Protocol Title: Evaluation of Novel Point of Care Coagulation System in Pregnant Women

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Unexpected obstetric hemorrhage continues to confront anesthesiologists, and obstetric hemorrhage spearheads as the leading cause of maternal mortality and morbidity. One of the hurdles confronting clinicians is the lack of a Point of the care coagulation system that can hasten the clinician management strategy in the right direction. Conventional time-tested coagulation systems take considerable time to obtain results. Thromboelastography (TEG®) and Rotational Throboelastometry (ROTEM®) are welcome additions to assess coagulation quickly. However, these two modalities require expertise and proficient training to perform test, and interpret results. This poses a hurdle for providing coagulation evaluation services around the clock. Therefore, many users of these technologies rely on the conventional laboratory personnel to use these technologies at remote location and thereby delaying expeditious acquisition of results by the clinicians. What is currently required for efficient and effective management of obstetric hemorrhage is a point of care coagulation system in the operating room or labor and delivery suite that is user friendly, easy and quick to perform by any clinician or support staff and does not require certified training. In this study, we evaluate a new point of the care, Quantra Hemostasis Analyzer system to assess coagulation rapidly with ease. The Quantra is a fully automated instrument that requires no sample handling steps from the user. The aim of the present study is to determine how Quantra system performs when compared to conventional coagulation tests at varying level of fibrinogen and clotting factors in the blood obtained from pregnant women.

Methods:

Details of the system:

The Quantra is a Point of the Care instrument where all components of test sequence, fluid handling, temperature control, ultrasound transmission, data processing and result output are automated. The device incorporates an embedded processor, a mechanical assembly that provides connection to a peristaltic pump and solenoid valves, heating elements to heat and maintain samples at 37°C, and ultrasound transducers operating in the megahertz range. Figure 1 shows the device used for the study. Quantra uses a plastic cartridge with embedded reagents. The cartridge has 4 test channels that perform 4 parallel and independent measurements using different reagent combinations in each channel. The cartridge has connection mechanism for attaching 3 ml syringe that can contain blood for coagulation analysis.

The reagents used in each channel are shown in the table 1.

Channel	Reagents
1	Kaolin, calcium, buffers, and stabilizers
2	Kaolin, heparinase I, calcium, buffers, and stabilizers
3	Thromboplastin, polybrene, calcium, buffers, and stabilizers
4	Thromboplastin, polybrene, abciximab, calcium, buffers, and stabilizers

The reagents in channel 1 are optimized for the measurements of Clot Time, whereas the channel 2 measures Clot Time without the effect of any potential heparin in the blood sample. Clot Times provides an indication of the functional status of the coagulation factors that lead to fibrin formation.

Furthermore, Clot Time and Heparinase Clot Time (channel 2) can be combined to a clot time ratio for determining the presence of residual heparin in the sample. Channel 3 is optimized to provide measurements of clot stiffness, which combines information about platelets and fibrinogen function. Finally, channel 4 is optimized to measure the Fibrinogen contribution to clot stiffness. Both channels 3 and 4 use hexadimethrine bromide to neutralize residual heparin. The difference between Channel 4 and 1 can provide platelet contribution to clot stiffness.

We designed an in vitro model of obstetric hemodilutional coagulopathy to assess the new technology. We also measured coagulation parameters after reconstituting the dilutional blood with undiluted blood to obtain serial increase in coagulation factors. The results obtained were compared to ROTEM®. The Quantra has been designed to provide results in 20 minutes, the fastest so far among available coagulation technologies.

Quantra uses SEER Sonorheometry. This is a patented technology that uses high-frequency ultrasound pulses to quantify the shear modulus (stiffness) of a blood sample during the process of coagulation. The shear modulus is a parameter that describes the elastic properties of a solid material. The shear modulus of bone tissue is approximately 3.3 hPa, whereas natural rubber is typically approximately 600 Pa.

A focused ultrasound pulse is transmitted into the blood sample to generate a shear wave, causing the sample to resonate once the clot begins to form. As the clot vibrates during resonance, a series of "tracking" ultrasound pulses are transmitted, and the returning echoes are analyzed to estimate the sample's motion. The shape of estimated displacement curve is directly related to the shear modulus of the sample. The time-displacement curve can be compared to theoretic models to determine the actual shear modulus for that specific point in time. Repeated acquisition over time produces a signature curve that shows dynamic changes in shear modulus of the sample during coagulation. From this curve, the start of the clot formation, or clot time, and the stiffness of the clot can be directly estimated. The combination of these two parameters provides information about the functional role of the coagulation factors, fibrinogen, and platelets in the sample. Shear modulus values are determined every 4 seconds by interrogating the blood sample. Clot time, expressed in minutes, is estimated by determining the time at which rate of change in stiffness exceeds a predefined value. Clot stiffness is estimated by identifying the shear modulus value at a specific time point after clot time. This parameter is expressed in units of hectopascals, or hPa (1 hPa= 100 Pa).

Validation and comparative studies

The validation of the results of Quantra have been performed using a plasma-based control material and Whole blood samples from healthy volunteers (blood samples obtained in 3.2% sodium citrate). Comparative studies were performed against TEG 5000 and the Clauss fibrinogen assay implemented in the StagoSTart4 Hemostasis analyzer (Stago, Asnieres-sur-Seine, France).

Protocol:

Twenty healthy parturients aged 18-40 years with uncomplicated pregnancies at term gestation, 37 to 41 weeks, presenting for labor, or cesarean delivery will be recruited. Exclusion criteria includes hypertension, preeclampsia, gestational diabetes, preexisting coagulopathy, history of deep vein thrombosis, medications that impair coagulation, or history of pulmonary embolism or thrombosis. Women in active labor receiving intravenous fluids, or oxytocin will be also excluded. After obtaining consent, one table spoon of blood (16.2 ml) will be obtained at the time placement of intravenous cannula along with blood obtained for routine blood work (Hemoglobin and platelet count). The blood will be collected into five citrated Vacutainers (Beckton Dickinson, Franklin Lakes, NJ) each with a maximum capacity of 2.7 mL of blood and containing 0.5 mL 0.109 molar, 3.2% sodium citrated. Citrated blood from each Vacutainer from a single patient will be pooled to eliminate variability in citrate concentration between samples.

Protocol Methodology:

Three ml (aliquot 1) of pooled blood will be used analyzed in the Quantra analyzer using 4 channel cartridge for obtaining clot time and clot stiffness. Second aliquot (2) will be used to determine CBC and conventional coagulation tests (PT, PTT, Fibrinogen, Factor VIII). These three sets (Quantra, CBC, and CL will provide baseline values for Quantra and conventional coagulation tests. 3 ml of pooled blood (aliquot 3) will be spun to yield plasma for additional process (PP).

Six ml (aliquot 4) will be mixed with 18 ml 0.9% normal saline to yield 24 ml of diluted blood (75%). Four ml will be used for Quntra and CL tests. Four additional aliquots of diluted blood sample will be mixed

with pregnant subject's plasma or non-pregnant plasma to yield 15%, and 30% PP aliquots, or 15% and 30% NP aliquots. These diluted blood enriched samples will be analyzed for Quantra and CL tests.

Details of the protocol flow are shown in table 2.

Statistical Analysis:

Results will be presented as Mean±SD for each parameter. Paired t test will be used to study differences in each parameter compared to the baseline for each method.

A scatter plot will be used to compare variations in parameter among three methods of coagulation assessment.

In addition, ANOVA (Analysis of Variance) for multiple comparisons will be used to assess changes in coagulation parameter of each methodology.

Flow Sheet:

Collect six blue tops

Run Quantra – One tube (Q1)

Mix 5 tubes = 15 ml

8 ml into four aliquot tubes and spin at 1000 rmp for 10 min.

One aliquot 1 ml for CL (1)

Balance 3.8 ml approx. for PP dilution.

Study protocol

7 ml of blood to 21 ml NS: 28 ml (Sample 2)

75% diluted sample:

3 ml Quantra – Q2

2m to aliquot tube for centrifuge CCL2 and transferred to CL2 (Draw from this CCL2 tube to fresh aliquot tube for CL2)

15% Reconstitution to diluted blood: NP Plasma (Sample 3)

4 ml DB + 0.7 (NP plasma) = 4.7

3ml Quantra Q3

1.7 ml CCL3 centrifuged and transferred to aliquot CL 3

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15 % Reconstitution to diluted blood: PP Plasma (Sample 4)

4 ml DB + 0.7 PP =4.7

3ml Quantra Q4

1.7 ml centrifuged in CCL 4 and transferred to aliquot CL 4

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30 % Reconstitution to diluted blood:NP Plasma (Sample 5)

4 ml DB + 1.7ml NP =5.7 ml

3 ml Qunatra Q 5

2 ml centrifuged in CCL5 and transferred to aliquot CL 5

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30 % Reconstitution to diluted blood:PP Plasma (Sample 6)

4 ml DB + 1.7ml PP =5.7 ml

3 ml Qunatra Q 6

2 ml centrifuged in CCL 6 and transferred to CL 6

CL P and Q P

Will be the IDs NP plasma

We will see if one bag will suffice into 20 aliquots, or multiple bags needed.

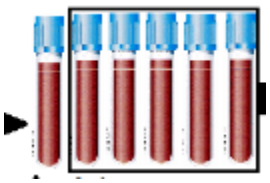
Our lab is able to provide the plasma

All set with approval for this.

FLOW SHEET

Thaw NP Plasma at 37 degree water bath Quantra P1 and CL P1

Draw 6 blue tubes



Q1

1 ml CL1

8 ml

8 ml into 4 aliquot tubes and spin

POOL 15 ml into a cup

7 ml

Glass tube 3ml

DB

7 ml + 21 ml NS =28 ml

3ml Q2

2 ml CCL centrifuge and transfer to CL2

CL P a

Balance PP

3.8 ml

Will b sma

We w will suffice into 20 alliquots, or multiple bags needed.

Our lab is able to provide the pla ma

All set with approval for t s.

s tube ml

15% NP

4ML DB +0.7 NP = 4.7

15% PP

4 ML DB + 0.7 PP= 4.7

30% NP

4 ML DB + 1.7 NP =5.7

30% PP

4 ML DB + 1.7 PP =5.7

3 ML Q3

1.7 CCL3 TO 1ML CL3

3 ML Q4

1.7 CCL4 TO 1ML CL4

3 ML Q5

1.7 CCL5 TO 1ML CL5

3 ML Q6

1.7 CCL6 TO 1ML CL6

Glass tube 3ml

Glass tube 3ml

Glass tube 3ml

Glass tube 3ml