HEMOSONICS

White Paper

QPlus® Platelet Contribution to Clot Stiffness

Principles of Design, Key Characteristics, and Clinical Utility

Executive Summary

- Platelets play a central role in hemostasis and their functional interactions with the coagulation proteins of the plasma is a key factor in determining the overall hemostatic balance. Proper hemostatic balance is required to avoid excessive bleeding or thrombosis.
- The Quantra® QPlus® System is the first viscoelastic testing device to provide a direct quantification of the Platelet Contribution to Clot Stiffness, or PCS.
- PCS is a key component of a comprehensive diagnostic panel designed to support clinical decisions in the management of acute bleeding in critical care settings.
- PCS was designed to mimic the platelets' (patho)physiology and functional interactions with other whole blood components.
- PCS is derived from direct measurements of the clot's elastic properties. It accounts for both platelet count and platelets' ability to aggregate, contract, and contribute to clot strengthening.
- PCS was tested in several single- and multi-center clinical studies. Results indicate high precision, generally strong correlation with available platelet assays, and high negative predictive value for thrombocytopenia.
- PCS, along with other information provided by the Quantra QPlus System, can be integrated into an effective and comprehensive algorithm for the management of perioperative bleeding.

1. Introduction – Platelets and Hemostasis

Hemostasis, the innate response of the body to stop bleeding upon injury, requires a delicate balance between the coagulation and the clot-dissolution (fibrinolytic) systems. The main functional components of hemostasis include the vasculature and endothelium, the coagulation proteins of the plasma (including fibrinogen), cellular components such as platelets, and the fibrinolytic proteins of the plasma. Figure 1 shows an illustration of this complex process.

Platelets play a critical role in the hemostatic process. First, by formation of a platelet plug at the site of injury, a process referred to as primary hemostasis. Mediated by von Willebrand factor, platelets adhere to exposed collagen, become activated and release granule contents that in turn attract more platelets into the growing clot.[1] Secondly, platelets control the formation of fibrin which is formed on the surface of activated platelets.[2] This step of fibrin formation, referred to as secondary hemostasis or coagulation, produces a meshwork of fibrin strands around the platelet plug to hold it in place. Platelets and the coagulation proteins of the plasma are therefore not separate and independent components but instead "need to be considered as highly reciprocal and interconnected processes".[2,3]

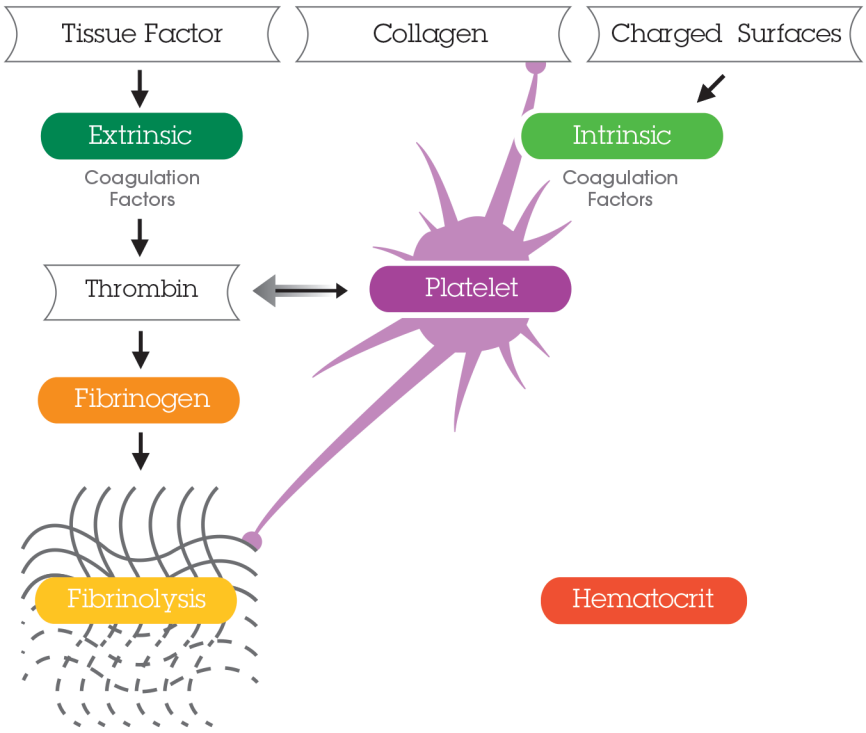

Figure 1. Schematic representation of the hemostatic process.

Once initiated, clot growth and clot stiffening are mediated through bridging of platelets by fibrinogen that binds to integrin $\alpha_{IIb}\beta_3$ receptors* that become available on the surface of activated platelets.[4] While fibrinogen generates a loose three-dimensional fibrin structure, the functional interaction of platelets removes "slack" from this structure with a resulting multiplicate effects on the overall viscoelastic properties. This is an important process since the developed clots need to have adequate "stiffness" to withstand the pressure and flow within the vasculature. It has been estimated that each platelet has between 40,000 and 50,000 fibrinogen anchoring $\alpha_{IIb}\beta_3$ receptors[5], which further exemplifies the complex and integrated functions that these components play under (patho)physiological conditions.

Platelets represent a target of several pharmacological therapies aimed at inhibiting pathological thrombosis and reduce the risk of ischemic stroke or myocardial infarction. These therapies modulate platelet adhesion and aggregation by interfering with specific physiological mechanisms that lead to platelet activation. For example, aspirin inhibits platelet activation by blocking cyclooxygenase (COX) enzymes, whereas clopidogrel and other ADP inhibitors bind to and inhibit platelet P2Y12 receptors involved in ADP-dependent platelet aggregation.

Conversely, when platelet count drops or platelets become non-functional, the physiological ability to clot can become impaired leading to excessive and potentially life-threatening bleeding. The Quantra QPlus System is a viscoelastic testing (VET) device designed to support clinical decisions in the management of acute bleeding by providing rapid and comprehensive measurement of the key functional elements of coagulation, including a quantitative measurement of the platelets function.† This is accomplished by a parameter termed Platelet Contribution to Clot Stiffness, or PCS, which is described in more detail below. PCS is unique to the Quantra platform and not available with other VETs.

2. Principles of Design

Platelet count is part of a series of lab-based assays routinely used in critical care settings to monitor coagulation function and guide therapeutic interventions. Measurements of platelet functions are seldomly used in these clinical settings and current assays test the

* Also referred to as Glycoprotein IIb/IIIa

† Fibrinolysis is not measured with the Quantra QPlus System since antifibrinolytics are routinely administered in the perioperative settings.

functional response of platelets independently from the activity of the coagulation proteins of the plasma. One of the main limitations of these functional assays is that they do not provide any information about the cross-functional interactions between the cellular and enzymatic components of coagulation. As previously described, these interactions are clinically important since the functional balance between platelets and fibrinogen is important in determining the overall hemostatic balance.

The Quantra QPlus System was developed to ameliorate and augment clinical diagnostic capabilities and therefore overcome the limitations of the current devices. The PCS parameter is an important component of the comprehensive information provided by this system. It was designed and optimized based on two overarching criteria: (1) to mimic the platelet (patho)physiology and its interactions with other whole blood components and (2) to achieve well defined clinical performance objectives. It is representative of the effects of cardiopulmonary bypass, hypothermia, hemodilution, coagulation consumption, as well as other factors that can affect overall platelet function in the perioperative setting.[‡] From a performance point of view, we required the following:

- Accurate and precise assessment of overall platelet contribution to coagulation across a broad measurement range
- Continuous, not quantized, measurements
- Rapid turnaround of results

As shown below, these goals were achieved by utilizing an innovative technology and measurement principle coupled with a cartridge design that facilitates parallel differential testing.

2.1 QPlus Cartridge and Platelet Contribution to Clot Stiffness (PCS)

The QPlus Cartridge is a multi-channel single-use disposable plastic component. The cartridge has four independent channels that can be tested simultaneously with each channel containing pre-filled lyophilized reagents that can enable differential testing without the need for reagent preparation or pipetting before testing. Figure 2 shows a schematic representation of the QPlus Cartridge and the reagents used in each channel. A detailed description of the cartridge and its output parameters is presented in various publications.[6,7]

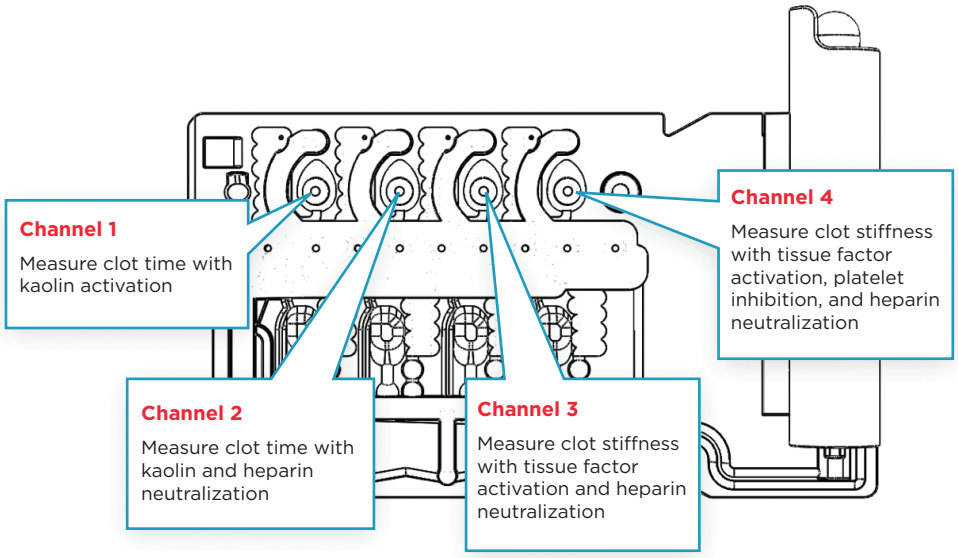

Figure 2. Schematic representation of the QPlus Cartridge. The cartridge consists of four parallel channels that have different sets of reagents. Four parameters are directly measured from the four channels of the cartridge and two parameters are calculated from the measured ones.[§]

[‡] Note, however, that PCS is not a universal test for platelet function but instead it has been developed based on its intended clinical use for the evaluation of blood coagulation in perioperative patients to assess possible hypocoagulable and hypercoagulable conditions in cardiovascular or major orthopedic surgeries. Therefore, PCS is not reflective of platelet inhibition caused by anti-platelet therapies such as aspirin and/or ADP inhibitors.

[§] The QPlus measured parameters include Clot Time (CT), Heparinase Clot Time (CTH), Clot Stiffness (CS), and Fibrinogen Contribution to Clot Stiffness (FCS). The calculated parameters are Clot Time Ratio (CTR) and Platelet Contribution to Clot Stiffness (PCS).

PCS is a calculated parameter that is derived from the combination of the measurements performed in channels #3 and #4. Channel #3 measures overall clot stiffness in the presence of thromboplastin, an activator of the extrinsic pathway of coagulation, and polybrene, a neutralizer of the anticoagulant heparin. The output parameter from this channel is Clot Stiffness (CS) which represents the stiffness (or elasticity) generated by all the components, cellular and enzymatic, of the whole blood sample. Channel #4 measures clot stiffness with the same reagents as channel #3, but with the addition of abciximab, a potent platelet aggregation\contraction inhibitor. The output parameter from this channel is Fibrinogen Contribution to Clot Stiffness (FCS) which represents the stiffness (or elasticity) generated by the fibrin network without the contribution of the platelets. PCS is a functional parameter that is calculated as the difference between the overall CS and FCS, reported in hectoPascals or hPa.

2.2 Elasticity Measurements – Implications and Benefits

The QPlus parameters CS, FCS, and PCS are based on direct measurements of the clot shear modulus, a well-known and objective parameter that describes the elastic properties of a solid material. Shear modulus is expressed in SI (Système Internationale) units of Pascals. These measurements are performed using SEER (Sonic Estimation of Elasticity via Resonance) Sonorheometry, an ultrasound-based technology that can measure shear modulus without direct contact with the sample and with minimal sample perturbation.[6,8] SEER is the only available VET technology that measures shear modulus.

The utilization of an absolute value of elasticity allows approximation of the overall clot stiffness as the linear sum of the relative contributions of fibrinogen and platelets. There is a growing number of publications demonstrating that calculation of the platelet component to clot stiffness should be based on measurements of elasticity and not on measurements of clot amplitude as done with the current VET devices based on thromboelastography or thromboelastometry.[9-11] This consideration forms the basis of the differential measurement approach utilized in the Quantra QPlus System to provide a direct quantification of PCS, which is shown in Figure 3.

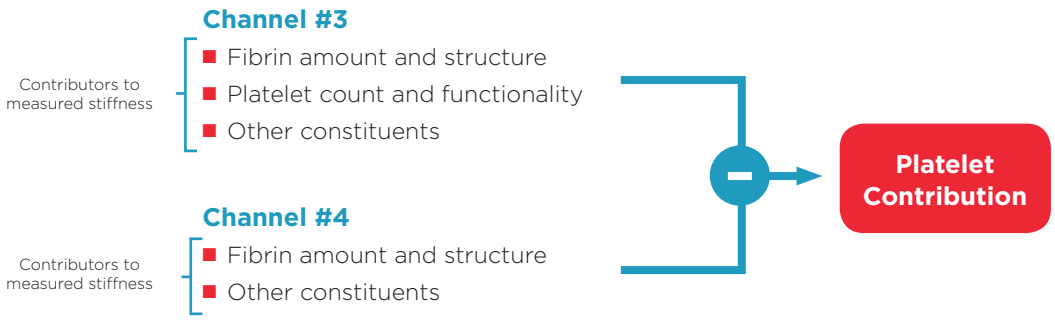

Figure 3. Differential strategy implemented to directly measure the platelet contribution to clot stiffness.

3. Clinical Relevance

Unbalanced hemostasis, known as coagulopathy, is often observed during and after major surgical procedures, such as in the case of cardiac surgery with cardiopulmonary bypass, major spine reconstruction, and major orthopedic surgery. These procedures can result in significant intra- and/or post-operative bleeding followed by a period of hypercoagulability.[12] The presence and severity of the coagulopathic state is multifactorial with likely factors including pre-surgical medications, intra-operative blood loss, tissue trauma, exposure to foreign surfaces, allogeneic blood product utilization, hypothermia, and hemodilution, among others.[12-15] Under these circumstances, platelets can often become damaged and unresponsive and be a major contributor to the acquired bleeding diathesis. If a platelet defect is properly and timely recognized, platelets concentrate can be administered to restore a physiological hemostatic balance.

Although platelet count is often used in clinical practice to guide transfusion of platelet concentrate, this measurement is severely limited as it considers only a single dimension with no functional information. This is particularly relevant in the cardiac surgery patient population since it has been previously shown that the exposure of the patient's blood to the external bypass circuitry can reduce the ability of platelets to function correctly.

Figure 4 illustrates the relationship between PCS and the available lab-based platelet assays. Both platelet count and platelet function testing provide useful, albeit incomplete information regarding the ability of platelets to contribute to clot stiffness. The Quantra-based assessment of platelet contribution takes into account not only the count but also their ability to aggregate and contract, as well as their interaction with the polymerized fibrin network. In a recent study in cardiac surgery, Baryshnikova et al demonstrated that the PCS parameter is independently associated with platelet count and ADP-dependent platelet function as measured by multiple electrode aggregometry, thus demonstrating association between a viscoelastic test result and a parameter of platelet function.[10]

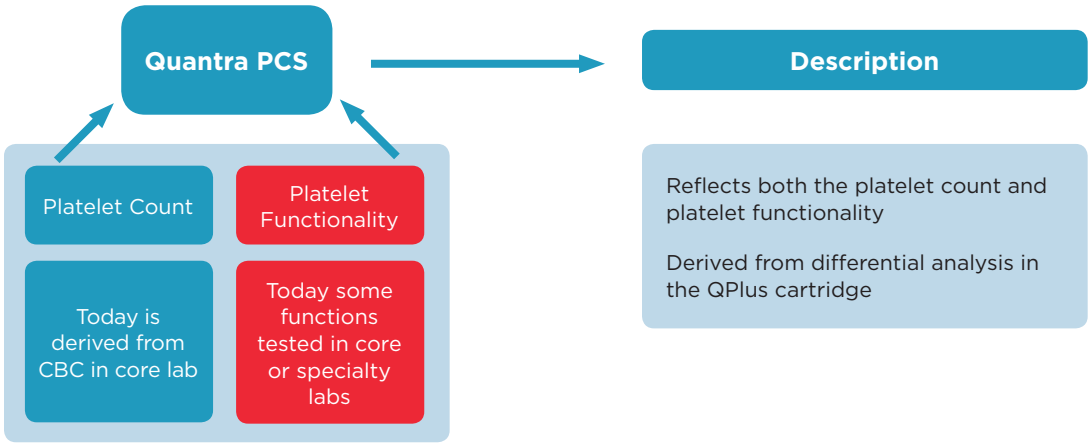

Figure 4. The Quantra-based PCS parameter measures the combined effect of platelet count and overall platelet functionality.

PCS offers a new dimension in the diagnosis and interpretation of platelets' ability to contribute to coagulation. The section below summarizes the available clinical data that further demonstrates the utility of this new and unique diagnostic parameter.

4. Supporting Data

The performance of the Quantra QPlus System was evaluated in multiple single-center and multi-center observational studies, primarily in patients undergoing either cardiac or major orthopedic (spine reconstruction) surgeries [7,10,16-18,23-25] as well as through extensive non-clinical testing. These studies demonstrated that PCS is measured with high precision, has generally strong correlation with well-established diagnostics, and exhibits a high negative predictive value for thrombocytopenia, which is often used as a trigger for platelet transfusion. A short summary of the key results is presented below.

Reference Range and Reportable Range

The normal reference intervals for PCS was determined in a reference range study conducted by collecting whole blood samples from 129 healthy donors across three sites in the United States. The data were evaluated as recommended in CLSI Guideline EP28-A3c. The reference range determined from this study, expressed as the central 95% confidence interval of the mean, is the following: **11.9 – 29.8 hPa**. The reportable range is: **2.0 – 50.0 hPa**.

Precision

Total imprecision (% coefficient of variability) of PCS was calculated in whole blood specimens that included normal, abnormal, and contrived samples to cover the assay measuring range (reportable range). Under these conditions, the total variability of PCS, which also included the effects of operators, instruments-cartridge lots, etc., was between 2.9% and 7.8%.

Correlation with Current Platelet Assays

Data obtained from the available studies is summarized in the table below.

Table 1. Summary of PCS correlation with platelets count and platelet function assays.

Clinical Setting	Comparator	r-value	Study Size	Ref
Major Orthopedic Surgery (spine)	Platelet Count	0.81	50 patients	18
Cardiac Surgery	Platelet Count	0.72	50 patients	17
Cardiac Surgery	Platelet Count	0.48	50 patients	16
Cardiac Surgery	Platelet Count	0.82	52 patients	24
Cardiac Surgery	Platelet Count	0.78	30 patients	23
Cardiac Surgery	Multiplate – ADP Platelet Count	0.67 0.71	30 patients	10
Cardiac and Major Ortho (spine)	Platelet Count	0.64	277 patients	25

Sensitivity/Specificity Analysis

Sensitivity / specificity analyses were performed based on the data obtained from two studies. The first study included 277 adult patients (total of 833 data points) undergoing cardiac and major orthopedic surgery. The second study consisted of a population of 52 adult patients undergoing cardiac surgery. Samples were obtained at multiple points before, during, and after surgery. The results are summarized in Table 2.

Table 2. Summary of PCS sensitivity and specificity analyses.

Platelet Level; N	PCS Cutoff	Sensitivity	Specificity	NPV	AUC	p-value
Study 1:						
<80,000/ul; N=31	12.1 hPa	93.5%	83.3%	99.7%	0.92	<0.001
<100,000/ul; N=84	14.1 hPa	89.2%	73.8%	98.4%	0.88	<0.001
<150,000/ul; N=376	18.0 hPa	84.5%	70.6%	84.8%	0.85	<0.001
Study 2:						
<100,000/ul; N=143	13.5 hPa	76.9%	85.4%	97.4%	0.83	<0.001

5. Translation into Clinical Algorithms

Several medical societies have recommended the use of goal-directed treatment algorithms guided by viscoelastic testing for managing acute bleeding in cardiovascular surgery, liver transplantation, trauma and obstetric hemorrhage. These societies include the American Society of Anesthesiologists (ASA), the European Society of Anesthesiology (ESA), and the International Society on Thrombosis and Haemostasis (ISTH), among others.[19-22] The algorithm is a flow chart intended to assist clinicians in interpreting the results of diagnostic tests (viscoelastic tests in this case) so that the appropriate and optimal interventions can be administered to the bleeding patient. In these charts, treatment decisions are based on trigger values derived from the diagnostic tests. Typically, some adjustments of these thresholds are required to account for specific clinical indications and the local patient population.

The outputs provided by the Quantra QPlus System are well-suited to support current clinical recommendations and to provide information that can be readily incorporated in a site's algorithm to manage perioperative bleeding. The Quantra QPlus System is the only VET device that provides a functional measurement of platelet activity that can be associated with the available therapeutic interventions. This allows a clinical site to develop a streamlined and more simplified treatment algorithm, which in turn would result in rapid and comprehensive patient management.

Figure 5 is an illustrative example of an algorithm for use in cardiac surgery which includes information related to a patient's coagulation status provided by the Quantra QPlus System. In this figure, the QPlus trigger and target values listed are for illustrative purposes

only. These values should be developed, reviewed, and validated by each clinical site. To facilitate this task at sites currently using a treatment algorithm based on TEG®, ROTEM®, or laboratory testing, it is possible to evaluate and define trigger and target values with comparable Quantra values using comparison charts available from HemoSonics, as shown in Table 3.

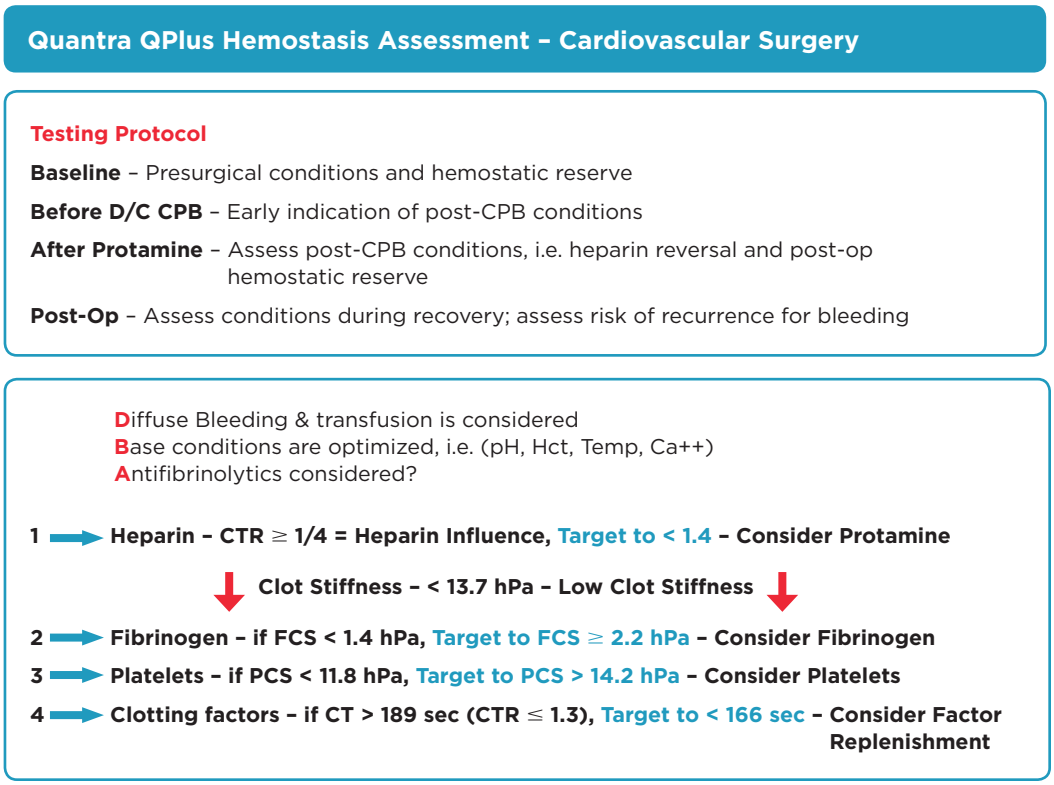

Figure 5. Example of a potential treatment algorithm for cardiac surgery that can be developed using information provided by the Quantra QPlus System.

This is an example based on published guidelines from other VET Systems. HemoSonics, LLC does not recommend specific therapeutic intervention. The values here have a statistical equivalence to other VET systems' published values. Any Quantra QPlus-directed guidelines must undergo comprehensive medical review and acceptance by each facility.

Table 3. Approximate Equivalent Values – ROTEM to Quantra QPlus

INTEM Clot Time to QPlus CT		*HEPTEM Clot Time to QPlus CTH*	
INTEM CT (sec)	**Clot Time (CT, sec)**	**HEPTEM CT (sec)**	**Heparinase Clot Time (CTH, sec)**
Ref Range 122-208 sec	Ref Range 113 – 164 sec	Ref Range 122-208 sec	Ref Range 109 – 150 sec
≥ 250	≥ 178	≥ 250	≥ 176
245	175	245	173
240	172	240	170
235	169	235	168
230	166	230	165
≤ 225	≤ 163	≤ 225	≤ 162

EXTEM A20 to QPlus CS		**FIBTEM A20 to QPlus FCS**	
EXTEM A20 (mm)	**Clot Stiffness (CS, hPa)**	**FIBTEM A20 (mm)**	**Fibrinogen Contribution (FCS, hPa)**
Ref Range 50 – 70 mm	Ref Range 13.0 – 33.2 hPa	Ref Range 7 - 24 mm	Ref Range 1.0 – 3.7 hPa
≤ 40	≤ 8.2	≤ 5	≤ 0.7
45	10.2	7	1.0
50	12.7	9	1.3
55	15.7	11	1.6
60	19.5	13	1.9
65	24.4	15	2.3
≥ 70	≥ 30.9	≥ 17	≥ 2.6

ROTEM A20 values approximate MCF. To compare early A"X" values to QPlus CS or FCS, make the following adjustments: EXTEM: A15 + ~5 mm; A10 + ~10 mm; A5 + ~20 mm
FIBTEM: A15 + ~4 mm; A10 + ~5 mm; A5 + ~6 mm

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