Thromboelastogram Platelet Mapping Procedure # 1679.1

PRINCIPLE

Thromboelastography is a method for measuring the viscoelastic properties of clotting blood or plasma. The TEG analyzer includes a sample cup that oscillates constantly at a set speed through an arc of 4°45'. The cup, containing a blood or plasma sample, and a stationary pin attached to a torsion wire is immersed in the sample. Once initiated, the cup rotates back and forth at 10 second intervals. When the first measurable clot forms, it begins to bind the cup and pin, causing the pin to oscillate in phase with the cup. The rate of increased movement of the pin is a function of clot development and is graphically displayed. The torque created by fibrin-platelet bonding that links the cup and pin together is transmitted to the immersed pin. Increased strength of the generated fibrin-platelet bonds translates to increased magnitude of pin motion, which is directly related to the strength of the formed clot. If lysis occurs, some bonds are broken and the degree of pin motion is diminished. The degree of rotational movement by the pin is converted by a mechanical-electrical transducer to an electrical signal, which is monitored by a computer then converted into a graphical tracing that reflects the hemostasis profile of clot formation.

For platelet mapping, the results of a heparin samples exposed to a snake venom, which activates the fibrinogen glycoprotein receptor (IIbIIIa), and a combination of venom with ADP and arachidonic acid agonists. The degree of inhibition is calculated using proprietary software using the parameters of maximum amplitude from kaolin activation, venom activation and platelet agonist activation.

EQUIPMENT, REAGENTS AND SUPPLIES

1. TEG 5000 analyzer, related software and dedicated computer
2. Pipets capable of dispensing 10µL, 20µL, 50µL, 100µL and 360µL with related disposable tips
3. TEG platelet mapping kit
    a. 4 disposable cups and pins
    b. 1 vial of kaolin
    c. Mapping reagents
        i. A:P1: activatorF
            1. Reconstitute with 50µL of provided distilled water
        ii. ADP:P2: ADP,2µM final concentration
            1. Reconstitute with 100µL of provided distilled water
        iii. AA:P3: arachidonic acid, 1mM final concentration
            1. Reconstitute with 100µL of provided distilled water
        iv. Distilled water

<u>Reconstituted reagents are stable for one hour at room temperature.</u>

SAMPLE REQUIREMENTS

One 1.8mL 3.2% sodium citrate samples and one 5.0mL sodium heparin tube are required for testing.

Once collected, the sample should equilibrate at room temperature for at least 15 minutes, but testing must be completed within two hours of collection. Sample should not be transported via pneumatic tube system.

DAILY MAINTENANCE

See Thromboelastograph procedure 1697 for performance of daily maintenance.

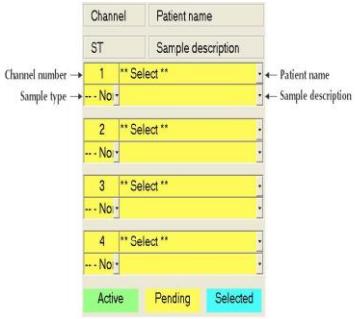
QUALITY CONTROL

Two levels of controls are required for every 24 hours of operation. The instrument will also provide alert by pop-up message when QC is due. Refer to procedure 1679 for instructions and procedure for performing daily electronic and commercial QC.

PROCEDURE

1. Prepare instrument and run QC as necessary (procedure 1679)
2. Assure instrument(s) is level
3. Load one cup and pin each channel on two TEG analyzers
    a. Slide the carrier down to the platform, with the lever in the Load position.
    b. Place a disposable cup, with the pin inside it, into the cupwell.
    c. Stabilize instrument by holding top portion of device while loading cup/pin, then carefully slide the carrier all the way up until it is flush with the bottom of the column. Make sure the pin stays upright in the cup so that it can fit over the tip of the spindle. Press upward on the pusher at the bottom of the carrier while using your other hand to apply counter-pressure to the top of the analyzer. This loads the pin on the spindle.
    d. Slide the carrier back down and push the cup firmly into the cupwell. When the cup is seated correctly, the flange of the cup touches the top of the carrier.
4. From Main Menu, In the TAS Main screen, click 

icon.
5. Complete the following fields in the channel sections that correspond to the appropriate TEG analyzer:



NOTE: It is possible to use only a single TEG instrument to perform platelet mapping, and channels 3 and 4 would refer to testing on channels 1 and 2 using a single device.
NOTE: For new patient data entry into "Patient Name", there will be a pop-up box that that a new patient requires additional data entry… MR#, gender and age are required.

Select the sample type (ST) using the following instructions:

Channel 1: select Kaolin Citrated Whole Blood
Channel 2: select Activated
Channel 3: select Activated + ADP
Channel 4: select Activated + Arachidonic acid

Select form the sample description:

Channel 1: Baseline
Channel 2: Activated
Channel 3: Activated + ADP
Channel 4: Activated + Arachidonic acid

6. Add 20µL of provided 0.2M calcium chloride to channel #1 cup.
7. Add 10µL of Activator to each cup for channel 2-4
8. Add 10µL of ADP to channel 3
9. Add 10µL of Arachidonic acid to channel 4
10. Using plastic transfer pipet, add 1.0ml of citrated whole blood to Kaolin vial. Mix by inversion 3-4 times.
11. Quickly pipette 340µL of kaolin enriched whole blood to into channel #1 cup.
12. Stabilizing instrument by holding top portion of device, with other hand quickly but carefully raise the carriers until they are flush with the bottom of each column.
    1. Move the levers to Test.
    2. In the TEG screen, select (highlighted blue) the appropriate channel, then press F10. Once the sample has been started, it will be backlit green indicating testing in progress. When selecting a sample that is currently running, the green "ACTIVE" icon will flash.
13. Add 360µL of well mixed lithium heparin blood to channel 2.
    1. Using pipet, mix sample 3-4 times within the cup. Avoid bubble generating while mixing sample within the cup.
    2. Move the levers to Test.
    3. In the TEG screen, select (highlighted blue) the appropriate channel, then press F10. Once the sample has been started, it will be backlit green indicating testing in progress. When selecting a sample that is currently running, the green "ACTIVE" icon will flash.
14. Repeat for step 13 for channel 3 and 4, using a new pipet top after each sample. NOTE: Refer to procedure 1697 for details about screen view and function.
15. Allow Parameters (R, K, etc) display in real time as the sample is running. Parameters with numerical values with "*****" indicate preliminary results, while parameters with just numerical values are final results.

16. Allow the test to continue to run maximum amplitude (MA) is allowed to complete for each channel.
17. Once testing is complete, highlight sample, then press F11 to stop testing.

CALCULATING PLATELET MAPPING RESULTS

To correctly calculate platelet mapping results, the sequence must be followed:

For ADP inhibition:

1. From Main screen, select "Multi" function icon
2. Select the results from the patient in the following sequence
    a. Baseline (kaolin activation)
    b. Activator
    c. Activator + ADP
3. Select "Done" icon
4. Print results

For Arachidonic acid inhibition:

1. From Main screen, select "Multi" function icon
2. Select the results from the patient in the following sequence
    a. Baseline (kaolin activation)
    b. Activator
    c. Activator + Arachidonic acid
3. Select "Done" icon
4. Print results

Record results into LIS

REFERENCE RANGE:

This test is only intended for use on patients with recent anti-platelet therapy (aspirin and P2Y12 inhibitors)

RESULT REPORTING

% inhibiton for both ADP and Arachidonic acid will be reported in LIS.

CRITICAL VALUES

There are no critical values for this test

RESULT INTERPRETATIONS

Interpretation of TEG platelet mapping must be performed in conjunction with patient's clinical presentation, medication history, transfusion history, etc.

LIMITATIONS AND INTERFERENCES

- The TEG analyzer must be level. A leveling bubble and leveling feet are built into the instrument.
- The TEG analyzer is sensitive to vibration and must be set up so that vibrations and jolting are avoided.
- Testing sensitivity of the TEG analyzer is affected if the following environmental specifications are not met:
    - Operating temperature must be between 15°C to 30°C. Storage temperature is from –30°C to 50°C. Mains supply fluctuations not to exceed ±10% of the nominal voltage. Maximum relative humidity is 80%. Over-voltage Category II.
- The maximum oscillation of the cup in the TEG instrument is approximately 5 degrees, as described in the Interference section below. Therefore, the maximum amplitude (MA parameter) cannot be measured beyond 96 mm.
- The eTest value of the TEG instrument determines the zero starting point of the graphical output tracing. Therefore, out of range conditions may prevent the TEG graph from reaching its maximum amplitude (the MA parameter may not reach its maximum value). The software issues a warning if the eTest value is out of range when a sample is started.
- As with any coagulation test, the TEG can be affected by pre-analytical variables associated with blood collection, transport, and temperature.
- The TEG platelet mapping is not intended to use for assessing or detecting platelet function defects.

INTERNAL PERFORMANCE VERIFICATION

Reference Range:

Published report (Bochsen, et al):
% inhibition for ADP: 0-58.1%
% inhibition for Arachidonic acid: 0-10.1%

Accuracy:

50% inhibition	ADP PRP NL	ADP PRP ABN
ADP TEG NL	0	18
ADP TEG ABN	3	1

10% Inhibition	AA PRP NL	AA PRP ABN
AA TEG NL	5	0
AA TEG ABN	8	9

25% Inhibition	AA PRP NL	AA PRP ABN
AA TEG NL	7	0
AA TEG ABN	6	9

REFERENCES

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Procedure History

Date	Written/ Revised By	Revision	Approved Date	Approved By
08/15	B. Gosselin	New	08/10/2015	L Howell, MD
09/15	B Gosselin	QC parameter change Sample mixing		