Learning Objectives:
1. Understand mechanism of Rapid TEG, Platelet Mapping and ROTEM as compared to classic Thromboelastography
2. Understand applications of Platelet mapping in the clinical setting
3. Describe clinical application and evidence for using Rapid TEG
4. Discuss how these modified POC tests fit into current transfusion models

Introduction:
This syllabus will attempt to provide the background information needed to discuss the newer modalities used in thromboelastography. The lecture will focus on clinical utility of these tests, while the syllabus will attempt to fill in the key details.

A basic understanding of platelet function and physiology is important in order to evaluate the effectiveness of the various Point of Care (POC) tests available on the market today. Given the complexities of the coagulation system, there are numerous methods available to test coagulation in the laboratory. The coagulation system as a whole has been thought of as the extrinsic (tissue factor) pathway and intrinsic (contact activation) pathway, which is conveniently measured by the prothrombin time (PT) time and activated partial thromboplastin time (aPTT) respectively (Figure 1). The common pathway was thought to represent the intersection point where thrombin is generated to produce widespread clotting. This entire process is now understood to happen in a more cohesive and dynamic process that is interdependent on activation of the various enzymes in the coagulation cascade, along with a synergy of cell signaling and activation that depends in large part on platelet function. The term cell based clotting is now frequently used to describe this process. Briefly, exposure of blood vessel endothelium initiates a cascade of proteolytic reactions that are localized to the platelet surface, allowing association of thrombin and fibrin in close association with activated platelets.

Platelet Function in Hemostasis:
There are three key steps in the hemostatic function for platelets: Activation, Adhesion and Aggregation. Activation results in a conformational shape change in the platelet and involves the release of α-granules and / or dense granules. α – granules contain adhesive ligands, PF4 and coagulation factors including Factor V and VIII, while dense granules contain calcium, ADP and serotonin, and require stronger signal for release. Major platelet activators are outlined in Table 1. The results of platelet activation include recruitment or coagulation factors and additional platelets, vasoconstriction of nearby vessels, release of messaging factors, acceleration of fibrin formation, and overall clot protection or strengthening. Adhesion occurs in a two-step process, with initial linkage through the low affinity GPIbα receptor, followed by high affinity bonding with vWF, GPIIbα, and GPIIb / IIIa. The platelet plug is a platelet – ligand – platelet matrix which relies on fibrinogen and vWF to serve as links. These bind at GPIIb / IIIa

Figure 1: Depiction of the classic coagulation cascade.
initiation, the priming, and the propagation phase[1]. The propagation phase is depicted in Figure 3 below. Key concepts for clot formation is that various complexes are formed between the coagulation factors and activated platelets, conferring a kinetic advantage that allows for the key step in clot formation – the Thrombin burst. Following this, thrombin is able to cleave fibrinogen into fibrin – enabling firm clot formation.
POC testing and Platelet Dysfunction: The key point in investigating the use of POC testing of platelet function is to predict if there will be perioperative implications — whether it is bleeding or thrombotic comorbidity. POC testing in the perioperative setting has been examined in various settings, and in general has been shown to reduce blood transfusion both in cardiac and non-cardiac surgery (2,3). In cardiac surgery it is felt that platelet dysfunction is a major player in coagulopathy, and there are multiple known causes for platelet dysfunction related to bypass which are demonstrated in figure 4. The main argument for utilizing POC testing is that many of these tests (thromboelastography, thromboelastometry, sonoclot) evaluate the whole blood clotting mechanism, whereas laboratory testing evaluates the individual pathways discussed above. The laboratory process takes more time, resulting in clinical decision making often based on empiric decision making. POC testing can offer results in under 30 minutes, with preliminary results often in less than 15 minutes, thus offering a rapid, bedside monitor for clinical use. The negative aspects of POC testing, is concerns for global standardization of the testing and quality control of the results as these tests do not need to go through the central standard lab. In addition, as there are multiple points of activation in the clotting cascade with many different tests to examine activity, many of which have not been able to adequately predict bleeding.

In the perioperative arena outside of cardiac surgery, POC testing is important to help determine which patients act as responders to antiplatelet therapy. For patients who have had recent percutaneous coronary interventions with stent placement, it is key to know if they are adequately inhibited by ASA or clopidogrel, and in addition if the platelet activity has returned to normal for elective surgery where these agents are help. Specific platelet activity monitors can assess platelet activity at any point of activation presented in table 1. Viscoelastic testing (Thromboelastography (TEG) and Thromboelastometry (ROTEM), as well as specific platelet function testing with platelet mapping will be addressed.

TEG / Platelet Mapping (4): (Haemonetics Corp) Standard TEG® technology was developed by Hartert in 1948 and is a point of care test that measures the rate of clot formation using thrombin as the main platelet activator (3,5-8). Thrombelastography gives a graphic representation of clot formation and lysis. The formation of the clot is a net result of interaction between the platelets and coagulation proteins. The test is performed in a small quantity (0.36 ml) of whole blood that is placed in a heated cup. A pin, that is suspended freely in the blood by a torsion wire, is monitored for motion. The torque of the rotation cup is transmitted to the immersed pin only after fibrin-platelet bonding has linked the cup and pin together. Initially, when no clot exists, the oscillation of the cup does not affect the pin and a straight-line trace is recorded. The strength and rate of formation of fibrin-platelet bonds affect the magnitude of the pin motion. As the clot lyses, these bonds are broken and the transfer of cup motion is again diminished. The rotation movement of
the pin is converted by a mechanical-electrical transducer to an electrical signal, finally being displayed as the typical TEG tracing. The clot’s physical properties may suggest whether the patient has normal hemostasis, a tendency to bleed or to develop thrombosis. In a standard TEG tracing like that seen in below, the R value reflects clotting factor activation via initial thrombin generation. The K value is a time interval from R until a predetermined level of clot firmness is reached. The Alpha Angle is generated by a tangential line from horizontal x-axis to the point when cross linking occurs as the tracing begins to flatten out. The larger the Angle, generally represents the larger the clot formation as the angle reflects the amount of fibrinogen being converted to fibrin. The maximum amplitude simulates the platelet contribution to the final point of coagulation. Rapid TEG (r-TEG) is a derivative of the standard TEG but the use of tissue factor as an activator in addition to kaolin to speed the clotting reaction, thus reducing the time to results.

Complete testing with r-TEG can be obtained in approximately 20 minutes, compared to 30-45 min with standard TEG. The addition of online display sent directly to a monitor in the OR, allows for real time use of the test as it is proceeding. Parameters generated by r-TEG are equivalent to standard TEG except for the use of TEG-activated clotting time (ACT) to replace the traditional reaction time (R), representing the status of clotting factors and/or the effect of anticoagulants. The ACT (normal range, 0–118 seconds) is the time in seconds between initiation of the test and the initial fibrin formation and is increased with factor deficiency or severe hemodilution (9).

Platelet Mapping™ is a modification of TEG allowing a specific examination of platelet function relating to two different agonists, arachidonic acid (AA) and adenosine-5-diphosphate (ADP). Thrombin activation (initiated in the TEG assay by contact with Kaolin) of platelets is so powerful that it masks any effect of secondary platelet activators. Therefore this reaction is carried out in the setting of heparinized blood to block thrombin activation. Factor 13 is added to generate a baseline fibrin meshwork (generates fibrin) and represents minimal platelet activation. The AA or ADP is then added to separate reactions to generate a curve and compared to standard TEG trace. Platelet Mapping™ has shown a statistically significant correlation to optical platelet aggregation as the gold standard assay (7,10).
ROTEM (11): (TEM Innovations GmbH) This system is based on the same concept as the TEG, however instead of the rotational cup, it is the pin / torsio wire that is physically rotating. This system has been used extensively in Europe, and has just recently become available for clinical use in the US following recent FDA approval. All images here from TEM Innovations GmbH.

The standard tracing is below and is very similar to the TEG tracing. Various reagents are available to test the coagulation parameters, which are reported as the CT (clotting time), the CFT (clot formation time), alpha, A10, MCF, LI30 and ML.