown to be stable for up to 72 h [27] and 7 days [28], respectively. The explanation for the longer acceptable storage times found in some published studies could be less strict acceptance criteria. The large variation in the guidelines (Table 1), and thereby the large variation in requirements in the laboratories, could be explained by this. It could also be that the laboratories have performed their own studies. Some published studies have used a mean difference of the results of 10% as a criterion for stability [5, 6], while others use more strict criteria, such as desirable bias based upon biological variation (APTT $\pm 2.8\%$, PT-sec $\pm 2\%$, fibrinogen $\pm 4.8\%$) [29].

The wide range in storage time requirements for PT-INR could in part be explained by the Nordic countries accepting longer storage time for PT-INR (median 24–48 h for the different sample materials) compared to the other countries (4–8 h). A similar tendency could be seen for fibrinogen, while accepted storage times for APTT were rather similar (data not shown). The longer storage time for PT-INR in the Nordic countries could be due to the use of the Owren method in these countries, not being affected by the fast decrease of the labile factor V [6], but this cannot explain the longer storage times accepted for fibrinogen.

Citrated blood for coagulation testing should not be stored in a cold environment because of possible cold activation of factor VII and thereby shorter coagulation times [30] and also loss of the von Willebrand factor and factor VIII [31, 32]. This is in agreement with the fact that many laboratories in the present study do not have requirements for acceptable storage times of refrigerated citrated blood samples (Table 2).

Most laboratories have a system for temperature control for sample storage in the laboratory, but not during transportation of samples. To avoid that samples are affected by low (cold activation during winter) or high temperatures, one could demand transportation of samples in specific boxes made for avoiding extremes of temperatures.

Although use of automated instruments with cap-piercing probably is increasing, 20% of the laboratories still store the samples uncapped for potential later requests. Prolonged storage of uncapped samples may lead to pH changes in the samples and thereby an increasing risk of erroneous results (prolonged clotting times with a pH decrease) [3, 33].

Clinical consequences of pre-analytical errors

Standardisation of the pre-analytical phase is important to avoid erroneous results released to the clinicians leading to wrong diagnoses and treatment. However, it is also important that the laboratory understands the effect of different pre-analytical errors, to help clinicians interpret a result from a non-optimal sample in case of an emergency. In the present study, as many as 84% and 72% of the responding laboratories would reject the samples if the requirements for tube fill volume and stability, respectively, were not fulfilled. In these cases, it is known that the clotting times will be falsely prolonged [4, 19, 34], and a result within the reference interval would potentially be

helpful for the clinician to avoid delay in diagnosis and treatment. However, it is important to analyse an optimally drawn blood sample as soon as possible to confirm the results. In other instances, the effect of the pre-analytical error is unpredictable, especially if several errors occur at the same time. In general, strict rules for pre-analytical handling of blood samples should be followed, but the laboratory should be able to give advice to clinicians if a pre-analytical error is known.

Pre-analytical guidelines

Some countries have national guidelines for pre-analytical issues (Table 1), but only France and the Netherlands have national guidelines (GHET [9] and SKML [10], respectively) which are widely known by the responding laboratories. The BSH guideline [11], was mentioned by a few, but it does not deal in detail with all the issues included in the present questionnaire. This could also be the reason why some reported the *international* guidelines from CLSI [7] and WHO [12] as their “national” guidelines.

The guidelines differ in some of their recommendations (Table 1) and none of them have discussed criteria for acceptable changes. The CLSI guideline is from 2008 and the WHO from 2002, so the several published studies in recent years should encourage a revised version of these guidelines including criteria used for deciding on acceptable pre-analytical handling.

Conclusions

Large variations in pre-analytical practices for routine coagulation testing were found in the European laboratories. This might be caused by the different recommendations in guidelines and results from published or own studies. Criteria for clinically acceptable changes are not included in the guidelines. The clinical consequences of the large variation in pre-analytical requirements are not known.

Acknowledgments: The authors thank EQALM and the national EQA organisations for distributing the questionnaire to their members, and all participating laboratories for their time and thoughtful completion of the questionnaire. We also thank Andreas Hillarp for valuable comments on the manuscript and the Norwegian Western Health Authority for post-doc scholarship for Ann Helen Kristoffersen.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organisation(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

- Favaloro EJ, Adcock Funk DM, Lippi G. Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. *Lab Med* 2012;43:1–10.
- Favaloro EJ, Lippi G, Adcock DM. Preanalytical and postanalytical variables: the leading causes of diagnostic error in hemostasis? *Semin Thromb Hemost* 2008;34:612–34.
- Adcock Funk DM, Lippi G, Favaloro EJ. Quality standards for sample processing, transportation, and storage in hemostasis testing. *Semin Thromb Hemost* 2012;36:576–85.
- Adcock DM, Kressin DC, Marlar RA. Minimum specimen volume requirements for routine coagulation testing: dependence on citrate concentration. *Am J Clin Pathol* 1998;109:595–9.
- Zurcher M, Sulzer I, Barizzi G, Lammler B, Alberio L. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. *Thromb Haemost* 2008;99:416–26.
- Linskens EA, Devreese KM. Pre-analytical stability of coagulation parameters in plasma stored at room temperature. *Int J Lab Hematol* 2018;40:292–303.
- CLSI. Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays, Approved Guideline – Fifth Edition. CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
- Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favaloro EJ. Quality standards for sample collection in coagulation testing. *Semin Thromb Hemost* 2012;38:565–75.
- Polack B, Schved JF, Boneu B, Groupe d'Etude sur l'Hemostase et la T. Preanalytical recommendations of the 'Groupe d'Etude sur l'Hemostase et la Thrombose' (GEHT) for venous blood testing in hemostasis laboratories. *Haemostasis* 2001;31:61–8.
- Sectie Stolling van de Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek. Preanalytische voorschriften voor stollingsbepalingen, 2012. Available at: <https://www.nvkc.nl/sites/default/files/11.pdf>. Accessed: 2019.
- Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M, et al. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *Int J Lab Hematol* 2013;35:1–13.
- Use of anticoagulants in diagnostic laboratory investigations and Stability of blood, plasma and serum samples, 2002. Available at: https://apps.who.int/iris/bitstream/handle/10665/65957/WHO_DIL_LAB_99.1_REV.2.pdf. Accessed: 2019.
- Lippi G, Plebani M, Favaloro EJ. Interference in coagulation testing: focus on spurious hemolysis, icterus, and lipemia. *Semin Thromb Hemost* 2013;39:258–66.
- Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thromb J* 2016;14:49.
- Stavelin A, Albe X, Meijer P, Sarkany E, MacKenzie F. An overview of the European Organization for External Quality Assurance Providers in Laboratory Medicine (EQALM). *Biochem Med (Zagreb)* 2017;27:30–6.
- Lippi G, Salvagno GL, Adcock DM, Gelati M, Guidi GC, Favaloro EJ. Right or wrong sample received for coagulation testing? Tentative algorithms for detection of an incorrect type of sample. *Int J Lab Hematol* 2010;32:132–8.
- Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs. 3.8% sodium citrate concentration on routine coagulation testing. *Am J Clin Pathol* 1997;107:105–10.
- Duncan EM, Casey CR, Duncan BM, Lloyd JV. Effect of concentration of trisodium citrate anticoagulant on calculation of the International Normalised Ratio and the International Sensitivity Index of thromboplastin. *Thromb Haemost* 1994;72:84–8.
- Reneke J, Etzell J, Leslie S, Ng VL, Gottfried EL. Prolonged prothrombin time and activated partial thromboplastin time due to underfilled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. *Am J Clin Pathol* 1998;109:754–7.
- Pretorius L, Janse van Rensburg WJ, Conradie C, Coetzee MJ. Minimum citrate tube fill volume for routine coagulation testing. *Int J Lab Hematol* 2014;36:493–5.
- Ver Elst K, Vermeiren S, Schouwers S, Callebaut V, Thomson W, Weekx S. Validation of the minimal citrate tube fill volume for routine coagulation tests on ACL TOP 500 CTS(R). *Int J Lab Hematol* 2013;35:614–9.
- Chuang J, Sadler MA, Witt DM. Impact of evacuated collection tube fill volume and mixing on routine coagulation testing using 2.5-mL (pediatric) tubes. *Chest* 2004;126:1262–6.
- Lippi G, Salvagno GL, Montagnana M, Manzato F, Guidi GC. Influence of the centrifuge time of primary plasma tubes on routine coagulation testing. *Blood Coagul Fibrinolysis* 2007;18:525–8.
- Sultan A. Five-minute preparation of platelet-poor plasma for routine coagulation testing. *East Mediterr Health J* 2010;16:233–6.
- Dimeski G, Solano C, Petroff MK, Hynd M. Centrifugation protocols: tests to determine optimal lithium heparin and citrate plasma sample quality. *Ann Clin Biochem* 2011;48:218–22.
- Nelson S, Pritt A, Marlar RA. Rapid preparation of plasma for 'Stat' coagulation testing. *Arch Pathol Lab Med* 1994;118:175–6.
- Baglin T, Luddington R. Reliability of delayed INR determination: implications for decentralized anticoagulant care with off-site blood sampling. *Br J Haematol* 1997;96:431–4.
- Heil W, Grunewald R, Amend M, Heins M. Influence of time and temperature on coagulation analytes in stored plasma. *Clin Chem Lab Med* 1998;36:459–62.
- Salvagno GL, Lippi G, Montagnana M, Franchini M, Poli G, Guidi GC. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. *Int J Lab Hematol* 2009;31:462–7.
- Palmer RN, Gralnick HR. Cold-induced contact surface activation of the prothrombin time in whole blood. *Blood* 1982;59:38–42.

31. Favaloro EJ, Soltani S, McDonald J. Potential laboratory misdiagnosis of hemophilia and von Willebrand disorder owing to cold activation of blood samples for testing. *Am J Clin Pathol* 2004;122:686–92.
32. Bohm M, Taschner S, Kretzschmar E, Gerlach R, Favaloro EJ, Scharrer I. Cold storage of citrated whole blood induces drastic time-dependent losses in factor VIII and von Willebrand factor: potential for misdiagnosis of haemophilia and von Willebrand disease. *Blood Coagul Fibrinolysis* 2006;17:39–45.
33. Narayanan S. Preanalytical aspects of coagulation testing. *Haematologica* 1995;80:1–6.
34. Cadamuro J, Simundic AM, Ajzner E, Sandberg S. A pragmatic approach to sample acceptance and rejection. *Clin Biochem* 2017;50:579–81.

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/cclm-2019-0214>).