Fibrinogen measurement in cardiac surgery with cardiopulmonary bypass: Analysis of repeatability and agreement of Clauss method within and between six different laboratories

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Summary
Plasma fibrinogen concentration is important for coagulopathy assessment, and is most commonly measured using the Clauss method. Several factors, including device type and reagent, have been shown to affect results. The study objective was to evaluate performance and repeatability of the Clauss method and to assess differences between measurements performed during and after cardiopulmonary bypass (CPB), by testing plasma samples from patients undergoing cardiac surgery with CPB. Samples were collected from 30 patients before surgery, approximately 20 minutes before weaning from CPB, and 5 minutes after CPB and protamine. Fibrinogen concentration was determined using the Clauss method at six quality-controlled specialised laboratories, according to accredited standard operating procedures. Regarding within-centre agreement for Clauss measurement, mean differences between duplicate measurements were between 0.00 g/l and 0.15 g/l, with intervals for 95% limits of agreement for mean Bland-Altman differences up to 1.3 g/l. Regarding between-centre agreement, some mean differences between pairs of centres were above 0.5 g/l. Differences of up to ~2 g/l were observed with individual samples. Increased variability was observed between centres, with inter-class correlation values below 0.5 suggesting only fair agreement. There were no significant differences in fibrinogen concentration before weaning from CPB and after CPB for most centres and methods. In conclusion, considerable differences exist between Clauss-based plasma fibrinogen measured using different detection methods. Nevertheless, the similarity between measurements shortly before weaning from CPB and after CPB within centres suggests that on-pump measurements could provide an early estimation of fibrinogen deficit after CPB and thus guidance for haemostatic therapy.

Keywords
Blood coagulation tests, clinical laboratory techniques, fibrinogen, nephelometry and turbidimetry, patient care management

Introduction
There are over 60 different methods available to determine plasma fibrinogen concentration (1), the most commonly used being the Clauss method, a form of thrombin time (2). In the Clauss method, diluted, citrated platelet-poor plasma (PPP) is activated with an excess of thrombin (~100 U/ml) and the time taken for the coagulometer to stop at defined points is determined; this time is converted into a measurement of the functional fibrinogen concentration using calibration standards (3, 4). Several methods have been developed to detect the fibrin clot endpoint generated in the Clauss assay; automated coagulation analysers incorporating mechanical, electromechanical or photo-optical detection are commonly used.

Multiple commercially produced Clauss assays are available, with marked differences in thrombin concentration and source (e.g. bovine or human) and in the concentration of stabilisers, inhibitors, and buffers, etc. (5, 6). Furthermore, the measurement of plasma fibrinogen concentration can be affected by a number of factors, including the type of device (7, 8), reagents (8), assay method (9), and calibrator (10, 11) used. In addition, a number of different reference plasmas are available for use as calibrators, and differences in composition exist between these products (12). The Clauss assay is influenced by the presence of colloid plasma ex-
panders such as hydroxyethyl starch (HES) (7, 13, 14), and both heparin and fibrinogen degradation products (FDPs) (15). Considering the number of factors that can affect the Clauss assay, it is perhaps not surprising that fibrinogen measurements have been shown to vary widely between laboratories (8, 10, 11, 16-18).

Historically, Clauss methods have been used in epidemiological investigations to evaluate the accuracy concerning fibrinogen as a thromboembolic risk factor (19). Recently, interest in fibrinogen measurement has increased due to its role in guiding fibrinogen supplementation in the perioperative setting (20). In this context, long laboratory turnaround times could delay the identification of coagulation defects such as hypofibrinogenemia (21, 22) and hence the administration of fibrinogen as haemostatic therapy. One option for minimising treatment delays would be to shorten the turnaround times for laboratory measurement of plasma fibrinogen concentration, and some centres have successfully implemented this (23). However, within centre, and more importantly between centre, variability of the Clauss methods used to guide haemostatic therapy in the perioperative setting have not been investigated so far. The question as to whether the Clauss assay could be used to measure fibrinogen concentration before weaning from cardiopulmonary bypass (CPB) i.e. on pump, has already been raised in the literature (24) and investigated in a single centre study (25).

The aim of this study was to evaluate, in a clinical setting, the performance and repeatability of the Clauss assay incorporating different reagent and coagulation analyser combinations with different detection methods. The same plasma samples, taken at several time points from patients undergoing cardiac surgery with CPB, were tested in six different laboratories.

Materials and methods

The study was approved by the local Ethics committee (protocol number 2627). Patients undergoing cardiovascular surgery involving CPB at IRCCS Policlinico San Donato, Milan, Italy were eligible for inclusion. All patients provided written informed consent to participate in the study and were interviewed regarding their intake of coagulation-influencing medication.

The primary outcome of the study was the reproducibility of a new fibrin-based thromboelastometry test, and this has been published elsewhere (26). The present manuscript describes the secondary outcome, i.e. measurement of plasma fibrinogen concentration in six quality-controlled laboratories to which the authors are affiliated, with the exception of CSL Behring and the laboratory in the Department of Anaesthesiology and Intensive Care, Skåne University Hospital and Lund University, Lund, Sweden. For reference, each centre was designated a number from 1 to 6.

Patient management

Patients were operated upon according to the clinic’s standard protocol. Detailed methodology has been published previously (26).

Coagulation analyses

Blood samples were collected at the start of surgery, approximately 20 minutes (min) before weaning from CPB, and 5 min after neutralisation of heparin (i.e. post-protamine, following weaning from CPB) from a radial artery catheter (20 Gauge) into commercially available pre-filled Monovette collection tubes (Sarstedt, Nuremberg, Germany), which contained citrate (0.106 mol/l) as anticoagulant at a ratio of 9:1 (blood:anticoagulant). Each whole-blood sample was centrifuged at 2,000 g for 15 min to obtain PPP, which was then separated into aliquots and stored at -80°C. Each laboratory received at least one aliquot of all 90 PPPs (three time points for each of 30 patients).

Fibrinogen measurements using the Clauss assay

The Clauss assay was performed according to accredited standard operating procedures. Details of the assays performed in each laboratory, along with the analysers and calibrators used are shown in Table 1. The clotting reaction was initiated with the addition of thrombin reagent and the time to clot formation was measured. The coagulation signal was detected by photo-optical, electromagnetic, or steel ball mechanical readout methods. The time to clot formation in seconds was converted into fibrinogen concentration (g/l) according to a local standard calibration curve prepared by testing calibrators of each Clauss assay according to the manufacturer’s instructions. The only exception to this was centre 4 where calibration was carried out using a barcode provided by the manufacturer. Plasma fibrinogen concentration was determined in duplicate at each time point.

Fibrinogen measurement using fibrinogen antigen levels

These were determined by photo-optical detection at two centres. Details of the analysers and calibrators used are shown in Table 1. Single fibrinogen antigen concentration measurements were performed using antiserum raised in rabbit against human fibrinogen.

Heparin levels

Heparin levels were deduced via chromogenic anti-Xa and anti-IIa assays (Biophen Heparin assays; Hyphen Biomed, Neuville-sur-Oise, France) at centre 1 only and have been published elsewhere (26).

Fibrinogen measurements in the 1st WHO International Standard

The 1st WHO International Standard for Fibrinogen Concentrate consists of aliquots of freeze-dried pooled fibrinogen concentrate from human plasma, (15 mg total protein; 10.4 mg clottable protein). For calibration of secondary and/or in-house working standards of fibrinogen concentrate the total contents of the aliquot...
were reconstituted at room temperature with 1 ml distilled water (27). To assess the comparability of the fibrinogen tests between different centres, the 1st WHO International Standard was distributed to each laboratory along with the patients’ plasma samples. The Clauss method was used to measure fibrinogen concentration in the 1st WHO International Standard in five of the six laboratories, while the fibrinogen antigen concentration was measured in two of the laboratories. All measurements were performed in duplicate.

Statistical analyses
For each variable, the data distribution was tested graphically using normal probability plots and histograms. Continuous variables with a normal distribution are presented as mean ± standard deviation (SD). Statistical calculations were performed using Stata 12.1 (StataCorp LP, College Station, TX, USA).

Within-centre agreement
Agreement between duplicate measurements within each centre at each time point was assessed firstly by the Bland-Altman limits of agreement method, which calculates the mean, SD and 95% limits of agreement for the difference between repeat measurements. A second assessment of within-centre agreement was the intra-class correlation (ICC), which examines the proportion of the total variation that is due to differences between the samples versus differences between repeat measurements of the same samples.

Between-centre agreement
Using only the first measurement from each centre at each time point, the Bland-Altman limits of agreement method was used as above to compare the agreement between pairs of centres using the same detection method (i.e. photo-optical or mechanical). The ICC was also used as above to assess agreement between centres using the same detection method.

Comparison between time points
Comparison of fibrinogen concentrations was made for plasma collected at the before weaning from CPB and post-protamine time points. Changes in values over time were found to be normally distributed; therefore, the paired t-test was used for all analyses. Only the first measurements were used from each centre, and separate analyses were performed for each method and each centre.

Table 1: Assays performed, and devices and reagents used by centre.

<table>
<thead>
<tr>
<th>Centre</th>
<th>Test</th>
<th>Detection method</th>
<th>Coagulation Analyser</th>
<th>Calibrator</th>
<th>Thrombin reagent</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre-1</td>
<td>Fibrinogen Clauss</td>
<td>Photo-optical</td>
<td>ACL Top 700</td>
<td>HaemosIL Normal Control</td>
<td>HemosIL QFA thrombin</td>
<td>Instrumentation Laboratory, Milan, Italy</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen Antigen</td>
<td>Photo-optical</td>
<td></td>
<td></td>
<td>Liaphen fibrinogen</td>
<td>Instrumentation Laboratory, Milan, Italy</td>
</tr>
<tr>
<td>Centre-2</td>
<td>Fibrinogen Clauss</td>
<td>Photo-optical</td>
<td>BCS XP</td>
<td>Fibrinogen Calibrator Standard 1–6</td>
<td>Multifibrin U reagent</td>
<td>Siemens Healthcare Diagnostics, Marburg, Germany</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen Antigen</td>
<td>Photo-optical</td>
<td>BN II</td>
<td>N Protein Standard PY</td>
<td>NAS FIB reagent</td>
<td>Hyphen Biomed, Neuville-sur-Oise, France</td>
</tr>
<tr>
<td>Centre-3</td>
<td>Fibrinogen Clauss</td>
<td>Photo-optical</td>
<td>Sysmex CA-7000</td>
<td>Plasma calibration kit Sclavo Diagnostics</td>
<td>Sclavo fibrinogen kit</td>
<td>Siemens Healthcare Diagnostics, Marburg, Germany</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen Antigen</td>
<td>Photo-optical</td>
<td></td>
<td></td>
<td></td>
<td>Sclavo Diagnostics, Sovicille Italy</td>
</tr>
<tr>
<td>Centre-4</td>
<td>Fibrinogen Clauss</td>
<td>Electro-mechanical</td>
<td>STA-Compact</td>
<td>Barcode calibration</td>
<td>STA Fib 2</td>
<td>Diagnostica Stago ASA, Asnieres sur Seine, France</td>
</tr>
<tr>
<td>Centre-5</td>
<td>Fibrinogen Clauss</td>
<td>Photo-optical</td>
<td>Sysmex CA-1500</td>
<td>Standard Human Plasma</td>
<td>Dade thrombin reagent</td>
<td>Siemens Healthcare Diagnostics, Marburg, Germany</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steel ball – mechanical</td>
<td>KC10A</td>
<td>Coagulation Control N</td>
<td>Fibrinogen reagent</td>
<td>Amelung GmbH, Lemgo, Germany</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Technoclone GmbH, Vienna, Austria</td>
</tr>
<tr>
<td>Centre-6</td>
<td>Fibrinogen Clauss</td>
<td>Photo-optical</td>
<td>ACL Top 700</td>
<td>Hemosil Calibration Plasma</td>
<td>Hemosil Q.F.A. thrombin</td>
<td>Instrumentation Laboratory, Milan, Italy</td>
</tr>
</tbody>
</table>
Results

Patient characteristics

Patient characteristics and surgical procedures have been described previously (26).

Clauss-derived fibrinogen concentration measurements

Mean (SD) values of fibrinogen concentration measurements for each centre and each type of detection method are shown in ▶Table 2 and ▶Figure 1, as the mean of the first measurement for each plasma sample from each centre.

Within-centre agreement

Bland-Altman method

Overall, there was little difference between duplicate measurements, as assessed using the Bland-Altman method (▶Table 3). At most centres mean differences between duplicate measurements were ≤0.05 g/l at all three time points. At centre 2 and centre 5, at all time points the intervals between the 95% limits of agreement for mean Bland-Altman differences were narrow, and the majority of repeat measurements were within 0.15 g/l of each other. At centre 3 (mean difference of 0.07 g/l, start of surgery and post-protamine) and centre 6 (mean difference of 0.15 g/l start of surgery) the intervals between the 95% limits of agreement for mean Bland-Altman differences were wider than for the other centres, with observed differences up to 1.3 g/l.

Table 2: Mean (SD) values of fibrinogen concentration measurements at each centre, and comparison of the differences in fibrinogen concentration measurement at the before weaning from CPB and post-protamine time points. CPB, cardiopulmonary bypass.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Centre</th>
<th>Start of surgery</th>
<th>Before weaning from CPB</th>
<th>Post-protamine</th>
<th>Mean change (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo-optical</td>
<td>1</td>
<td>2.04 (0.61)</td>
<td>1.29 (0.38)</td>
<td>1.30 (0.58)</td>
<td>0.01 (-0.14, 0.17)</td>
<td>0.86</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>2</td>
<td>2.81 (0.72)</td>
<td>1.61 (0.67)</td>
<td>1.74 (0.68)</td>
<td>0.13 (0.01, 0.24)</td>
<td>0.03</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>3</td>
<td>2.67 (1.03)</td>
<td>1.85 (0.64)</td>
<td>1.68 (0.79)</td>
<td>-0.17 (-0.42, 0.08)</td>
<td>0.18</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>5</td>
<td>3.04 (0.76)</td>
<td>1.96 (0.52)</td>
<td>1.95 (0.63)</td>
<td>-0.01 (-0.16, 0.14)</td>
<td>0.89</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>6</td>
<td>2.73 (0.87)</td>
<td>1.67 (0.59)</td>
<td>1.65 (0.69)</td>
<td>-0.02 (-0.21, 0.17)</td>
<td>0.85</td>
</tr>
<tr>
<td>Electromechanical</td>
<td>4</td>
<td>3.42 (1.08)</td>
<td>1.94 (0.81)</td>
<td>1.92 (0.92)</td>
<td>-0.02 (-0.25, 0.22)</td>
<td>0.87</td>
</tr>
<tr>
<td>Steel ball</td>
<td>5</td>
<td>3.68 (1.07)</td>
<td>2.34 (0.71)</td>
<td>2.22 (0.73)</td>
<td>-0.11 (-0.30, 0.07)</td>
<td>0.23</td>
</tr>
<tr>
<td>Antigen</td>
<td>1</td>
<td>3.13 (0.87)</td>
<td>2.05 (0.78)</td>
<td>2.09 (0.80)</td>
<td>0.04 (-0.10, 0.19)</td>
<td>0.55</td>
</tr>
<tr>
<td>Antigen</td>
<td>2</td>
<td>3.62 (1.03)</td>
<td>2.10 (0.87)</td>
<td>2.17 (0.86)</td>
<td>0.07 (-0.10, 0.25)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

ICC method

Similar results were found with this method (▶Table 3). At centres 1, 2, 4 and 5, ICC values close to 1 were observed at all time points. At centres 3 and 6 ICC values were lower, with values below 0.75 at centre 3 (before weaning from CPB and post-protamine). Thus, in a few cases, reduced repeatability of measurements was apparent.

Between-centre agreement

To evaluate the impact of the centre, the agreement between Clauss assays using similar detection methods but run at different centres was analysed (e.g. photo-optical Clauss at centre 1 vs photo-optical Clauss at centre 2). The Suppl. Figure 1 (available online at www.thrombosis-online.com) shows fibrinogen concentration measurements at each centre, for each patient, at all three time points. Variability was apparent between centres using the same detection method, and between centres using different detection methods.

Bland-Altman method

Variation was found between all pairs of centres at all time points (▶Table 4). Although some mean differences between pairs of centres were below 0.15 g/l, others were above 0.5 g/l, indicating that clinically significant differences were likely. Moreover, the intervals between the 95% limits of agreement were wide underlining the potential for significant variability. Accordingly, differences of up to ~2 g/l were observed with individual samples (see Suppl. Figure 1, available online at www.thrombosis-online.com). With
the photo-optical method, values from centre 1 were consistently lower than those from all other centres. In addition, the steel ball mechanical method (centre 5) gave higher values than the electromechanical method (centre 4) at all time points.

For the antigen measurement of fibrinogen levels, small average differences were found between the two centres before weaning from CPB and post-protamine. However, at the start of surgery time point, values from centre 2 were, on average, 0.5 g/l lower than values from centre 1. As with the Clauss assays, intervals between the 95% limits of agreement suggested a potential for clinically significant differences between the two centres at all time points.

Table 3: Within-centre agreement of Clauss-derived fibrinogen concentration measurements. CPB, cardiopulmonary bypass; ICC, intra-class correlation.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Centre</th>
<th>Time point</th>
<th>Start of surgery</th>
<th>Before weaning from CPB</th>
<th>Post-protamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bland-Altman mean difference (95% limits of agreement)</td>
<td>ICC</td>
<td>Bland-Altman mean difference (95% limits of agreement)</td>
<td>ICC</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>1</td>
<td>0.05 (-0.27, 0.36)</td>
<td>0.961</td>
<td>-0.02 (-0.22, 0.18)</td>
<td>0.962</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>2</td>
<td>-0.01 (-0.09, 0.06)</td>
<td>0.998</td>
<td>0.00 (-0.11, 0.10)</td>
<td>0.997</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>3</td>
<td>-0.07 (-0.84, 0.70)</td>
<td>0.932</td>
<td>0.00 (-0.91, 0.91)</td>
<td>0.690</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>5</td>
<td>0.00 (-0.13, 0.12)</td>
<td>0.997</td>
<td>-0.01 (-0.16, 0.14)</td>
<td>0.994</td>
</tr>
<tr>
<td>Photo-optical</td>
<td>6</td>
<td>0.15 (-0.92, 1.24)</td>
<td>0.788</td>
<td>0.04 (-0.35, 0.43)</td>
<td>0.942</td>
</tr>
<tr>
<td>Electromechanical</td>
<td>4</td>
<td>0.05 (-0.19, 0.30)</td>
<td>0.992</td>
<td>0.02 (-0.18, 0.21)</td>
<td>0.992</td>
</tr>
<tr>
<td>Steel ball</td>
<td>5</td>
<td>0.02 (-0.13, 0.17)</td>
<td>0.997</td>
<td>0.02 (-0.07, 0.12)</td>
<td>0.997</td>
</tr>
</tbody>
</table>
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ICC method

ICC values suggested only fair agreement between centres, when either photo-optical or mechanical detection methods were used for fibrinogen measurement (Table 4). For both methods, ICCs below 0.5 were observed at all time points, with the exception of mechanical methods at the start of surgery time point, for which an ICC of 0.681 was calculated. For fibrinogen antigen levels, ICC values were 0.75 or above at all time points (Table 4).

Overall, the results indicate more variation between centres than between duplicate measurements within a centre. There was a higher level of agreement between centres using the antigen method than between centres using the Clauss assay with different detection methods.

Comparison between time points

The differences between fibrinogen concentration measurements made before weaning from CPB and post-protamine are summarised in Table 2. There was no significant difference in fibrinogen measurements between the two time points for the different centres and methods with the exception of centre 2.

Fibrinogen standard

The fibrinogen concentrations obtained with the 1st WHO International Standard are shown in Table 5. The values from centre 6 were omitted due to technical issues during measurement. As with the patient samples, variations between centres were much greater than those between duplicate measurements within centres. The difference between the two centres measuring levels of fibrinogen antigen was large, with a mean of 4.5 g/l for centre 1 compared with 12.8 g/l for centre 2.

Discussion

The results of this study show that the measurement of plasma fibrinogen concentration using the Clauss assay or antigen levels is

<table>
<thead>
<tr>
<th>Method</th>
<th>Time point</th>
<th>Start of surgery</th>
<th>Before weaning from CPB</th>
<th>Post-protamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bland-Altman mean difference (95% limits of agreement)</td>
<td>ICC</td>
<td>Bland-Altman mean difference (95% limits of agreement)</td>
<td>ICC</td>
</tr>
<tr>
<td>ICC</td>
<td>–</td>
<td>–</td>
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<td>ICC</td>
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<td>ICC</td>
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<tr>
<td>ICC</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

Table 5: Fibrinogen concentration values obtained with the 1st WHO International Standard.

<table>
<thead>
<tr>
<th>Fibrinogen concentration (g/l)</th>
<th>Photo-optical</th>
<th>Mechanical</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre 1</td>
<td>Centre 2</td>
<td>Centre 3</td>
<td>Centre 5</td>
</tr>
<tr>
<td>7.9</td>
<td>6.5</td>
<td>6.9</td>
<td>7.9</td>
</tr>
<tr>
<td>8.5</td>
<td>6.4</td>
<td>6.9</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Plasma fibrinogen concentration is important for coagulopathy. Although agreement between duplicate fibrinogen measurements within centres was generally observed, examples of clinically significant differences were apparent. Variability of measurements between centres was greater than the variability of duplicate measurements within centres, with clinically significant differences likely to be encountered. This study also indicated that there is no significant difference within centres between fibrinogen measurements made shortly before and after weaning from CPB, with a significant increase observed only in one centre.

Prompt availability of coagulation test results at the end of CPB is mandatory for appropriate bleeding management, in particular for patients with severe bleeding for whom delays to treatment must be minimised. In practice, turnaround times for the Clauss assay are typically around 30–60 min (28, 29), and longer times of around 88 min have also been reported (21). The current solution, recommended in European guidelines, is to implement viscoelastic devices (30, 31) to assist in characterising the coagulopathy and in guiding haemostatic therapy (30). These tests can be performed at the point-of-care and the results can be available in 5–10 min, much quicker than the turnaround times for fibrinogen concentration (21, 22). However, further standardisation of these methods is needed (32), and it should be kept in mind that not all centres can afford this technology or they do not have the personnel or the infrastructure to implement it. Nevertheless, as the measurements before and after weaning from CPB in our study showed no significant difference in the majority of centres, it is possible that fibrinogen measurement could be carried out during CPB. This could provide an early estimation of a fibrinogen deficit after CPB, which may potentially impact the time point at which haemostatic therapy is administered.

Considerable variation in plasma fibrinogen measurements between laboratories has been shown previously (8, 10, 11, 16–18), in line with the results from this study. The laboratories in this study used five different Clauss brands, which are different in relation to source material (e.g. bovine or human thrombin) (5, 6) and this, together with the use of different coagulation analysers, is likely to have caused some of the observed variability. The type of reagent used has been shown to have the greatest effect on accuracy and the instrument type the greatest effect on precision (8). The type of calibrator used may also impact the agreement between measurements. In our study, centres 1 and 6 used the same Clauss reagent and the same instrument, but different calibrators. The differences between duplicate measurements within these centres were 0.05 and 0.15 g/l, respectively, while the Bland–Altman mean difference between the two centres was among the highest observed in the study (0.69 g/l). The use of a common calibrator has been shown to improve both the overall between-laboratory and between-reagent variation (10, 11). However, it has also been shown that variability still exists (16) and it has been suggested that a degree of variation between laboratories would persist regardless of whether a common calibrator were used (16). Results with the 1st WHO International Standard also highlight the potential for differences between detection methods (e.g. higher values with mechanical vs photo-optical detection) with only centre 5 using steel ball mechanical detection giving an accurate result. This may also be true for the antigen method; although both centres used photo-optical detection, centre 1 performed turbidimetry with latex enhancement while centre 2 used a nephelometer designed for protein measurements.

Most previously published quality control investigations were carried out on pooled plasma or plasma samples from healthy volunteers (33, 34). To our knowledge, this is the first study to compare the performance and repeatability of Clauss assays across multiple laboratories in a real-life perioperative cardiovascular surgery setting. In contrast to fibrinogen testing in congenital hypo- or afibrinogenaemia, or epidemiological studies, in this setting the measurement might be influenced by a heavily impaired coagulation system caused by CPB (35), and potential confounding factors such as FDPs and assay-influencing drugs like heparin or colloids. A potential effect of heparin may be suspected when the values obtained after CPB are higher than the ones obtained on CPB (e.g. a mean increase of 0.13 g/l in centre 2, which used Multifibren U as reagent). Multifibren U has been shown to be sensitive to heparin levels of ≥2 IU/ml (36), and in our study, levels at the before weaning from CPB time point exceeded this. The mean anti-factor Xa activity was 2.8 U/ml and the mean anti-factor IIa activity was 2.1 U/ml, with a maximum of 4.55 U/ml and 2.7 U/ml, respectively (26). To minimise heparin interference, some commercially available fibrinogen reagents contain heparin neutralisers and high concentrations of thrombin (4, 5). In addition, the majority of the Clauss brands used in this study are sensitive to increased levels of FDPs; their presence may prolong clotting and give falsely low fibrinogen concentrations. However, it must be noted that it is highly unlikely that a difference between measurements pre- and post-CPB is explained by FDP influence. It must be noted that in centre 3 a decrease in fibrinogen concentration of 0.17 g/l was observed between the before weaning from CPB and...
post-protamine time points. As centre 3 showed the largest difference between duplicate measurements, one may speculate that the difference is related, at least partly, to within-centre variability. It is unclear what other factors may have influenced these differences, but it is notable that the mean difference between these time points did not exceed 0.2 g/l.

The differences between measurements of fibrinogen in this study may be clinically significant, as they could potentially lead to inappropriate treatment decisions. For example, the plasma fibrinogen concentration of a patient sample could be measured by the Clauss assay in two centres as 1.4 g/l and 2.0 g/l, respectively. When institutions are following guidelines with clear trigger levels for fibrinogen supplementation this would result in therapy to supplement fibrinogen in the first instance but not the second (30, 31). This indicates a need for standardisation of fibrinogen testing in the perioperative field, a requirement which could be investigated by the ISTH/SCC Fibrinogen Subcommittee. However, absolute consistency is unlikely to be achieved even with a single, standardised, reproducible assay and the use of a single, common calibrator, as differences related to the readout method and the coagulometers may still exist. Further studies are required to compare these methods in different clinical settings, such as trauma, transplant surgery, and peripartum. [As a note, consistency in the nomenclature of the Clauss method should be a goal as in the past it has been erroneously referred to as the "von Clauss" method (19, 37). In the original publication in German (2), "von" is not part of the name (i.e. a title of nobility), but simply means "written by" A. Clauss (Arnold Clauss).]

In conclusion, this study found clinically significant differences between the performance of assays for measuring plasma fibrinogen concentration, particularly between different laboratories. Improved standardisation of fibrinogen measurement may reduce this variability, although it is unclear how absolute consistency could be achieved. Nevertheless, within-centre comparability between fibrinogen measurements before and after weaning from CPB suggests that on-pump measurements could provide an early estimation of fibrinogen deficit expected after CPB allowing clinicians to make earlier decisions on, and potentially provide earlier administration of, the appropriate haemostatic therapy.

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Conflicts of Interest
CS is an employee of CSL Behring and previously received speaker honoraria and research support from Tem International and CSL Behring and travel support from Haemoscope Ltd (former manufacturer of TEG®). CJS has received research support and speaker fees from CSL Behring and research support from Tem International. HS has received study grants and speaker fees from CSL Behring and Tem International. LA has received speaker honoraria or research support from Axon Lab, Bayer, Boehringer Ingelheim, CSL Behring, GlaxoSmithKline, Pfizer, Sanofi Aventis, and Vifor. MR received speaker honoraria and research support from CSL Behring and Grifols, speaker honoraria from Medtronic and Haemoscope, research support from Tem International and was on the Steering committee of a FXIII study (until 2010) for Novo Nordisk. EB, AT, JC and DW have no conflicts of interest to declare.

References