

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K062025

B. Purpose for Submission:

To obtain clearance for a new device.

C. Measurand:

11 dehydro thromboxane B₂ (TxB₂)

D. Type of Test:

Enzyme linked immunoassay (ELISA)

E. Applicant:

Corgenix, Inc

F. Proprietary and Established Names:

AspirinWorks[®] Test Kit

G. Regulatory Information:

1. Regulation section:

21 CFR 864.5700

2. Classification:

Class II

3. Product code:

OBW

4. Panel:

81 Hematology

H. Intended Use:

1. Intended use(s):

The AspirinWorks[®] Test Kit is an enzyme-linked immunoassay (ELISA) to determine levels of 11-Dehydro Thromboxane B₂ (11dhTxB₂) in human urine, which aids in the qualitative detection of aspirin effect in apparently healthy individuals post ingestion. For professional use only.

2. Indication(s) for use:

The AspirinWorks[®] Test Kit is an enzyme-linked immunoassay (ELISA) to determine levels of 11-Dehydro Thromboxane B₂ (11dhTxB₂) in human urine, which aids in the qualitative detection of aspirin effect in apparently healthy individuals post ingestion. For professional use only.

The AspirinWorks[®] test Kit is intended for use in clinical (hospital and reference) laboratories.

3. Special conditions for use statement(s):

4. Special instrument requirements:

I. Device Description:

A competitive ELISA for the detection of urinary 11-Dehydro Thromboxane B₂ (11dhTxB₂). Each AspirinWorks kit contains goat anti-mouse IgG polyclonal antibody coated onto 96-microwell plates, Sample Diluent, Reference Solution, three levels of control, purified 11dhTxB₂ conjugated to alkaline phosphatase, purified anti-11TxB₂ antibody, chromogenic substrate, stopping solution and wash concentrate. Purified mouse monoclonal antibody and purified 11dhTxB₂ conjugated to alkaline phosphatase compete for binding with the 11dhTxB₂ present in the sample. The intensity of color development following addition of chromogenic substrate (pNPP) is inversely proportional to the concentration of 11 dhTxB₂ in the urine sample read at 405 nm. Results (pg/ml) are calculated against a reference curve. Final patient results are normalized by urine creatinine concentration, as measured by a separate assay.

J. Substantial Equivalence Information:

1. Predicate device name(s):

VerifyNow[™] Aspirin Assay

2. Predicate 510(k) number(s):

K042423

3. Comparison with predicate:

| Similarities | | |
|---------------------|--|---|
| Item | Device | Predicate |
| Assay Principle | Detects a metabolite of thromboxane A ₂ , a direct inducer of platelet aggregation. | Measures <i>ex vivo</i> platelet aggregation caused by thromboxane A ₂ . |
| Measurement | Qualitative | Qualitative |

| Differences | | |
|--|--|---|
| Item | Device | Predicate |
| Intended Use | An enzyme-linked immunoassay (ELISA) to determine levels of 11-Dehydro Thromboxane B ₂ (11dhTxB ₂) in human urine, which aids in the qualitative detection of aspirin effect in apparently healthy individuals post ingestion. For professional use only. | A qualitative assay to aid in the detection of platelet dysfunction due to aspirin ingestion in citrated whole blood for the point of care or laboratory setting. |
| Assay Principle | An enzyme-linked immunosorbent assay (ELISA) for the detection of 11dhTxB ₂ in human urine | A turbidimetric based optical detection system which measures platelet induced aggregation. |
| Sample Matrix | Human urine | Whole blood |
| Cutoff - qualitative detection of aspirin effect | 1500 pg/mg creatinine | 550 Aspirin Resistance Units (ARU) |
| Methodology | Manual ELISA | Automated assay |

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

CLSI EP-6 *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*

CLSI EP7-A2 *Interference Testing in Clinical Chemistry; Approved Guideline – Second Ed*

CLSI EP17-A *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*

L. Test Principle:

The AspirinWorks[®] Test Kit measures urinary 11 dhTxB₂ and is performed as a competitive ELISA. Diluted samples (Reference Solution, controls and patient urine), purified 11dhTxB₂ conjugated to alkaline phosphatase (AP), and purified mouse monoclonal antibody directed to 11dhTxB₂ are combined and incubated in microwells coated with a polyclonal anti-mouse antibody. Incubation allows the endogenous 11dhTxB₂ present in the samples to compete with the purified AP-conjugated 11dhTxB₂ for binding to the mouse monoclonal anti11dhTxB₂ antibody. The monoclonal antibody then binds to the polyclonal anti-mouse antibody coated on the microtiter plate. The complex formed on the plate is composed of monoclonal antibody and endogenous or AP-conjugated 11dhTxB₂. After removal of unbound complexes by washing, the bound AP-11dhTxB₂ conjugate is assayed by the addition of para-nitrophenylphosphate (pNPP) chromogenic substrate. The intensity of color development in the wells is inversely proportional to the sample urine concentration of 11dhTxB₂, and is read at 405 nm. Results (pg/mL) are calculated against a reference curve prepared from the Reference Solution provided in the kit. Final results are reported as pg 11dhTxB₂ per mg creatinine to normalize results for urine concentration.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The AspirinWorks Test Kit was evaluated for repeatability and within-laboratory precision. Three urine samples were run on 24 wells per plate over three plates per lot, repeated on three lots for a total of 216 measurements per urine sample. A test outcome is defined as the average of two measurements, so the study design results in 108 test measurements (12 per plate over three plates per lot run on three lots of plates) on which to base the precision calculations shown in the table below.

| Urine # | Mean 11-DehydroThromboxane B ₂ Concentration | Repeatability as %CV | Within-Laboratory Precision as %CV |
|---------|---|----------------------|------------------------------------|
| 1 | 424 pg/mL | 8% | 14% |
| 2 | 1399 pg/mL | 5% | 7% |
| 3 | 3380 pg/mL | 5% | 10% |

Repeatability and within-laboratory precision for the AspirinWorks Test Kit is acceptable for each of the three pilot lots according to the Product Specifications set by Corgenix (all CV% less than 20%).

b. *Linearity/assay reportable range:*

Linearity was performed following the CLSI EP-6 guideline. A low and high value urine sample were diluted together and the samples were run in quadruplicate on the AspirinWorks Test Kit. Mean values of the range of dilutions for linearity assessment were 352 – 4475 pg/ml. The detection range for 11-Dehydro Thromboxane B₂ assay is 300-4000 pg/ml urine.

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

Stability testing was performed in real time and tested throughout the shelf life of the kit. Lot specific components were used for testing. Data was provided for 11 months real time stability to date. Acceptable ranges for the lot of urine-based controls were 216-416 pg/ml for Control 1, 598-916 pg/ml for Control 2, and 1480-2220 pg/ml for Control 3. Values were determined to be within acceptable ranges.

Room temperature, 4° C, and freeze-thaw stability testing was performed as follows: five urine samples collected from apparently healthy volunteers. Aliquots of the samples were then stored either at room temperature or 4° C for the times specified, or frozen and thawed. The samples were run on the AspirinWorks Test Kit immediately (time zero) or after the times/freeze-thaws.

Real time stability testing on samples has also been performed. For these studies, 37 samples spanning the range of the assay were collected from apparently healthy volunteers. Aliquots were made and samples were frozen at -70° C. These samples were then measured for 11dhTxB₂ levels (in pg/ml) quarterly over a period of one year. Results reveal that frozen samples remain viable for 11dhTxB₂ measurement for up to one year at -70° C. Stability analysis will be monitored for up to two years.

Value assignment of Reference Solution was performed using a panel of 12 urine samples (obtained from three bulk urine samples) with lot specific plates from the beginning, middle and end of each coating of each of three pilot lots. Two operators each ran four assays using beginning, middle and end plates of the coating run. The raw data was transferred into a controlled spreadsheet and the mean OD, standard deviation, and %CV were calculated for each sample on each plate. A minimum of six of the eight assays must pass all specifications for R², %CV, control values, assay blank and non-specific binding according to the kit Product Specifications. The Reference Solution correction factor was then calculated from this data.

d. Detection limit:

The Limit of Blank (LoB) was determined to be 151 pg/ml and the Limit of Detection (LoD) was determined to be 222 pg/ml based on the protocol in CLSI EP17-A.

To determine the LoB, two urine samples with minimal levels of 11dhTxB₂ were used as blanks and run by 3 operators across 3 plate lots for a total of 72 measurements. The mean of the blank measurements was 91.4 pg/ml with a pooled standard deviation of 36.1 pg/ml. The Limit of Blank (LoB) was calculated based upon the blank mean plus the pooled standard deviation multiplied by the c_{β} of 1.651 to obtain the σ_{β} and was determined to be 151.0 pg/ml.

To determine the LoD, four urine samples with low levels of 11dhTxB₂ were run by 3 operators across 3 plate lots for a total of 144 measurements. The pooled mean of the low samples was 204.4 pg/ml with a pooled standard deviation of 43.1 pg/ml. The Limit of Detection (LoD) was calculated based upon the LoB plus the pooled standard deviation multiplied by the c_{β} of 1.648 to obtain the σ_{β} and was determined to be 222.0 pg/ml.

e. Analytical specificity:

Two urines were measured without the presence of any interferent (labeled “Control”), as well as in the presence of two different concentrations of interferents (in mg/dL, 1x and 0.5x). The difference in values (“diff”) is the amount of 11dhTxB₂ (in pg/mL) different from the control value. The percent recovery is in relation to the control value. The raw data was calculated from duplicate well from each interferent. The spiked samples used for interference testing include common agents that could be found in the urine of patients submitting specimens for the AspirinWorks test acetaminophen, acetylsalicylic acid, ascorbic acid, caffeine, gentisic acid, glucose, hemoglobin, protein (BSA), and salicylic acid. No significant interference ($\leq 25\%$ difference from control) was caused by physiologically excessive concentrations of the substances for the levels tested.

Recovery experiments were performed using four different samples containing a high value of 11dhTxB₂ diluted into the range of the assay. The samples were then serially diluted 1:1.25 in sample diluent for a final panel of 11 – 12 serial dilutions spanning the range of the assay and run on the AspirinWorks Test Kit pilot lots. The expected concentrations of each dilution were calculated based on the value obtained for the top dilution of each sample. Observed values were compared to expected values, and a ratio of observed/expected values was calculated.

f. *Assay cut-off:*

11-Dehydro Thromboxane B₂ concentrations were measured and normalized in 405 patients (210 samples from individuals on aspirin and 204 samples from individuals not on aspirin). Analysis of the frequency distribution of the samples resulted in a cutoff of 1500 pg 11dhTxB₂ per mg urinary creatinine.

2. Comparison studies:

a. *Method comparison with predicate device:*

Results for the AspirinWorks Test Kit compared the predicate device at one study site for detection of aspirin effect in apparently healthy subjects. In total, 173 urine samples were analyzed both on and off aspirin, and all were used to compare the AspirinWorks Test Kit to the predicate device. In the study, 44/49 (89.8%) of the AspirinWorks Baseline 81 mg samples were above cutoff compared to 47/49 (95.9%) with the predicate. For the Baseline 325 mg samples, 34/38 (89.5%) were above cutoff for the AspirinWorks kit, while 38/38 (100%) were above cutoff for the predicate. Of those that had ingested 81 mg aspirin per day, 3/47 (6.4%) of the AspirinWorks samples were above 1500 pg/mg creatinine, while 1/48 (2.1%) of the samples measured by the predicate were above the assay cutoff. In patients taking 325 mg ASA per day, 4/37 (10.8%) of the samples were above cutoff for the AspirinWorks kit, while 3/38 (7.9%) were above cutoff for the predicate.

The data is presented in the table below.

| | | VerifyNow™ Result | |
|---------------------------------|--------------------------------------|------------------------|------------------------|
| | | Positive (<550 ARU) | Negative (≥550 ARU) |
| AspirinWorks® Result | Positive (≤1500 pg/mg creatinine) | 77 | 11 |
| | Negative (>1500 pg/mg creatinine) | 7 | 78 |
| Total | | 84 | 89 |

Overall Percent Agreement = 89.6%
 Positive Percent Agreement = 91.7%
 Negative Percent Agreement = 87.6%

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

The clinical performance of the Corgenix AspirinWorks® test was evaluated at two study sites with apparently healthy individuals, before and after aspirin ingestion. Samples were tested to evaluate and validate the AspirinWorks Test Kit. AspirinWorks® results are presented as positive or negative, based on a cutoff of 1500 pg 11-Dehydro Thromboxane B₂ per mg urinary creatinine. A table is presented for both 81 mg and 325 mg aspirin doses.

| | | Aspirin Ingestion | |
|---|---|--------------------------|------------|
| | | Present | Absent |
| AspirinWorks® Result 81 mg | Positive (≤1500 pg/mg creatinine) | 156 | 20 |
| | Negative (>1500 pg/mg creatinine) | 7 | 146 |
| Total | | 163 | 166 |

Overall Percent Agreement = 91.8%
 Positive Percent Agreement = 95.7%
 Negative Percent Agreement = 88.0%

| | | Aspirin Ingestion | |
|--|---|--------------------------|-----------|
| | | Present | Absent |
| AspirinWorks® Result 325 mg | Positive (≤1500 pg/mg creatinine) | 34 | 4 |
| | Negative (>1500 pg/mg creatinine) | 4 | 34 |
| Total | | 38 | 38 |

Overall Percent Agreement = 89.5%
 Positive Percent Agreement = 89.5%
 Negative Percent Agreement = 89.5%

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

A cutoff was established at 1500 pg 11dhTxB₂ per mg urinary creatinine.

5. Expected values/Reference range:

Performance characteristics of the AspirinWorks® Test Kit were evaluated in a study involving 166 apparently healthy adults before and/or after receiving controlled doses of aspirin (201 samples from individuals on aspirin and 204 samples from individuals not on aspirin). 11-Dehydro Thromboxane B₂ concentrations were measured and normalized by dividing by the concentration of creatinine. A frequency distribution graph of the 405 samples is shown below. Based on these frequencies, a cutoff was established at 1500 pg 11dhTxB₂ per mg urinary creatinine.

N. Proposed Labeling:

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

O. Conclusion:

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