Materials Required but Not Provided

**Impedance cell counter**
- Blood collection materials (syringes, blood collection set, etc.)

**Step-by-Step Method**

**Note:** Allow Plateletworks tubes to equilibrate to room temperature (20-24°C) before adding sample.

1. Obtain the desired fresh whole blood sample. A 1.0 cc whole blood sample is required for each agonist tube and each baseline tube.

2. Gently dispense 1.0 cc of blood into each of the baseline tube and the agonist tube.

3. Mix each tube (baseline and agonist) vigorously 15 to 20 times to ensure adequate mixing.

4. The baseline tube is then run on the cell counter, recording the platelet count.

5. Continue to mix the AA tube by holding it in the hand and inverting it every 8-10 seconds for 2 minutes. Place the tube in a rack and allow to stand for 5 to 8 minutes.

6. The AA tube is then inverted gently 2 times to mix. Place the tube in the cell counter and record the platelet count.

**Note:** If running AA tube as part of the Combo Kit, count the baseline tube, then the ADP, the collagen tube and last the Arachidonic Acid tube. All counts can be completed in 10 minutes.

7. The percent platelet aggregation is then calculated
   - a) from the % Aggregation/Inhibition Chart supplied in the packaged tubes, or
   - b) using the Plateletworks Calculation Wheel, and
   - c) calculated by the appropriate formula.

**Baseline Platelet - Agonist Platelet**

**Count**

<table>
<thead>
<tr>
<th>Count</th>
<th>% Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>X 100%</td>
<td></td>
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</tbody>
</table>

**Quality Control**

Quality control testing of the cell counter used to perform the Plateletworks assay should be completed during each shift the system is used. These results will ensure that the instrument is functioning properly and that the results obtained are valid. It is suggested that each laboratory establish its own normal range. No commercial controls for platelet aggregation testing are available. Blood drawn from healthy adults may be used as reference controls for the Plateletworks assay. These individuals must not be free from any medication known to affect platelet function for a minimum of 7 to 9 days and should have prior platelet aggregation tests that fall within the normal range established by the laboratory. If the first normal control value is outside the normal reference interval, a second normal control should be run. If the second normal control values are also outside the normal reference interval, the assay should be considered out of control and no testing should be performed. In this case, contact Helena’s Technical Support for assistance.

**REFERENCE VALUES**

The reference value for Plateletworks AA agonist tube was determined on samples collected from healthy volunteers. The data are as follows:

**Aspirin**
- Non-Aspirin
- Total

<table>
<thead>
<tr>
<th>Normal (positive) = 360% Aggregation</th>
<th>Abnormal (negative) = &lt;60% Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Agreement 90.7%</td>
<td>Positive Agreement 87.6%</td>
</tr>
<tr>
<td>Negative Agreement 97.0%</td>
<td></td>
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</tbody>
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**LIMITATIONS**

- Only fresh human whole blood should be added to the Plateletworks tubes. Do not collect samples into blood collection tubes containing anticoagulant (i.e., sodium citrate, EDTA, or heparin) prior to addition to the Plateletworks tubes.
- It is recognized that many drugs and compounds (prescription and non-prescription) may affect platelet aggregation. The most common of these is aspirin. Therefore, a complete medical history that includes a list of drugs taken for 7-10 days prior to testing should be obtained.
- The validity of the Plateletworks assay is dependent on the accuracy of the platelet counts obtained. Multiple factors may potentially interfere with the accuracy of the platelet count when performed on an automated cell counter. Therefore, platelet counts obtained should be scrutinized in light of the patient’s clinical circumstance and previous platelet count results. Platelet results should always be interpreted in light of the patient’s medical history and condition of the patient.
- It may be beneficial for any abnormal baseline results to be further investigated using additional platelet testing methodology, such as platelet count, bleeding time, measurement of platelet morphology, and others.
- Do not use Plateletworks tubes past their expiration date or those which have been improperly stored.
- Plateletworks results may be affected by poor technique (e.g., improper blood sample volume, delayed test performance beyond recommended procedure, etc.).

**INTERFERENCES**

Platelet thrombocytopenia, though infrequent, can result from EDTA-dependent platelet agglutination. Pseudothrombocytopenia may be suspected with the Plateletworks assay if the platelet count determined using the agonist tube is higher than the platelet count determined using the baseline tube (containing EDTA anticoagulant). If pseudothrombocytopenia is suspected, common laboratory practice is to re-draw the blood sample and retest.
sample into a sodium citrate collection tube and perform the blood count; the results should be corrected by a factor of 1.1 to account for the sample dilution that occurs with the use of sodium citrate as an anticoagulant. This procedure should be followed using the sodium citrate tube in lieu of the Plateletworks baseline tube, followed by the Plateletworks agonist tube, to determine percent platelet aggregation.

- Cell counters utilizing electronic impedance cell counting principles may be subject to known interfering substances which can impact platelet count results. These include:
  - Microcytes, schizocytes, and WBC fragments, which may interfere with the proper counting of platelets and cause elevated platelet counts.
  - Agglutinated erythrocytes, which may trap platelets and cause an erroneously low platelet count.
  - Giant platelets, which may cause an erroneously low platelet count since they may exceed the upper limit threshold for the platelet parameter.
  - Chemotherapy, which may increase the fragility of platelets and cause low platelet counts.
  - Hemolysis, which contains red cell stroma and may elevate platelet counts.
  - Acid-citrate-dextrose (ACD) blood, which may contain platelet aggregates that could depress the platelet count.
  - RBC inclusions, which may produce a spuriously increased platelet count.
  - Platelet agglutination, due to poor collection techniques or EDTA activation, which may cause a decreased platelet count.

**PERFORMANCE CHARACTERISTICS**

**Correlation Study**

Correlation of the Plateletworks assay to platelet aggregometry on platelet rich plasma (PRP) is supported by data generated by testing male and female adults, greater than 18 years of age, at three clinical sites. This includes normal, healthy volunteers, and patients and volunteers who were taking aspirin. All blood samples were acquired from in-dwelling lines or venipuncture using established methods. For the Plateletworks assays and PRP aggregometry, the manufacturers’ recommendations were adhered to as per instructions provided in the package insert. A positive result was equal to or greater than 60% aggregation and a negative result was less than 60% aggregation. A comparison study of 337 specimens gave an overall agreement of 87.5%; positive agreement of 93.2%; and negative agreement of 85.0%.

**Note:** Thrombocytopenic specimens may be tested using the Plateletworks assay. As this system utilizes electrical impedance cell counting principles (i.e., Ichor Hematology Analyzer), instrument platelet counts >10^11/L can be accurately obtained. Agonist platelet counts can be measured in samples meeting the limits of aggregation detection (>27 x 10^3/µL). Although EDTA-induced thrombocytopenic samples may be tested using the Plateletworks assay, no actual testing was performed on this sample type.

**Precision**

Precision of the Plateletworks assay was determined using duplicate samples from a patient who was taking aspirin. For the Plateletworks assay, no actual testing was performed on this sample type.

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