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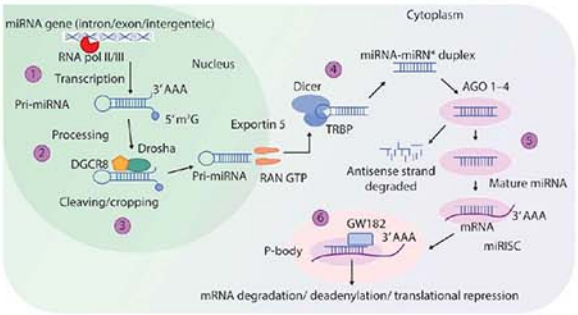
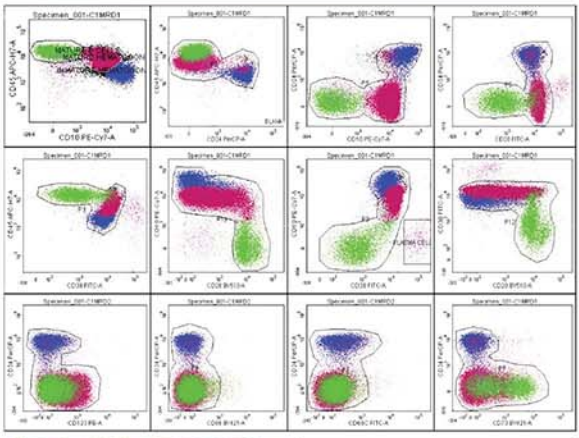
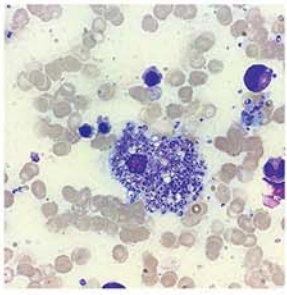
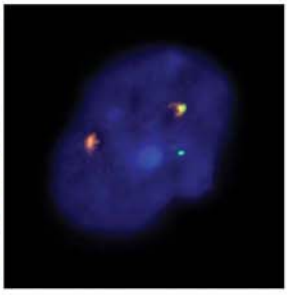
HEMATOLOGY

Recent Advances in HEMATOLOGY-5









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CHAPTER 10

Point-of-Care Testing in Coagulation

Vandana Puri, Jasmita Dass

ABSTRACT

Point-of-care testing (POCT) devices in coagulation provide rapid, bedside evaluation of hemostatic function, measuring parameters such as prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), activated clotting time (ACT), and comprehensive viscoelastic tests like thromboelastography (TEG) or ROTEM. These devices are invaluable in critical care, trauma, cardiac surgery, and anticoagulation clinics, where immediate results guide urgent interventions, transfusion decisions, or anticoagulant dosing. POCT minimizes delays by eliminating sample transport and lab processing, reduces blood volume requirements—beneficial for pediatric or ICU patients—and enables decentralized testing, including at-home monitoring for patients on long-term anticoagulation therapy. This enhances patient compliance, improves safety, and may lower overall healthcare costs by preventing complications and enabling early intervention.

INTRODUCTION

Point-of-care (POC) testing is defined as testing performed at or near the patient's bedside with the aim that results will possibly change the patient's therapy/affect patient care. POC testing is sometimes performed by patients themselves or by staff not primarily trained for laboratory testing. The tests in general have a higher reagent cost per test than laboratory assays but offer advantages in certain situations such as managing anticoagulant therapy with warfarin, perioperative heparin management in cardiac surgeries, patient blood management in situations like trauma, obstetric care, liver transplant surgeries, etc., managing antiplatelet therapy, etc., as the measurement durations (25–45 minutes) are shorter as compared to conventional coagulation tests (CCTs) (40–60 minutes). There are lesser chances of sample degradation as no transport or minimal transport time is required. There is also a potential for round-the-clock patient monitoring if required.

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PHASES OF COAGULATION

The process of coagulation in vivo begins after endothelial injury that exposes von Willebrand factor antigen (vWF) which binds to platelets by the glycoprotein (Gp)Ib-IX-V receptor causing platelet activation. This is referred to as primary hemostasis. Simultaneously, the tissue factor exposure activates the extrinsic pathway of coagulation and forming small amounts of thrombin by extrinsic tenase complex and is referred as the initiation phase of coagulation. The small amount of thrombin produced activates the factor IX and VIII leading to formation of the intrinsic tenase complex leading to a much higher formation of thrombin as by this time the extrinsic tenase complex is already inhibited by tissue factor pathway inhibitor. This is the amplification phase. The last phase is propagation which results in the formation of a stable fibrin clot. Platelets play an important role as the intrinsic and extrinsic tenase complex and prothrombinase complex are formed on their surface.

CONVENTIONAL TESTS OF COAGULATION AND THEIR DRAWBACKS

Traditionally CCTs—prothrombin time (PT) with international normalized ratio (INR) which measure extrinsic and common pathway, activated partial thromboplastin time (APTT) that measures intrinsic and common pathway, thrombin time (TT), fibrinogen are used as an initial screen. However, these CCTs suffer from some drawbacks and do not measure the entire process of hemostasis. These drawbacks are listed in the following text:

- Cannot predict which patients will bleed.
- Not proportional to the severity of the bleed.
- Does not take into account other components of coagulation system such as the platelets and cellular components.
- Does not take into account fibrinolytic pathway and the effect of factor XIII.
- Do not yield to monitoring of newer anticoagulants.
- False negative for mild factor deficiencies.
- Does not explain why hemophiliacs bleed despite normal extrinsic pathway.
- Patients with similar PT and APTT results may have significantly different bleeding profiles.

Some of these drawbacks are taken care of by viscoelastic testing such as thromboelastography (TEG) and rotational thromboelastometry (ROTEM) where the contribution of fibrinogen, platelets, and fibrinolytic pathway can be assessed within an hour of sample collection.

Point-of-Care Tests in Coagulation

Point-of-care devices and their functions are listed in **Flowchart 1**.

POC market in coagulation is expanding at a rapid pace and is expected to grow from $1.53 billion in 2021 to $2.44 billion in 2028 at a compound annual growth rate (CAGR) of 6.9% with the largest market share occupied by anticoagulant monitoring devices followed by platelet function monitoring.

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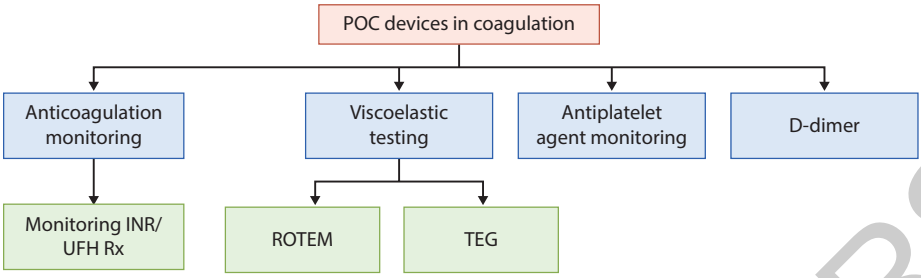

FLOWCHART 1: Point-of-care (POC) testing devices that can be used for platelet and coagulation testing.

(INR: international normalized ratio; ROTEM: rotational thromboelastometry; TEG: thromboelastography; UFH: unfractionated heparin)



FIG. 1: Point-of-care (POC) devices for international normalized ratio (INR) monitoring.

(PT: prothrombin time; APTT: activated partial thromboplastin time)

We will look at the utility and details of these devices by looking at four case scenarios.

Case 1

A 41-year-old doctor, posted at primary healthcare center (PHC) is diagnosed with right lower limb deep venous thrombosis (DVT). He was started on warfarin, INR stabilized at 2.6. He is having problems in getting frequent INR done.

Vitamin K antagonists (VKA) including warfarin have a narrow therapeutic window and therefore require frequent monitoring of INR. Traditionally, this is done by the citrated samples tested in the laboratory. There are multiple POC devices that can monitor INR. These devices and their principle are shown in **Figure 1**.

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The POC INR devices are approved for monitoring of VKA therapy. These offer significant advantages for outpatient monitoring as the need to travel to give samples can be obviated. These devices are also useful in the thrombosis clinic to take quick decisions to manage VKA therapy for patients. The use of POC INR devices for at-home management are recommended for patients who can self-adjust their VKA therapy. A meta-analysis has shown that the use of POC INR led to a significant rise of 6.14% in the time in INR in therapeutic range compared to laboratory PT/INR measurements.

These devices typically require 10–30 μL of blood mostly the capillary blood collected by fingerprick. This is applied to a test strip which is inserted in the INR device. Testing is performed on the whole blood (WB) rather than plasma as in the CCT with testing optimized to function across a hematocrit range of 25–55%. Of these systems, the ones based on optical/mechanical clot detection require WB samples while electrochemical devices do not necessarily require WB samples. Calibration is performed and International Sensitivity Index (ISI) for these devices is assigned by the manufacturers. Calibration data and mean normal PT (MNPT) is encoded in the test strips or in a batch-specific code chip. The POC INR devices have a coefficient of variation (CV) of 3–5%. Quality control (QC) on these systems involve the electronic QC by testing the optical/electronic system within the device. Some of the manufacturers also have liquid QC (LQC) material and are recommended for all POC instruments at the start of a new batch of strips, when results are in doubt or every 6 months. LQC is sometimes waived for at-home testing. Devices used for anticoagulation use should be enrolled in external quality assurance program with the caveat that the samples sent as a part of external quality assessment (EQA) is a citrated sample while most of these devices test WB, and hence, measure precision rather than accuracy. The devices usually overestimate INR at a value of ≥3.5 while at low INR values, they have a tendency to underestimate. POC INR devices are not approved for anticoagulation monitoring for lupus anticoagulant (LA) positive patients. A detail of these devices with ISI and QC are given in **Table 1**.

The patient bought a CoaguChek XS and comfortably could monitor INR on fortnightly basis.

Case 2

A 68-year-old male with triple vessel disease, due for coronary artery bypass grafting (CABG). Monitoring of unfractionated heparin (UFH) is required during surgery.

Activated clotting time (ACT) was first described by Hattersley in 1966 and is used for monitoring UFH therapy in patients on cardiopulmonary bypass (CPB), on extracorporeal membrane oxygenation (ECMO), undergoing percutaneous transluminal coronary angioplasty, on hemofiltration or hemodialysis. ACT is a one-stage WB clotting coagulation test for intrinsic pathway using celite/kaolin/particulate glass as activators with platelets as the source of phospholipids. CPB is performed with systemic anticoagulation to prevent both thrombosis and consumptive coagulopathy. Dependent on the circuits, an ACT of 300–600 seconds is targeted with a dose of UFH of 300–400 IU/kg with boluses thereafter.

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TABLE 1: Point-of-care (POC) devices with thromboplastin and quality control (QC) details.

POC devices with thromboplastin					
Instrument, manufacturer	**Sample**	**Thrombo-plastin, ISI, heparinase**	**Clot detection method**	**Calibration method**	**QC**
CoaguChek XS, Roche	Capillary/ venous whole blood (WB) noncitrated	Recombinant human; 1.0; Yes	Electrochemical cleavage (amperometric)	Code chip	On board QC, strip integrity check; LQC NA
CoaguChek Pro II, Roche	Capillary/ venous WB noncitrated	Recombinant human; 1.0; Yes	Electrochemical cleavage (amperometric)	Code chip	On board QC, strip integrity check; liquid QC (LQC) available
Coag-Sense, CoagSense Inc	Capillary WB/ recalcified plasma	Rabbit brain; 1.0; No	Optical/ mechanical	Barcode on cuvette, automatic	Additional QC strips
Hemochron Signature Elite, Werfen	Capillary/ venous WB citrated/ noncitrated	Rabbit brain; 1.0; No	Optical/ mechanical/ microfluidics flow	Barcode on cuvette, automatic	Electronic QC and LQC available
I-STAT, Abott	WB noncitrated	Human recombinant; 1.05; Yes	Electrochemical cleavage	On board software	Electronic QC and LQC

ACT is done 60 minutes before starting CPB and then every 30–60 minutes after heparinization. At such doses of UFH, PT and APTT are unmeasurable and therefore ACT is used for UFH monitoring. For uncoated circuits, an ACT value of >480 seconds is the target. For patients who have a contraindication to heparin, bivalirudin can be used with a target ACT of 2.5 times the baseline value. ACT is also used to check for completeness of protamine reversal after CPB is concluded. The reference range for unheparinized blood is 70–180 seconds with a linearity of the test for approximately 8.0 U/mL heparin. There are multiple factors that influence ACT including antithrombin deficiency, factor or fibrinogen deficiency, thrombocytopenia, anemia, hemodilution, hypothermia, and LA. ACT is sensitive to deficiencies in FVIII (<25% of normal), IX, X, XI, XII deficiency, in patients receiving oral anticoagulants or with liver disease. The ACT is insensitive to deficiencies of factor VII and factor XIII. ACT is also prolonged by direct thrombin inhibitors and lesser prolongation is seen with anti-Xa drugs.

Direct thrombin inhibitors will prolong the ACT. The direct Xa inhibitors (apixaban, edoxaban, and rivaroxaban) have a lesser effect on the ACT although this is dependent upon the precise drug being used. Various devices that monitor ACT are listed in **Table 2**.

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The ACT-low range is typically used for monitoring of ECMO, percutaneous coronary intervention (PCI), and hemodialysis as these use a lower concentration of UFH (0.5–3.0 U/mL). It is recommended that LQC is done for ACT devices daily along with electronic QC.

Modifications of ACT include Xa-ACT, MAX-ACT, and plasma ACT. Xa-ACT uses bovine Xa instead of the contact activator and was designed to monitor low molecular weight heparin (LMWH) therapy. MAX-ACT uses a combination of kaolin, celite, and glass to maximally activate factor XII making the test more sensitive to heparin and less affected by hypothermia and hemodilution.

The patient was put on CPB after checking the baseline ACT and could achieve an ACT of 500 seconds at 30 minutes post the first UFH bolus. His ACT continued to be >480 seconds throughout the procedure and after protamine reversal, fell to 100 seconds. His surgery was uneventful and he made a complete recovery.

Case 3

A 40-year-female presents with acute abdomen and fever. He has received 2L NS and colloid, noradrenaline and meropenem and was on mechanical ventilation.

TABLE 2: Point-of-care (POC) devices for activated clotting time (ACT).

POC devices for ACT					
Instrument, manufacturer	**Sample**	**ACT activator**	**Clot detection method**	**Analytical imprecision**	**Other assays available**
Hemochron Signature Elite, Werfen	Capillary/ venous whole blood (WB) citrated/ noncitrated	ACT+ uses a mixture of silica, kaolin and phospholipids; ACT-LR uses a celite activator	Optical/ mechanical/ microfluidics flow	≤10%	ACT+, ACT low range, POC INR, POC aPTT
I-STAT, Abott	WB noncitrated	Kaolin, celite	Electrochemical cleavage (amperometry)	<6%	ACT, POC INR
Actalyke Mini II and Actalyke XL, Helena Laboratories	Venous WB, noncitrated	Celite, kaolin, glass particulate	Magnet rotation	0.1% mean difference of duplicates	ACT, MAX-ACT, ACT-low range, heparinase
ACT plus, Medtronic cardiac surgery	Venous WB, native and citrated	Kaolin	Plunger motion	~15% duplicate values showed >12% difference	ACT, heparin dose response,
HMS plus, Medtronic cardiac surgery	Venous WB, noncitrated	Kaolin	Plunger motion		Heparin protamine titration

(ACT-LR: low range activated clotting time; APTT: activated partial thromboplastin time; INR: international normalized ratio)

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He was posted for exploratory laparotomy. Her CBC is hemoglobin (Hb): 8.2 g/dL, platelets: 80,000/μL, INR: 1.4.

To predict the risk of bleeding in this patient, viscoelastic testing can be used. The common viscoelastic tests are TEG and ROTEM which are global tests of hemostasis. TEG was first described by Hartert in 1948. Pentapharm GmbH (Munich, Germany) marketed a modified instrumentation using the terminology ROTEM. Both TEG and ROTEM provide continuous information from initial fibrin formation to clot retraction and fibrinolysis to assess clot formation, strength, and dissolution by measuring the effect of a continuously applied rotational force on WB that is transmitted to an electromechanical transduction system (TEG) or optical detection system (ROTEM). **Figure 2** shows the principle of TEG and ROTEM.

The tracings produced by these look similar and have fairly similar parameters. These are shown in **Figure 3**.

TEG and ROTEM can be performed on WB postrecalcification called native-TEG and nonactivated thromboelastometry (NATEM) respectively or can be performed using different agonists as shown in **Table 3**.

MA/MCF measure the contribution of platelets and fibrinogen, R time or CT are dependent on the clotting factors and fibrinolysis is measured by the CL30/Ly30 and CL60/Ly60. Various parameters and their measurement are shown in **Table 4**.

The ROTEM images from Case 3 and the management done are shown in **Figure 4**. The EXTEM and INTEM both show a reduced maximum clot firmness (MCF) while FIBTEM was normal indicating that fibrinogen was not the cause of reduced MCF. Despite a platelet count of 80,000/μL, the patient was at a risk of increased bleeding and the same was managed by giving pooled platelets to the patient following which ROTEM normalized.

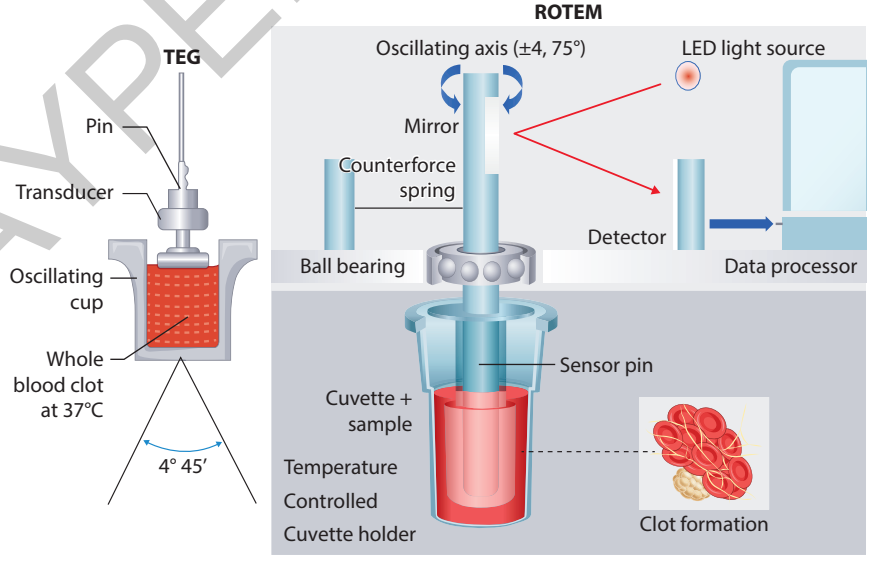

FIG. 2: Principles of thromboelastography (TEG) and rotational thromboelastometry (ROTEM).

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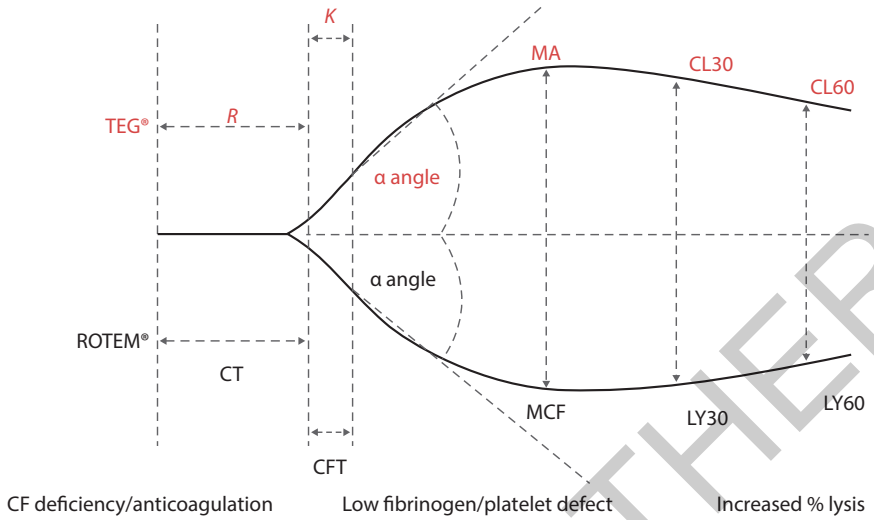

FIG. 3: Thromboelastography (TEG) and rotational thromboelastometry (ROTEM).
(CT: clotting time; CFT: clot formation time; MCF: maximum clot firmness)

TABLE 3: Pathways assessed by various agonists using TEG and ROTEM.

Pathways assessed by various agonists		
	TEG	**ROTEM**
Intrinsic pathway	Kaolin-TEG	INTEM—ellagic acid activator
Extrinsic pathway	-	EXTEM—tissue factor activator
Both extrinsic and intrinsic pathway	Rapid TEG—TF and kaolin	-
Heparin	Heparinase TEG—kaolin + heparinase	HEPTEM—kaolin + heparinase
Fibrinogen	Functional fibrinogen TEG—TF + abciximab	FIBTEM—TF + cytochalasin D
Fibrinolysis inhibition	-	APTEM—TF + aprotinin/tranexamic acid
Recalcification alone	Native TEG	NATEM

(APTEM: anti-plasminogen-activated-tissue-factor; EXTEM: extrinsic thromboelastometry; HEPTEM: heparinase thromboelastography; NATEM: nonactivated thromboelastometry; ROTEM: rotational thromboelastometry; TEG: thromboelastography; TF: tissue factor)

There are various algorithms to guide patient management based on TEG/ROTEM results for trauma, obstetric emergencies, cardiac surgery, etc. Also, their usage in many settings has led to reduction in the overall units transfused and optimization of blood product usage.

The ROTEM sigma and TEG-6s are cartridge-based systems that will require minimal pipetting and are available in the market. A soon-to-be launched system is the Quantra which will be a purely automated system with no manual pipetting based on ultrasound to detect clot. The measurements will be clotting time, clot stiffness, and platelet and fibrinogen contributions to clot stiffness.

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TABLE 4: Various parameters measured by TEG and ROTEM.

Various parameters measured by TEG				
ROTEM	**TEG**	**Unit**	**Explanation**	**Significance**
Clotting time (CT)	Reaction time (R)	Seconds	Time from test start to an amplitude of 2 mm	Velocity of thrombin generation
Clot formation time (CFT)	Kinetic time (K)	Seconds	Time between 2 and 20 mm clot amplitude	Kinetics of clot formation
Alpha-angle (α)	Alpha-angle (α)	0	Tangential angle at 2 mm amplitude (ROTEM®) or slope between R and K (TEG®)	Velocity of clot formation
Amplitude at 5, 10 minutes (A5, 10)	Amplitude at 30, 60 minutes (A30, 60)	mm	Amplitude at set time	Clot strength
Maximum clot firmness (MCF)	Maximum amplitude (MA)	mm	Greatest width achieved	Maximal cloth strength
Lysis index at 30, 60 m (L130, 60)	Clot lysis at 30, 60 m (CL30, 60)	%	Residual clot firmness at set time, as % of MCF/MA	Clot stability and fibrinolysis

(TEG: thromboelastography; ROTEM: rotational thromboelastometry)

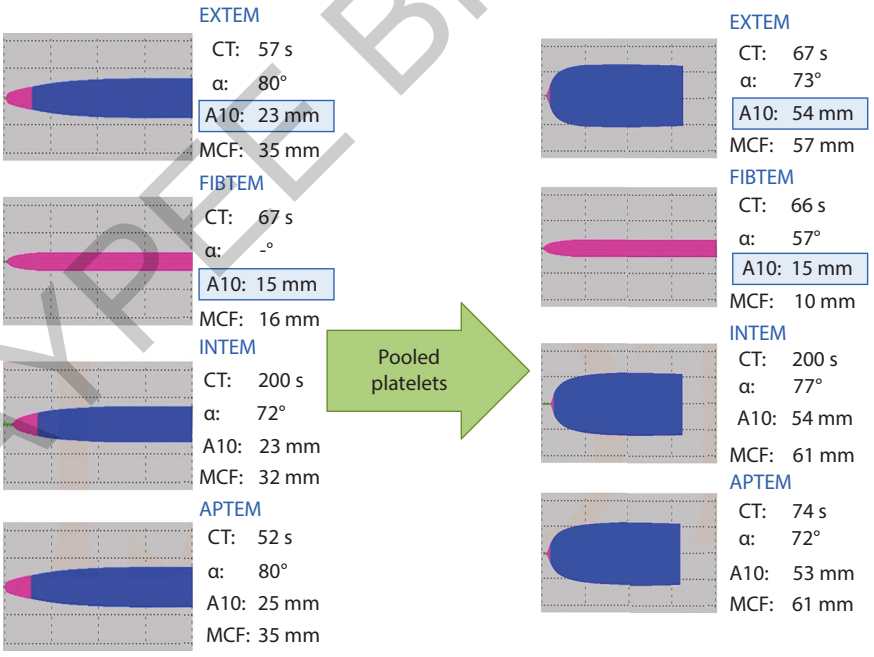

FIG. 4: Baseline rotational thromboelastometry (ROTEM) traces from case 3 and the effect after transfusion of pooled platelets.

(APTEM: anti-plasminogen-activated-tissue-factor; CT: clotting time; FIBTEM: fibrinogen-based ROTEM test; EXTEM: extrinsic thromboelastometry; MCF: maximum clot firmness)

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Case 4

A 62-year-male presented with pain in right leg. He had history of deep vein thrombosis (DVT) 2 years back. On examination, swelling of right leg is seen. Clinically, he is suspected to have recurrent DVT. Can any POC test be used to predict recurrence?

D-dimer along with a low pretest probability score is the parameter that has a high negative predictive for DVT in the outpatient setting. In this scenario, referrals for D-dimer testing to central laboratories take time and delay the diagnosis. There are multiple POC devices that can measure D-dimer and can aid in diagnosis especially at the primary care or emergency setting. Some of the POC devices for D-dimer are listed in **Table 5**.

TABLE 5: Point-of-care (POC) devices for D-dimer.

Test	Quantitative				Qualitative
	Pathfast D-dimer	***Vidas D-dimer test***	***Cardiac D-dimer***	***Triage D-dimer***	***Clearview simplify D-dimer***
Sample volume	100 µL citrated plasma	200 µL of citrated plasma	150 µL whole blood anticoagulated with Li-heparin	250 µL of EDTA whole blood	35 µL of capillary whole blood
Methodology	Chemiluminescent enzyme immunoassay with two D-dimer monoclonal antibodies, one labeled with alkaline phosphatase and the other coated with magnetic particles	Two-step enzyme immunoassay sandwich method with fluorescent detection. It uses an alkaline phosphatase–labeled anti-FbDP monoclonal antibody (P2C5A10)	Dual monoclonal antibody sandwich comprising a poly-(streptavidin)-biotin capture system with a gold particle label	Single-marker fluorescence sandwich immunoassay uses the 3B6 D-dimer antibody	Immunochromatography, and the test uses two D-dimer–specific murine monoclonal antibodies, one of which (DD3B6/224) is conjugated to colloidal gold particles
Results	The analyzer displays a quantitative result within a range of 5–5000 µg/L FEU, with a cutoff value of 570 µg/L FEU	The Vidas analyzer displays a quantitative result within a range of 45–10,000 µg/L FEU, with a cutoff value of 500 µg/L FEU	The cardiac D-dimer displays a quantitative result within a range of 100–4,000 µg/L FEU, with a cutoff value of 500 µg/L FEU	The Triage Meter Plus displays a quantitative result within a range of 56 –2,800 µg/L FEU (or 100 –5,000 µg/L D-DU), with a cutoff value of 196 µg/L FEU (or 350 µg/L D-DU)	A visible pink-purple colored line is formed at the test zone (T) when D-dimer concentrations exceed 80 µg/L (positive test result). Test results are valid if a pink-purple line is present at the control zone (PC)

(EDTA: ethylenediaminetetraacetic acid; FEU: fibrinogen equivalent units; FbDP: fibrin degradation products)

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Case 5

A 62-year-male, case of ischemic stroke was started on clopidogrel 75 mg + aspirin. He suffered one more stroke while on therapy. The neurologist wants to assess if antiplatelet therapy is working or not.

The gold standard technique to measure platelet function is light transmission aggregometry (LTA). However, it requires patient preparation, fasting state, operator skill-intensive, is laborious, and requires a large volume of blood. Some POC devices that are used to assess platelet function are described in **Table 6**.

TABLE 6: Point-of-care (POC) devices of platelet function monitoring.

System	PFA-100 (Siemens Healthcare)	VerifyNow assay (Werfen)	Multiplate analyzer (Roche Diagnostics)	TEG platelet mapping (Hemonetics corp.)	ROTEM platelets system (Werfen)
Methodology	WB is drawn through a small capillary tube and an aperture coated with collagen and other agonists under high shear. The reading stops when platelet adhesion and aggregation obstruct blood flow through the aperture (closure time). Three cartridges are available (collagen/ADP, collagen/epinephrine and collagen/prostaglandin E1), which enable detection of the effects of aspirin or P2Y12 receptor inhibitor	Turbidometry-based optical detection system that assesses the antiplatelet response of aspirin and P2Y12 receptor inhibitors in whole blood. Agonist-stimulated platelets bind to fibrinogen-coated polystyrene beads, leading to aggregation and changes in light transmission in citrated whole blood after addition of different activators, including lyophilized ADP and prostaglandin E1. The result is shown as platelet reaction units (PRU) calculated from changes in light transmission	WB using electrical impedance aggregometry. Hirudin-anticoagulated blood is mixed with saline in a disposable test cuvette containing two independent highly conductive metallic wires. Specific platelet activators (e.g., AA, ADP + PGE1, or TRAP) are added to induce platelet aggregation. Electrical impedance between the wires increases, which is monitored for 6-minutes. Results are averaged to a calculated area under the curve and are expressed as aggregation units	Evaluation of platelet function through direct activation of AA and ADP receptors, with analysis of the resulting formation of TEG curves. Comparison of these curves with the standard kaolin activated TEG assay allows for the quantification of platelet aggregation and inhibition at both AA and ADP receptors	Impedance aggregometry to assess platelet function in citrated whole blood. ADP-TEM for Clopidogrel, ARA-TEM for Cox inhibitors, TRAP-TEM for GpIIb/IIIa inhibitors

(AA: arachidonic acid; ADP: adenosine diphosphate; PGE1: prostaglandin E1; TEG: thromboelastography)

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Of these devices, VerifyNow is a cartridge-based system that lends itself to platelet function monitoring in a very simplified manner.

Verify, Now was used for our case 5. Platelet reaction unit (PRU) was 240 in the P2Y12 cartridge and indicated inadequate inhibition of platelet function. The antiplatelet therapy was modified to aspirin 100 mg and clopidogrel dose was increased to 150 mg. Reassessment revealed a PRU of 145 and the patient did not suffer any subsequent events on the revised doses.

CONCLUSION

POC tests in coagulation are an expanding market. The biggest share is the monitoring of vitamin K antagonist therapy with many devices such as CoagChek XS and Pro, Xpercia Stride, etc. These devices are of use for patients who can monitor and self-adjust their therapy. In addition, they are also used in the anticoagulation clinics to reduce turn-around time. The second large market is to monitor UFH therapy and for that ACT is used with a target value of >480 seconds for nonheparin bound circuits and is also used for protamine reversal. The third segment is viscoelastic testing with use of ROTEM and TEG which study all the phases of coagulation and also the contribution of platelets and fibrinolytic pathway. They have led to an optimization of patient blood management during many operative procedures. D-dimer testing at primary care settings can also be done using multiple POC devices. The last segment is for assessing antiplatelet therapy for which VerifyNow, MultiPlate, etc., are available and can be used to tailor therapy in patients started on antiplatelet therapy.

Future directions: The POC devices are going to expand with additions in the automated equipment such as Quantra, higher uptake of TEG-6s, ROTEM Sigma, INR monitoring devices, as well as antiplatelet therapy adjustment.

Take Home Points

- POC testing in coagulation is expanding.
- POC tests have a higher cost per test but can be performed with less skilled individuals and some tests even by patients themselves.
- VKA monitoring by INR estimation is the largest component of POC market.
- ACT-UFH monitoring for cardiac surgeries.
- Viscoelastic testing—TEG/ROTEM/(Quantra).
- Antiplatelet monitoring by VerifyNow, MultiPlate. Platelet function modules on TEG and ROTEM are also available.
- D-dimer estimations at primary care centers—Vidas®, Pathfast™, Cardiac®, and Triage®, LumiraDx, AQT90 Flex, etc.

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