Point-of-care coagulation testing

Amit Srivastava FRCA FFICM
Andrea Kelleher MB BS FRCA

Revalidation
FOR ANAESTHETISTS
RCA Revalidation matrix
Matrix reference
1A03, 2A04, 3G00

Key points

The range of available point-of-care tests continues to increase; clinicians must review developing technologies relevant to their practice, ensuring that their patients benefit from new advances.

Whole blood analysis forms the mainstay of perioperative coagulation point-of-care testing (POCT) allowing insight into the complex interactions within the clotting system, reducing turnaround test times, and helping minimize inappropriate transfusion.

Preoperative platelet function testing is useful in identifying responders and non-responders to antiplatelet agents, thus allowing optimization of the timing of surgery.

Any POCT facility must be supported by rigorous quality control and oversight by a multidisciplinary committee responsible for maintaining standards.

Amit Srivastava FRCA FFICM
Consultant in Anaesthesia and Intensive Care
Glenfield Hospital
University Hospitals of Leicester NHS Trust
Leicester LE3 9QP
UK

Andrea Kelleher MB BS FRCA
Consultant Cardiothoracic Anaesthetist
Department of Anaesthesia and Intensive Care
Royal Brompton Hospital
London SW3 6NP
UK
Tel: +44 20 7351 8247/+44 20 7351 8526
Fax: +44 20 7351 8524
E-mail: a.kelleher@rbht.nhs.uk
(for correspondence)

Point-of-care testing (POCT) may be defined as the rapid specific testing of bodily fluids at the bedside. There is an ever-increasing array of POCT available, most of which are beyond the remit of this article which will concentrate upon those commonly used and relevant to the management of perioperative coagulation.

Owing to the relatively long turnaround time of conventional laboratory tests, and with the aim of reducing inappropriate transfusion, there is increasing interest in point-of-care coagulation testing (POCCT) which can provide results within minutes. In addition, the wide acceptance of the current cell-based model of coagulation has led to an emphasis on the pivotal role of platelets and particular interest in devices testing whole blood, rather than plasma used in laboratory-based tests. The latter include the prothrombin time (PT) and activated partial thromboplastin time (aPTT) which provide information on the first phase of coagulation up to fibrin formation. Conversely, viscoelastic POCCT devices produce information on all phases of coagulation, providing insight into interactions between the cellular and plasma components of whole blood and the activity of the fibrinolytic system; all of which are vital for successful haemostasis.[1]

The advantages and disadvantages of POCT are listed in Table 1.

Viscoelastic point-of-care devices

Thromboelastography (TEG®; Haemonetics Corporation, Braintree, MA, USA) and rotational thromboelastometry (Rotem®; Tem International GmbH, Munich, Germany) provide the continuous measurement and display of the viscoelastic properties of a whole blood sample from the initial phase of fibrin formation to clot retraction and ultimately fibrinolysis.[2]

In the TEG® device, 360 μl of whole blood is added to activators in two disposable heated (37°C) cups. A pin attached to a torsion wire is immersed into the blood and the cup rotates through 4°45′ in either direction, each rotation lasting 10 s. The pin initially remains stationary generating a straight line on the tracing, but as the blood clots, the rotational movement of the cup is transmitted to the pin. A mechanical–electrical transducer converts the torsion on the pin into the characteristic TEG tracing from which a number of parameters are derived.

The ROTEM® analyser uses a modification of this technology; 300 μl of whole blood with activators is incubated in a disposable cuvette (four parallel channels) and placed in a heated (37°C) holder. A pin fixed on a steel axis stabilized by a ball bearing is immersed into the blood. A spring rotates the pin in either direction while the cuvette stays stationary. The initial unrestricted rotation of the pin starts to encounter increasing impedance as the clot strength increases. This is detected by an optical system consisting of a light-emitting diode, a mirror on the steel axis, and an electronic camera, and is translated into the characteristic tracing from which again various parameters are derived.

A characteristic TEG®/ROTEM® tracing is shown in Figure 1. Although the tracings for TEG® and ROTEM® look very similar, the nomenclature and reference ranges are different. It is vital not to confuse the two or to use treatment algorithms developed for one device, with the other. The parameters derived from the tracings reflecting the various components of coagulation are detailed in Table 2.

In both TEG® and ROTEM®, clotting within the cuvettes is accelerated by incorporating contact activators. The TEG® incorporates kaolin and the ROTEM® uses tissue factor in the EXTEM® cuvette (extrinsic pathway allowing faster assessment of clot formation and fibrinolysis), and contact activator in the INTEM® cuvette (intrinsic pathway). Both devices have heparinase-containing cuvettes which allow monitoring of the coagulation system while a patient is fully heparinized, for example, during cardiopulmonary bypass, by removing the effects of heparin on the tracing, and also detecting residual heparin or heparin rebound after reversal with protamine.

doi:10.1093/bjaceaccp/mks049 Advance Access publication 13 September, 2012
Continuing Education in Anaesthesia, Critical Care & Pain | Volume 13 Number 1 2013
© The Author [2012]. Published by Oxford University Press on behalf of the British Journal of Anaesthesia.
All rights reserved. For Permissions, please email: journals.permissions@oup.com

Downloaded from http://ceaccp.oxfordjournals.org/ by John Vogel on January 30, 2013

Table 1 Advantages and disadvantages of POCCT

Advantages	Disadvantages
Fast turnaround compared with laboratory-based testing	Measure coagulation under artificial conditions in a cuvette rather than flow within an endothelialized blood vessel
Whole blood often used allowing interaction between plasma clotting factors, platelets, and red cells	Training and competency of non-haematological staff members required
Real-time visual display of clot evolution at the point of care	Rigorous quality assurance standards more difficult to institute outside the laboratory
Reduction in non-evidence-based transfusion	May be more expensive than conventional testing

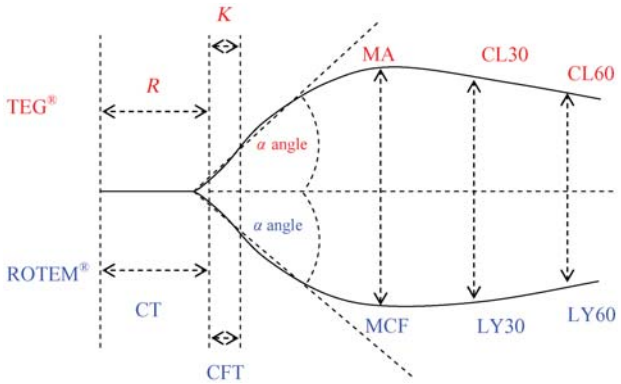

Fig 1 Typical TEG® and ROTEM® tracings. TEG®, thromboelastography; ROTEM®, rotational thromboelastometry; *R*, reaction time; *K*, kinetics; α angle, slope between *R* and *K* (TEG®) or slope of the tangent at 2 mm amplitude (ROTEM®); MA, maximum amplitude; CL30, clot lysis at 30 min; CL60, clot lysis at 60 min; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; LY30, lysis at 30 min; LY60, lysis at 60 min.

Several additional assays are also available (see below for a discussion of the TEG® Platelet Mapping Assays). The ROTEM® FIBTEM® cuvette contains the platelet inhibitor cytochalasin D and calcium, so that the MCF generated, when compared with the EXTEM® baseline tracing, represents the fibrinogen contribution to clot strength. The TEG® system includes a cuvette containing tissue factor and a glycoprotein IIb/IIIa receptor blocker which similarly fully inhibits platelets in the sample excluding their contribution to clot strength (MA) and thus revealing the fibrinogen contribution. By subtracting this from a baseline kaolin tracing, the MA then represents the platelet contribution to clot strength.

In addition, the ROTEM® APTEM® cuvette uses a combination of aprotinin and calcium to allow rapid detection of fibrinolysis and provide guidance for the appropriate use of antifibrinolytic agents.

The strengths and limitations of TEG® and ROTEM® are shown in Table 3.

Platelet function testing

Platelets play a pivotal role in haemostasis. When a vessel is damaged, platelets adhere to the exposed subendothelium resulting in activation, continued recruitment, and the formation of a primary platelet plug. Platelets also provide the phospholipid surface rich in receptors necessary for the activation of soluble coagulation factors. This produces the well-localized thrombin burst necessary for stable fibrin clot formation. It is therefore easy to appreciate that both quantitative and qualitative defects in platelet function can lead to major bleeding after surgery or trauma. Moreover, with the widespread use of antiplatelet agents in the management of cardiovascular, cerebrovascular, and peripheral vascular diseases, there has been increasing interest in the POCT of platelet function, both to confirm the effectiveness of these drugs and to assess recovery of function when they are stopped. Historically, most patients were advised to stop their antiplatelet

Table 2 Interpretation of TEG® and ROTEM® parameters. Normal ranges shown in italics. Those quoted for TEG® refer to whole blood (WB) with kaolin activator and those for ROTEM® citrated and recalcified samples (10); INTEM contact activator and EXTEM tissue factor

TEG®	ROTEM®	Definition	Significance
R (reaction time): *WB 4–8 min*	CT (clotting time) *INTEM 137–246 s* *EXTEM 42–74 s*	Time until initiation of fibrin formation, taken as a period to 2 mm amplitude on the tracing	Indicates concentration of soluble clotting factors in the plasma
K time: *WB 1–4 min*	CFT (clot formation time) *INTEM 40–100 s* *EXTEM 46–148 s*	Time period for the amplitude of the tracing to increase from 2 to 20 mm	Measurement of clot kinetics
α angle: *WB 47–74°*	α angle *INTEM 71–82°* *EXTEM 63–81°*	Angle between a tangent to the tracing at 2 mm amplitude and the horizontal midline	Rapidity of fibrin build up and cross-linking
MA (maximum amplitude): *WB 55–73 mm*	MCF (maximum clot firmness) *INTEM 52–72 mm* *EXTEM 49–71 mm*	Greatest vertical width achieved by the tracing reflecting maximum clot strength	Number and function of platelets and fibrinogen concentration
CL30	LY30	Per cent reduction in amplitude 30 min after MA	Clot stability and fibrinolysis
CL60	LY60	Per cent reduction of clot firmness 1 h after MCF	Clot stability and fibrinolysis

Downloaded from http://ceaccp.oxfordjournals.org/ by John Vogel on January 30, 2013

medication before surgery to reduce the risk of haemorrhage; now the balance of risk for many types of surgery has swung in favour of continuing these drugs to avoid an unacceptably high risk of perioperative thrombosis.

Several whole blood point-of-care platelet function tests have been developed with specific activators to detect cyclooxygenase inhibitors (e.g. aspirin), P2Y12 antagonists such as thienopyridines (e.g. clopidogrel, prasugrel, ticagrelor), and GPIIb/IIIa (glycoprotein IIb/IIIa) antagonists (e.g. abciximab, tirofiban, eptifibatide).[3] It should be remembered, however, that platelet physiology is extremely complex and all devices measuring function are limited by the use of *in vitro* non-physiological stimuli focusing on one aspect of platelet function; agreement between different devices and data confirming the predictive value of these devices on clinical outcome remains incomplete. Table 4 summarizes the utility of current common POCT platelet function testing devices.

Platelet function analyzer

Platelet function analyzer (PFA-100®) (Siemens, Deerfield, IL, USA) measures platelet adhesion and aggregation under high shear conditions. A vacuum induces flow of citrated whole blood across a collagen-coated membrane with a 150 μm aperture to which the platelet activators epinephrine or ADP are bonded. This results in platelet activation and aggregation. The time taken for a platelet plug to occlude flow through the aperture is defined as the closure time (CT) (normally 5–8 min) and reflects platelet function.[4] Both epinephrine and ADP CTs are prolonged in qualitative and quantitative abnormalities of von Willebrand factor which is the most common inherited disorder of coagulation affecting ~1% of patients. The epinephrine CT is prolonged in the presence of aspirin, while the ADP CT remains normal, making the PFA-100® useful in assessing aspirin sensitivity. The CT for both cartridges is prolonged by GPIIb/IIIa antagonists; however, the sensitivity is

Table 3 Strengths and limitations of TEG®/ROTEM®

Strengths	Limitations
Viscoelastic point-of-care tests such as TEG® and ROTEM® provide a rapid assessment of the overall coagulation status of the patient and the derived parameters can be used to guide the administration of specific blood components	The tracing cannot reflect the contribution of the endothelium to coagulation, therefore is very poor at detecting conditions affecting platelet adhesion, e.g. von Willebrand's disease
TEG® and ROTEM® analyse all three phases of coagulation, initiation, amplification, and propagation, reflecting the interactions of the cellular and plasma components of coagulation and the activity of the fibrinolytic system	Preoperative baseline TEG®/ROTEM® are poor predictors of postoperative bleeding
TEG® and ROTEM®-based transfusion algorithms have been shown to reduce rates of transfusion of blood components and reduce rates of surgical re-exploration	Will not reflect the effects of hypothermia as the measurement is undertaken at 37°C
Viscoelastic testing has also been shown to be of benefit in detecting a hypercoagulable state in postoperative patients; the best predictive parameter being an increased maximum amplitude (MA or MCF)	TEG®/ROTEM® tracings are insensitive to aspirin and clopidogrel
The TEG® kaolin and ROTEM® INTEM cartridges are exquisitely sensitive to residual heparin (0.005 IU ml^{-1}) which may be useful in the detection of inadequate heparin reversal or heparin rebound	TEG® and ROTEM® methodology is yet to be standardized in terms of sample collection and processing (native or citrated, time delay), activators used and other modifications, making it difficult to compare results between institutions
	There may also be concerns about adequate maintenance, quality control, and supervision of personnel running the tests away from the controlled environment of the laboratory
	Although the tracings appear similar, they are not interchangeable and agreement between ROTEM and TEG is only moderate

Table 4 Utility of point-of-care platelet function testing devices in assessing the effects of antiplatelet medications

Device	Antiplatelet agent		Strengths	Limitations
PFA-100®	Aspirin	√	Cheap, rapid, sensitive assessment of von Willebrand disease and aspirin effects	Wide normal range, influenced by thrombocytopenia and anaemia, not recommended for monitoring of thienopyridines and GPIIb/IIIa inhibitors
	P2Y12 antagonists	X		
	GpIIb/IIIa antagonists	X		
Plateletworks	Aspirin	√	Cheap, rapid, provides a platelet count, assessment of all three classes of antiplatelet agents	Requires a cell counter, blood must be tested within 10 min, limited evaluation to date
	P2Y12 antagonists	√		
	GpIIb/IIIa antagonists	√		
TEG® Platelet Mapping	Aspirin	√	Assessment of all three classes of antiplatelet agents	Relatively expensive, relatively slow, limited evaluation to date
	P2Y12 antagonists	√		
	GpIIb/IIIa antagonists	√		
VerifyNow	Aspirin	√	Rapid, assessment of all three classes of antiplatelet agents	Relatively expensive, effects of thrombocytopenia unknown, interpretation may be complex
	P2Y12 antagonists	√		
	GpIIb/IIIa antagonists	√		
MultiPlate	Aspirin	√	Cheap, assessment of all three classes of antiplatelet agents, good agreement with light transmission aggregometry	Manuel pipetting required (increased staff training), relatively short shelf life of reagents
	P2Y12 antagonists	√		
	GpIIb/IIIa antagonists	√		

Downloaded from http://ceaccp.oxfordjournals.org/ by John Vogel on January 30, 2013

so high that a quantitative assay is not possible. P2Y12 cartridges have been recently introduced to assess the effects of thienopyridines; however, these have not yet been fully evaluated and cannot currently be recommended to monitor clopidogrel.

Plateletworks®

Plateletworks® (Helena Laboratories, Beaumont, TX, USA) platelet aggregation system uses an impedance cell counter to perform a platelet count in a baseline whole blood EDTA sample. This is compared with the platelet count in a citrated sample plus agonist (arachidonic acid or ADP). The agonist causes platelet activation, leading to the formation of aggregates which exceed the threshold for platelet size and are not counted. Thus, if platelet function is normal, the count decreased to zero. Any other ratio of the count between the baseline and test samples can be used to calculate the percentage of platelet aggregation or inhibition in response to drugs such as aspirin (agonist arachidonic acid) and clopidogrel or GPIIb/IIIa antagonists (agonist ADP).[2]

The VerifyNow® system

The VerifyNow® system (Accumetrics, San Diego, CA, USA) measures the change in light transmission over time through an anticoagulated whole blood sample. The sample is agitated with fibrinogen-coated polystyrene beads and a platelet agonist designed to detect inhibition by aspirin (arachidonic acid), clopidogrel (ADP), or glycoprotein IIb/IIIa receptor antagonists (thrombin receptor-activating peptide). The activated platelets bind to the beads causing aggregation. The greater the platelet activation and aggregation, the greater the light transmission through the sample, thus the degree of platelet inhibition in response to an antiplatelet agent can be calculated.

TEG® Platelet Mapping Assay

TEG® Platelet Mapping Assay measures platelet inhibition relative to the patient's baseline viscoelastic profile. It involves up to four testing cups. The first runs a baseline kaolin-activated TEG® to measure thrombin-induced clot strength ($MA_{thrombin}$). The second tests a heparinized blood sample to which Reptilase and Factor XIIIa are added to generate a cross-linked fibrin clot without thrombin-mediated platelet activation (MA_{fibrin}). The third and fourth cups similarly require a heparinized blood sample with Reptilase and factor XIIIa; in addition, a selective platelet agonist (arachidonic acid for aspirin and ADP for thienopyridines or GPIIb/IIIa antagonists) is added to activate the platelets (MA_{AA} or MA_{ADP}). Percentage platelet aggregation is then calculated by the device software using the formula $=[(MA_{AA}-MA_{fibrin})/(MA_{thrombin}-MA_{fibrin})]\times 100$.

The Multiplate® device

The Multiplate® device (Dynabyte, Munich, Germany) has five parallel test cells using impedance aggregometry. Each cell has two pairs of silver-coated electrical wires and a stirring magnet. Hirudin anticoagulated blood and a platelet agonist are added to each cell. As the platelets are activated, they aggregate on the wires increasing the electrical impedance between them. This impedance is continuously plotted against time on a computer screen. The two sets of wires in each cell serve as controls for each other and the software calculates the mean area under the two curves, representing the overall platelet reactivity. The agonists used in the principal assays are arachidonic acid (ASPItest), ADP (ADPtest), and thrombin receptor-activating peptide (TRAPtest). Comparison of the area under the curve developed by each of these cartridges allows the detection of effects due to aspirin (ASPItest), thienopyridines (ADPtest), and GpIIb/IIIa antagonists (TRAPtest).

Clotting factor tests

Less commonly used in the operating suite but widely available for patients taking coumarin anticoagulants are POCCT for PT, aPTT, and INR. The CoaguChekProDM (Roche Basel, Switzerland) is one of several such systems using whole blood activated with soybean and phospholipids to derive all three. It has been shown to provide results comparable with a laboratory performed INR. The Hemochron Response system (International Technidyne Corporation, Edison, NJ, USA) can also be used to test the activated clotting time (ACT), PT, and aPTT. The latter two measurements are less commonly used in theatre as viscoelastic devices provide a flexible alternative.

Fibrinogen concentration may be assessed as described above using the TEG® fibrinogen cartridge[5] or the FIBTEM® cartridge of the ROTEM® analyser.[6] Both have been reported to show moderate agreement with the gold standard laboratory-based Von Clauss method of fibrinogen assessment.

Heparin tests

The ACT was first described in 1966 and remains virtually universal in the monitoring of anticoagulation with unfractionated heparin during cardiac surgery, extracorporeal oxygenation, angioplasty, and dialysis. Whole blood is incubated with kaolin at 37°C resulting in the activation of coagulation via the intrinsic pathway.[7] The normal range is wide (90–150 s) and prolonged by many factors, including hypothermia, haemodilution, coagulation factor deficiencies including hypofibrinogenaemia, group IIb/IIIa inhibitors, warfarin, lupus antibodies, thrombocytopenia, and abnormalities of platelet function. Aspirin and clopidogrel have a variable effect on the ACT and agreement between the ACT and the gold standard of heparin measurement (anti-Xa assay) is very poor.

A refinement of the ACT is provided by the Hepcon® HMS Plus system (Medtronic, Minneapolis, MN, USA) in which a three-point dose–response curve is constructed before bypass using 0,

Downloaded from http://ceaccp.oxfordjournals.org/ by John Vogel on January 30, 2013

1.5, and 2.5 IU ml^{-1} heparin plotted against the ACT generated. This curve allows derivation of the heparin concentration required to produce a target ACT. The heparin concentration during bypass is then maintained at this level using a heparin-protamine titration cartridge. There is evidence however that this system lacks precision and has poor agreement with the anti-Xa assay.[8] It has not been widely adopted as it is relatively expensive and the simple ACT is perceived as simple to perform, reasonably effective, and very cheap.

Algorithms and quality control

POCT is more expensive than laboratory testing; however, it is very useful in situations where speed is important. Multiple POCCT-guided transfusion protocols have been developed for the various devices and when used correctly may decrease inappropriate transfusion and product wastage, making these tests cost-effective.[9] It is important to remember that many of these algorithms, however, are not yet fully evaluated and are certainly not transferable between different devices.

It is essential that any POCCT facility is supported by rigorous systems of staff training, quality control, and audit in order to achieve consistently high standards. There must also be oversight of all POCCT devices by an appropriately comprised committee ensuring clear lines of accountability.[10] Devices constantly become available and clinicians must review new technologies to ensure that their patients benefit from the analyses that they offer. However, a sound awareness of the strengths and limitations of developing POCCT devices is essential before they can be used intelligently to guide clinical practice.

Declaration of interest

None declared.

References

1. Levy JH, Dutton RP, Hemphill JC *et al.* Multidisciplinary approach to the challenge of hemostasis. *Anesth Analg* 2010; **110**: 354–64
2. Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. *Anesth Analg* 2008; **106**: 1366–75
3. Gibbs NM. Point-of-care assessment of antiplatelet agents in the perioperative period: a review. *Anaesth Intensive Care* 2009; **37**: 354–69
4. Enriquez LJ, Shore-Lesserson L. Point-of-care coagulation testing and transfusion algorithms. *Br J Anaesth* 2009; **103**: i14–22
5. Kalina U, Stöhr HA, Bickhard H *et al.* Rotational thromboelastography for monitoring of fibrinogen concentrate therapy in fibrinogen deficiency. *Blood Coagul Fibrinolysis* 2008; **19**: 777–83
6. Coakley M, Reddy K, Mackie I, Mallett S. Transfusion triggers in orthotopic liver transplantation: a comparison of the thromboelastometry analyzer, the thromboelastogram, and conventional coagulation tests. *J Cardiothorac Vasc Anesth* 2006; **20**: 548–53
7. Rhee AJ, Khan RA. Laboratory point-of-care monitoring in the operating room. *Curr Opin Anaesthesiol* 2010; **23**: 741–8
8. Garvin S, Fitzgerald DC, Despotis G, Shekar P, Body SC. Heparin concentration based anticoagulation for cardiac surgery fails to reliably predict heparin bolus dose requirements. *Anesth Analg* 2010; **111**: 849–55
9. Ak K, Isbir CS, Tetik S *et al.* Thromboelastography-based transfusion algorithm reduces blood product use after elective CABG: a prospective randomized study. *J Card Surg* 2009; **24**: 404–10
10. Briggs C, Guthrie D, Hyde K *et al.* Guidelines for point-of-care testing: haematology. *Br J Haematol* 2008; **142**: 904–15

Please see multiple choice questions 9–12.

Downloaded from http://ceaccp.oxfordjournals.org/ by John Vogel on January 30, 2013