510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k122162

B. Purpose for Submission:

New device

C. Measurand:

Arachidonic acid assay (AA)

D. Type of Test:

Platelet aggregation

E. Applicant:

Aggredyne, Inc.

F. Proprietary and Established Names:

AggreGuide A-100 instrument

AggreGuide A-100 AA assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.5700, Automated Platelet Aggregation System

2. Classification:

   Class II

3. Product code:

   JOZ, System, Automated Platelet Aggregation

4. Panel:

   81 Hematology

H. Intended Use:

1. Intended use(s):

   The AggreGuide A-100 Aspirin 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. For professional use.

   This test is not for use in patients with underlying congenital 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.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

   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 risk 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 subjects were on chronic aspirin, and forty eight (n=48) 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.

4. Special instrument requirements:

   The AggreGuide A-100 instrument is required to test the AggreGuide A-100 AA assay.

I. Device Description:

The AggreGuide A-100 system consists of a stand-alone AggreGuide A-100 instrument and single use AggreGuide A-100 AA cartridge. Sodium citrated venous whole blood is added to the cartridge in the reaction chamber containing freeze dried arachidonic acid. The amount of platelet aggregation is measured by detecting the laser light scattered. Result is reported as Platelet Activity Index (PAI). The PAI is related to the number of platelets aggregated.

Higher PAI values represent a higher level of platelet aggregation. Lower PAI values represent a lower level of platelet aggregation.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   VerifyNow® Aspirin Assay, k042423

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The AggreGuide A-100 Aspirin 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. For professional use. This test is not for use in patients with underlying congenital 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.	The VerifyNow® Aspirin Test is a qualitative test to aid in the detection of platelet dysfunction due to aspirin ingestion in citrated whole blood for the point of care or laboratory testing. This test is not for use in patients with underlying congenial platelet abnormalities, patients with non-aspirin induced acquired platelet abnormalities or in patients receiving non-aspirin anti-platelet agents (may be used in patients treated with selective COX-2 inhibitors, e.g., celecoxib (Celebrex®)
Principle of Operation	Platelet aggregation with optical detection	Same
Type of Specimen	3.2% citrated whole blood	Same
Sample Preparation	None	Same
Calibration	Factory	Same
Test Agonist	Arachidonic acid	Same
Quality Control	QC cartridge	Same

Differences		
Item	Device	Predicate
Results	Platelet Activity Index (PAI)	Aspirin Reactive Units (ARU)
Cut-off	< 4 PAI (platelet dysfunction detected)	<550 ARU (platelet dysfunction detected)
External Quality Controls	None	Wet Quality Controls (WQC), level 1 and level 2
Test Procedure	Manual addition of sample to reagent cartridge	Placement of blood collection tube onto reagent cartridge

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI C28-A How to Define and Determine Reference Intervals in the Clinical Laboratory

L. Test Principle:

The AggreGuide A-100 is a laser light scattering system that detects the level of platelet aggregation induced by arachidonic acid agonist when whole blood is added to the AggreGuide A-100 AA assay cartridge. Arachidonic acid in the platelet is oxygenated and rearranged by cyclooxygenase (COX-1) into endoperoxide and subsequently into thromboxane. When thromboxane is released from the platelets and binds to the thromboxane receptors, platelet aggregation occurs. Aspirin irreversibly inactivates COX-1 which renders the platelets unresponsive to arachidonic acid, causes platelet dysfunction. The amount of platelet aggregation is measured by detecting the laser light scattered. Result is reported as Platelet Activity Index (PAI).

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   i. Simple Precision: Blood samples from three donors with low, mid, and high PAI levels were tested. Twenty replicates of each donor were used to assess the repeatability of the AggreGuide A-100 system.

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

ii. Precision-Over-Time: To evaluate the long term precision of the AggreGuide A system, blood samples from three normal donors were tested for twenty days, two replicates per run, and one run per day.

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

iii. Instrument-to-Instrument Variability: The study was conducted using three AggreGuide A-100 instruments and blood from a single normal donor. Twenty replicates were tested on each of three instruments using a single lot of the AA assay cartridge.

	Instrument 1	Instrument 2	Instrument 3	All instruments
N	20	20	20	60
Mean PAI	8.50	8.45	8.05	8.33
SD	0.76	0.69	0.89	0.80
%CV	8.95	8.12	11.02	9.55

iv. QC Test Verification for AggreGuide A-100 instrument: The technical QC cartridge was tested over three instruments for 13 days, one replicate per day.

AggreGuide A-100	Mean Electronic QC Test Count	SD	%CV
Instrument 1	1699	17	1%
Instrument 2	1704	9	0.5%
Instrument 3	1704	6	0.3%

v. Lot-to-Lot Variability for AggreGuide A-100 AA Assay Cartridges: To evaluate the lot-to-lot variability of the AA assay cartridge, three cartridge lots were used to test the blood of a single normal donor. Twenty replicates were tested on each cartridge yielding a total of 60 test results.

	Lot 1	Lot 2	Lot3	All Lots
N	20	20	20	60
Mean PAI	9.95	10.20	10.05	10.07
SD	0.83	1.15	1.10	1.02
%CV	8.30	11.29	10.94	10.16

b. Assay reportable range:

2-12 PAI

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:
Not applicable

Stability:
AggreGuide A-100 AA Assay Cartridge: To support a 12 month shelf life, the study was conducted at different time points for a 14 month period. Multiple lots were used in the study. At each time point, the aged cartridge was tested and results were compared to a freshly made cartridge using split fresh whole blood samples. Results were within acceptable limits.

Controls:

- Each reagent cartridge is checked by the AggreGuide instrument to ensure the cartridge is functioning properly.
- The electronic QC cartridge is performed weekly to verify that there have been no changes in the light scattering signal from the factory calibration.
- There are no manufactured external controls for AggreGuide A-100 AA assay. The manufacturer recommends that laboratories should establish their own donor reagent controls and may consider the following when establishing control donor groups:

    1) Normal PAI value: Blood drawn from healthy adults may be used for normal controls. The donor must not have taken any medication that is known to affect platelet function for at least 7 days and should have prior platelet aggregation tests that have fallen within the normal range established by the laboratory. The manufacturer recommends using donors whose PAI value is >5.

    2) Abnormal PAI value: Blood drawn from a donor who has been on aspirin may be used for abnormal controls. The donor must be on chronic aspirin treatment. The manufacturer recommends using donors whose PAI value is <4.

Aggredyne recommends that normal and abnormal donor control checks be performed for each new lot of AA assay cartridges. Additional QC check may be performed on a monthly, weekly, daily or shift basis based on the laboratory's

quality control policies. The end user should follow the recommendations of local and state regulatory agencies as well as facility policies.

Calibrators:
No calibrators are provided. Calibration is established by the manufacturer.

d. Detection limit:

To determine the blank/noise level for the AggreGuide A-100, three instruments and two assay cartridges specifically prepared (without reagents, without agonist) were used in the study. Blood samples from three donors were tested on each type of cartridge for 28 to 30 replicates. The study demonstrated that the PAI values 0-2 represent the blank/noise level, no detectable aggregation.

e. Analytical specificity:

i. Bilirubin: Blood was collected from 3 donors and spiked with bilirubin solution yielding control and elevated bilirubin samples. The AA assays were conducted in duplicate. The study demonstrated that there is no significant difference in platelet aggregation between the control samples and elevated bilirubin samples up to 10 mg/dL.
ii. Hemolysis: Blood was collected from 3 donors and spiked with hemoglobin. The AA assays were performed in singlicate. The results indicate that there is no significant difference in platelet aggregation between the control samples and samples with elevated hemoglobin up to 600 mg/dL.
iii. Lipid: Measurements of PAI from the AA assay were compared to the baseline and elevated triglyceride from 3 donors subsequent to a high fat meal. The study demonstrated that there is no significant difference in platelet aggregation between the control samples and samples with elevated triglyceride up to 360 mg/dL.

f. Assay cut-off:

Not applicable

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:
At one study site, results for the AggreGuide A-100 AA assay were compared to the predicate device VerifyNow® Aspirin assay for detection of aspirin effect in 63 adult subjects regardless of their aspirin status who were not taking warfarin or other anticoagulant medications.

- Demographics
  - N = 63
  - Gender: 51% males, 49% females
  - Ethnicity: 81% Caucasians, 8% Hispanics, 9% Asians, 2% African-Americans

- Outcome Variables
  - AggreGuide A-100 AA assay: Platelet Aggregation Activity (PAI)
  - VerifyNow® Aspirin assay: Aspirin Reaction Units (ARU)

- Measurement cut-off values
  - PAI < 4 indicates low platelet activity or platelet dysfunction
  - ARU < 550 indicates low platelet activity or platelet dysfunction

The method comparison study used split samples drawn from a single venipuncture to compare the results generated from the AggeGuide A-100 AA assay and VerifyNow® Aspirin assay.

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

Positive percent agreement (PPA) = 32/40 = 80%, 95% CI: (64%; 91%)
Negative percent agreement (NPA) = 20/23 = 87%, 95% CI: (66%; 97%)
Total Agreement = 52/63=82.5%, 95% CI: (71%; 91%)

b. Matrix comparison:

Not applicable

3. Clinical studies

a. Clinical Sensitivity and Specificity:
The clinical performance of the AggreGuide A-100 AA assay was evaluated at three sites with 169 healthy adults for pre and post 325 mg aspirin ingestion. Sixteen (16) subjects were not evaluable because pre-aspirin ingestion PAI results were below 4. Thirteen (13) subjects did not return for post-aspirin ingestion, two (2) subjects did not pass inclusion criteria, yielding 138 evaluable subjects. Each test subject had a baseline AA assay measurement. Then subjects took a single dose of 325 mg non-

coated aspirin and had an AA measurement after 2-30 hours of aspirin ingestion. The pre-established cut-off value, PAI < 4, was used to determine sensitivity, indicating low platelet activity or platelet dysfunction.

Results:
Demographic:

- Gender: 56% males, 44% females
- Race: 44% Hispanics, 40% Caucasians, 9% Black/African Americans, 3% Asians, 1% American Indian/Alaska native, 1% Native Hawaiian/Islander, 2% Other

Pre 325 mg aspirin ingestion:

≥4 PAI (Platelet dysfunction not detected)	<4 PAI (Platelet dysfunction detected)	Total
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

Post 325 mg aspirin ingestion:

Aspirin 325 mg Present			
≥4 PAI (Platelet dysfunction not detected)	<4 PAI (Platelet dysfunction detected)	Missing PAI (due to non-return)	Total
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 with PAI < 4 at baseline were not evaluated.

Based on this study, clinical sensitivity of the AggreGuide A-100 AA assay is 83% (115/138) (95%CI: 76%-89%).

b. Other clinical supportive data (when a. is not applicable):

4. Clinical cut-off**:**

Aspirin studies were used as a training set to establish the cut-off of 4 PAI. Cut-off was calculated using ROC curve and the intersection of the sensitivity and specificity obtained in the training studies.

5. Expected values/Reference range:

The reference range of the AggreGuide A-100 AA assay was established in a study involving 128 healthy adult subjects who were not receiving drug therapy with aspirin.

The cohort consisted of 65% females and 45% males. Test subjects included 66% Caucasians, 34% Blacks and 21% Hispanics.

	PAI
Mean	6.30
SD	2.03
5^{th} – 95^{th} percentile	3-9

N. Instrument Name:

AggreGuide A-100

O. System Descriptions:

1. Modes of Operation:

   The AggreGuide A-100 instrument is semi-automated. The software manages data collection.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Manual entry

4. Specimen Sampling and Handling:

   Manual sampling: manual addition of sample to reagent cartridge.

5. Calibration:

   The AggreGuide A-100 instrument is calibrated at manufacture. There are no user calibrations.

6. Quality Control:

   A technical QC check of the AggreGuide A-100 is performed by the end user by running a QC cartridge weekly that verifies there have been no changes in the light scattering signal from factory calibration. The electronic QC test count is the detector raw signal count that is the source data used for factory calibration and QC.

There are no commercial external quality control materials to verify the performance of the AggreGuide system. The manufacturer recommends the end user to use normal and abnormal donor controls for each lot of AA assay cartridges or on a monthly, weekly, daily or shift basis, based on the laboratory's quality control policies and according to the state, local, and regulatory guidelines of the testing facility.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.