AggreDYNE A-100 Platelet Aggregation Test

AA Assay

INTENDED USE

The AggreGuide A-100 AA Assay is a qualitative system to aid in the detection of platelet dysfunction due to aspirin ingestion by those 18 years of age or older in 3.2% citrated venous whole blood using the AggreGuide A-100 instrument. For professional use. This test is not for use in patients with underlying congenital platelet abnormalities, patients with non-aspirin induced acquired platelet abnormalities or in patients receiving non-aspirin anti-platelet agents. The test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

LIMITATIONS

Pre and Post 325 mg Aspirin study - The study results used to demonstrate the performance of the AA assay are based predominantly on healthy subjects (i.e. having 2 or less cardiovascular disease risks factors) ingesting non-coated aspirin. Ten percent (10%) of study subjects showed platelet dysfunction at baseline without ingesting aspirin.

Post-81 mg aspirin study - sixty (n=60) healthy subjects were studied at one time point post-aspirin ingestion of non-coated aspirin. Twelve (n=12) of the sixty subjects were on chronic aspirin. Forty eight (n=48) of the sixty subjects took aspirin for one week. Eighty-two percent (82%) of the subjects showed platelet dysfunction post aspirin ingestion. The prevalence of platelet dysfunction at baseline for patients taking 81 mg aspirin has not been established.

PRODUCT DESCRIPTION

The AggreGuide A-100 ("AggreGuide") is a laser light scattering system that detects the level of platelet aggregation induced by arachidonic acid (AA) agonist in whole blood in motion. The system consists of an instrument and disposable assay cartridge with a pre-loaded freeze dried agonist. A whole blood sample is added to a disposable cartridge that is preloaded with freeze dried arachidonic acid agonist (AA) in a reaction chamber for each individual test. The amount of platelet aggregation is measured by detecting the laser light scattering caused by platelet aggregates. Aspirin is known to inhibit the level of platelet activity when blood is mixed with arachidonic acid.[2]

PRINCIPLE

The AggreGuide AA assay is designed to measure platelet aggregation when a patient's whole blood is mixed with arachidonic acid (AA). Arachidonic acid in the platelet is oxygenated and rearranged by cyclooxygenase (COX-1) into endoperoxide and subsequently into thromboxane. When thromboxane is released from within the platelet and binds to the surface thromboxane receptors, platelet aggregation occurs. Aspirin irreversibly inactivates COX-1 which renders the platelets unresponsive to AA.[1]

MATERIALS PROVIDED

AggreGuide AA assay cartridges come individually sealed in Mylar pouches. Each cartridge contains a lyophilized mixture of trehalose, FD&C Red 40 dye, and arachidonic acid.

REAGENT STORAGE AND HANDLING

When a box of cartridges is opened, check the temperature indicator located on the outside of the box. If the indicator spot has turned black, this indicates exposure to elevated temperatures and should not be used. Call your distributor or customer support for a replacement.

- Store cartridges at room temperature, 59° F to 77° F (15° C to 25° C).
- Cartridge should remain sealed in the Mylar pouch until ready for use to prevent damage by humidity.

MATERIALS REQUIRED BUT NOT PROVIDED

- AggreGuide A-100 Instrument.
- QC cartridge (Provided with the instrument)
- Blood collection tubes containing 3.2% sodium citrate.
- Blood collection materials
- Electronic pipettor (See AggreGuide A-100 User's Manual)
- Pipette tips (200 µl with Gel-Load 0.6 mm OD orifice)

PRECAUTIONS

- For *in-vitro* diagnostic use.
- The AggreGuide and its components should only be used as directed in the AggreGuide User's Manual.
- Do not use the AggreGuide AA assay cartridge beyond the expiration date.
- Cartridges should remain sealed in the Mylar pouch until ready for use to prevent damage by humidity.
- All patient samples should be handled as if capable of transmitting disease.
- Samples should be treated as bio hazardous material and handled according to the institution's policies.
- The reagents are manufactured with a material derived from non-animal sources.

SAMPLE COLLECTION PRECAUTIONS

- Improper blood collection techniques may cause erroneous results. If unexpected or questionable results are reported, repeat the test with a new sample and a new cartridge.
- Fresh whole blood samples in the appropriate collection device are required for use with the AggreGuide A-100.
- Do not freeze or refrigerate sample. Samples should be stored at room temperature, 59° F to 77° F (15° C to 25° C).
- Collection of the blood sample should be performed with care to avoid hemolysis or contamination by tissue factors. Samples with any evidence of clotting should not be used.
- Do not shake or agitate samples. Hand carrying of samples is preferred. Pneumatic tube systems should be avoided.
- Always ensure collection tubes are filled to the indicated fill volumes. At altitudes greater than 2500 feet above sea level, blood collection tubes may not fill to the specified volume, which results in an incorrect ratio of blood to anticoagulant. Users at these elevations should refer to their facility's blood collection protocols for instructions to properly fill blood collection tubes.
- Samples should be collected and handled according to the institution's policies and procedures pertaining to bio hazardous material.
- Testing should not be performed until 10 minutes after blood collection.
- The cap of the collection tube should be replaced promptly after blood has been sampled to avoid pH changes in the blood sample.

SAMPLE COLLECTION AND HANDLING

- All whole blood samples must be assayed from vacuum collection tubes containing sodium citrate (3.2%).
- Blood should be tested within 10 minutes - 4 hours of the blood draw.

Instructions for Sample Collection Directly Into Vacuum Collection Tubes:

1. Whole blood may be collected from venous sites using a 19 or 21 gauge needle in 3.2% citrate vacuum collection tube. Blood samples should be obtained from an extremity free of peripheral venous infusions.
2. Gently invert the sample tube at least 5 times to ensure complete mixing of the contents.

Special Instructions if blood is obtained from an indwelling catheter:

1. Whole blood samples that are obtained from an indwelling catheter should be collected after sufficient discard (approximately 5 mL) has been drawn to clear the line. Ensure indwelling catheter is free of clots.
2. When using a syringe, transfer blood to the appropriate blood collection tube immediately after collection.
3. Gently invert the sample tube 5 times to ensure complete mixing of the contents.

TEST PROCEDURE

1. Refer to the AggreGuide A-100 User's Manual for complete operating instructions.
2. Open the Mylar pouch and remove the cartridge.
3. Insert the cartridge into the AggreGuide.
4. The AggreGuide will prompt user to prepare to pipette blood and press the READY button. Gently invert the sample tube 3 - 5 times before aspirating with pipettor.
5. The AggreGuide will prompt user to introduce blood and press RUN button.
6. The platelet activity index (PAI) will be displayed once the test has completed.
7 Remove the cartridge and check for any visible bubbles larger than 1mm. If bubbles greater than 1mm are present, abort test and repeat with a new cartridge.

REPORTED RESULTS

- Results are reported as Platelet Activity Index (PAI). The PAI is related to the number of AA induced platelets aggregated in the blood sample. The PAI represents the level of platelet aggregation. Higher PAI values represent higher levels of platelet aggregation and lower PAI values represent lower levels of platelet aggregation (or platelet dysfunction due to aspirin ingestion).
- The analytical measurement range has been determined to be from 2 to 12 PAI. The instrument will report a "LOW" result for values at or below the lower limit of the measuring range. The instrument will report a "HIGH" result for values above the upper limit of the measuring range. Numeric results will not be reported for values outside of the measurement range.
- With AA as the agonist, PAI values lower than 4 indicate platelet dysfunction.

CALIBRATION

The AggreGuide is calibrated at the factory. There are no user calibrations.

QUALITY CONTROL

The manufacturer recommends that laboratory quality control testing be performed by the user to verify the assay reagent is performing to expectation, as part of a regular quality control program including those that the user may be required to perform to comply with any local and state regulations, or other accrediting bodies' requirements.

Certificates of Conformance are provided for each lot of AA Assay Cartridges which certify that lot release testing for precision and accuracy of the cartridges have met the release criteria.

Internal Controls: The AggreGuide A-100 system utilizes controls that are internal to the instrument and act in connection to the AggreGuide A-100 AA Assay cartridges. These internal controls are checked at instrument start up, on an ongoing basis when the instrument is waiting for an assay to be performed, and during the beginning and ongoing portions of the actual assay.

External Controls: The "QC2" quality control cartridge and included software features allow the user to test for any gross changes to the system that might diminish quality of the AggreGuide A-100 measurements. Using a special quality control device called the "QC2 Cartridge" the A-100 tests the system that detects platelet aggregates. The QC2 quality control test is not a replacement for clinical laboratory validation, but does serve as a method of detecting gross failures of the A-100 system. The A-100 system software requires that the QC2 test is performed every thirty days, or every 200 assays, whichever occurs first. Additional A-100 assays cannot be performed unless a QC2 test has been successfully performed. The laboratory can elect to perform the QC2 test more frequently, for example, daily, as part of its laboratory quality control program.

The manufacturer recommends that laboratory quality control testing be performed by the user to verify the AA Assay cartridge is performing to expectation. This is recommended each time a new lot or shipment of assay cartridges is received, or every thirty days.

Since there are no commercially available standard control materials for platelet aggregation testing, it is recommended that the laboratory establish their quality control program using suitable elements from these approaches.

1. Aggredyne provides a liquid quality control kit, the AggreGuide A-100 Liquid Quality Control (LQC) Kit. It is suggested that each laboratory establish a testing program that utilizes this product as part of its quality control program. This LQC Kit is used without blood, and permits the User to verify correct operation of the AggreGuide A-100 AA Assay cartridges in the AggreGuide A-100. The LQC Kit contains both negative control and positive control liquid samples. When pipetted into Assay cartridges the negative control shows behavior that is representative of aggregation of platelets, indicative of the absence of platelet dysfunction due to anti-platelet medications. The positive control shows behavior representative of the absence of platelet aggregates, indicative of the presence of platelet dysfunction due to anti-platelet medications.
2. Blood drawn from healthy adult donors may be used for normal controls. It is suggested that each laboratory establish a control donor group. Such donors must not have taken any medication that is known to affect platelet function for at least 5 days and should have prior platelet aggregation tests that have fallen within the normal reference range established by the laboratory. The manufacturer recommends using donors whose PAI value is > 6.7. If the results do not fall within the expected range, a second donor should be tested. If the second control donor's results are also considered outside the reference range, the assay should be considered out of control and no further clinical testing should be performed. Please call technical support for assistance.

It is the responsibility of the laboratory director to develop an appropriate laboratory quality control program.

Please call technical support for assistance.

ASSAY LIMITATIONS

The lyophilized reagent is hygroscopic and can degrade after prolonged exposure to room air. Therefore, the cartridge should be used immediately after removal from the Mylar pouch.

With erroneous test results, the possibility of improper specimen collection or handling should be investigated. Repeat the test using a new cartridge and specimen.

Patients with inherited platelet disorders such as Von Willebrand Factor Deficiency, Glanzmann's Thrombasthenia and Bernard-Soulier Syndrome have not been studied with the AggreGuide.

Patients with a known history of platelet counts < 110,000/µL have not been studied.

It is known that many medications or compounds (prescription and non-prescription) may affect platelet aggregation. Therefore a thorough medication history of the patient should be taken and reviewed.

Thienopyridines e.g. clopidogrel (Plavix®), prasugrel (Effient®), and ticagrelor (Brilinta®) are known to inhibit platelet function by a mechanism (ADP blockade) different from aspirin and should not be evaluated with the AggreGuide A-100 AA assay. The AggreGuide A-100 AA assay is not recommended for patients taking thienopyridines.

AggreGuide test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

PERFORMANCE CHARACTERISTICS

Expected Values

A reference range for the AggreGuide AA Assay was calculated from 128 healthy subject's PAI who were not receiving drug therapy with Aspirin. This is summarized in Table 1.

Table 1 – Reference Range Determination

	PAI for Healthy Subjects
N	128
Mean	6.30
SD	2.03
5th – 95th percentile	3 - 9

Note: Each laboratory should establish its own expected values

Cut Point

The high dose and low dose aspirin studies served as the training studies to select a pre-determined cut-off of 4 PAI. The cut-off was calculated using an ROC curve and the intersection of the sensitivity and specificity obtained in the training studies. The intersection of sensitivity and specificity indicate that the cut point for determining whether platelet dysfunction due to aspirin is present is four, i.e., patients with PAI below 4 have platelet dysfunction due to aspirin, i.e., are responsive, while those equal to or greater than 4 do not have dysfunction, i.e., do not respond or have not taken aspirin

Sensitivity

One hundred sixty nine (n=169) healthy subjects were screened for study of the sensitivity of the AggreGuide A-100 in identifying platelet dysfunction due to aspirin ingestion. One hundred thirty eight (n=138) subjects were given an aspirin dose of 325 mg (1 adult noncoated tablet) and tested with the AggreGuide for aspirin induced platelet dysfunction within 2-30 hours. A summary of the patient demographics is provided in Table 2. A summary of the results is provided in Table 3, 4 and 5.

Table 2 – Demographics

Subject category	**Count**
Subjects Enrolled	169
Subjects excluded for not meeting the protocol's inclusion criteria	2
Subjects excluded for pre aspirin PAI < 4	16
Subjects dropped for not returning for post aspirin evaluation	13
Net evaluable subjects	138
Gender	**Percent**
Female	44%
Male	56%
Race	**Percent**
American Indian/Alaska native	1%
Asians	3%
Black/African American	9%
Hispanic/Latino	44%
Native Hawaiian/islander	1%
White	40%
Other	2%
Age	**Count**
Mean Age	37.19
Standard Deviation	11.22
Minimum	18
Maximum	68

Table 3 – Sensitivity Results

Site	Sensitivity	CI (95%)
All Sites	115/138 (83%)	76% - 89%

Table 4 – Pre 325 mg Aspirin

Aspirin 325 mg Absent		**Total**
≥ 4 PAI (Platelet dysfunction not detected)	< 4 PAI (Platelet dysfunction detected)	
151	16	167*
True Negative: 90% (151/167) 95% CI: (85%; 94%)	False Positive: 10% (16/167) 95% CI: (6%; 15%)	

*2 subjects were excluded due to inclusion/exclusion criteria

Table 5 – Post 325 mg Aspirin

Aspirin 325 mg Present			**Total**
≥ 4 PAI (Platelet dysfunction not detected)	< 4 PAI (Platelet dysfunction detected)	Missing PAI (due tp non-return)	
23	115	13	151*
False Negative: 17% (23/138) 95% CI: (11%; 24%)	True Positive: 83% (115/138) 95% CI: (76%; 89%)	Missing 9% (13/151) 95% CI: (5%; 14%)	

*16 subjects with PAI < 4 at baseline were not evaluated

Method Comparison

Agreement to the Accumetrics VerifyNow® is shown in Table 6 below.

Table 6 – Concordance Table

AggreGuide A-100	Accumetrics VerifyNow (VN)		Total
	< 550 (Platelet dysfunction detected)	**≥ 550** (Platelet Dysfunction Not Detected)	
≥ 4 (Platelet Dysfunction Not Detected)	8	20	28
< 4 (Platelet dysfunction detected)	32	3	35
Total	40	23	63

Agreement = 52/63=82.5% 95% CI: (64%; 91%)
Positive percent agreement (PPA) = 32/40=80%, 95% CI: (64%; 91%)
Negative percent agreement (NPA) – 20/23=87%, 95% CI: (66%; 97%)

The provided information and reference ranges should only be used as a guide for interpretation together with other clinical and laboratory data available to the clinician.

Precision

Simple precision was studied in three subjects with twenty replicates each. Table 7 shows the results of the simple precision study.

Table 7 – Simple Precision Results

PAI Type	Mean (PAI)	ST DEV	CV (%)
Low	3	0.39	12.88
Mid	5	0.83	15.47
High	9	0.96	10.14

Precision over time was studied in three subjects over twenty days. Table 8 shows that there was no drift over time.

Table 8 – Precision Over Time Results

Donor	Mean (PAI)	S_r (Repeatability Standard Deviation)	B (Standard Error of the Daily Means)	S_t (Device Precision Standard Deviation)	CV (S_t)
1	8	1.35	0.82	1.25	14.85
2	9	0.72	0.73	0.90	9.70
3	8	1.32	0.71	1.17	14.55

Inter-instrument variability was tested with three AggreGuide A-100 instruments on a single donor's blood. Twenty replicates were tested using AA Assay cartridges from a single lot. A general linear model with 'instrument' as a random variable was used. The mean was 8.3 and instrument variability (sd) was 0.17. The repeatability standard deviation was 0.78.

Lot to Lot variability was tested using sample AA Assay cartridges from each of three different lots on a single donor's blood. Twenty replicates were tested on each of the three AA Assay cartridge lot samples. A general linear model with 'lot' as a random variable was used. The mean was 10 and lot variability (sd) was 0. The repeatability standard deviation was 1.02.

Interfering Substances

- The AggreGuide A-100 has only been tested in subjects with hematocrit values ranging from 24.8% to 49.1%.
- Assay performance is not affected by hemolysis up to 600 mg/dL.
- No interference was observed when samples spiked with bilirubin up to 10 mg/dL were tested with the AggreGuide A-100 AA Assay.
- Assay performance was not affected by triglyceride levels between 66 and 361 mg/dL.
- Thienopyridines e.g. clopidogrel (Plavix®), prasugrel (Effient®), and ticagrelor (Brillinta®) are known to inhibit platelet function by a mechanism (ADP blockade) different from aspirin and should not be evaluated with the AA assay. The AA assay is not recommended for patients taking thienopyridines.

ERROR MESSAGES

If the instrument displays an error message or there is a technical error while running a test, the assay may be aborted by selecting "abort" instead of "save" after running a test. Please refer to the AggreGuide A-100 User's Manual for a more detailed explanation of errors and troubleshooting.

SERVICE

The AggreGuide is not intended to be serviced by the user. Instruments in need of service must be returned to Aggredyne, Inc. If there are problems related to the AggreGuide, call your distributor or call Aggredyne Customer Support. Customer/Technical Support 866-800-1955

BIBLIOGRAPHY:

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2. Zheng Y, Solen KA and Mohammad SF. *The light-scattering whole blood aggregometer: a novel device for assessment of platelet aggregation in undiluted blood.* Arch Pathol Lab Med 1998; 122:880-886.

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AA Assay
US Patents 6,043,871, 7,393,690 B2, 7,262,059 B2

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