

**Corgenix 11dhTx<sub>B</sub><sub>2</sub> Test Kit  
(11-Dehydro Thromboxane B<sub>2</sub>)****For In Vitro Diagnostic Use****INTENDED USE**

The 11dhTx<sub>B</sub><sub>2</sub> Test Kit is an enzyme-linked immunoassay (ELISA) to determine levels of 11-Dehydro Thromboxane B<sub>2</sub> (11dhTx<sub>B</sub><sub>2</sub>) in human urine, which aids in the qualitative detection of acetylsalicylic acid (ASA) effect in apparently healthy individuals post ingestion. For professional use only.

**SUMMARY AND EXPLANATION OF THE ASSAY**

Activated and aggregated platelets play a key role in clot formation. Activated platelets produce Thromboxane A<sub>2</sub> (Tx<sub>A</sub><sub>2</sub>), a potent vasoconstrictor and inducer of platelet aggregation.<sup>1-2</sup> Tx<sub>A</sub><sub>2</sub> is generated by Thromboxane synthase from molecules derived from arachidonic acid by cyclooxygenase-1 (COX-1).<sup>1,3</sup> Tx<sub>A</sub><sub>2</sub> has a short half-life in plasma and is rapidly hydrolyzed to Thromboxane B<sub>2</sub> (Tx<sub>B</sub><sub>2</sub>). Tx<sub>B</sub><sub>2</sub>, in turn, is metabolized to 11-Dehydro Thromboxane B<sub>2</sub> (11dhTx<sub>B</sub><sub>2</sub>), 11-Dehydro 2,3 dinor Thromboxane B<sub>2</sub> (11dh2,3DTx<sub>B</sub><sub>2</sub>), a truncated form of 11dhTx<sub>B</sub><sub>2</sub>), and a number of other minor Tx<sub>B</sub><sub>2</sub> metabolites which are excreted by the kidney.<sup>4-7</sup> Thus, 11dhTx<sub>B</sub><sub>2</sub> is a stable metabolite of Tx<sub>A</sub><sub>2</sub> and an *in vivo* indicator of platelet activity.

ASA has been known for many years to have antiplatelet activity.<sup>8</sup> ASA functions by acetylating and irreversibly inhibiting COX-1, thus inhibiting the production of Tx<sub>A</sub><sub>2</sub> and its metabolites.<sup>9-11</sup> Low dose ASA blocks more than 95% of platelet COX-1 activity.<sup>12-13</sup> The measurement of stable metabolites of Tx<sub>A</sub><sub>2</sub>, such as urinary 11dhTx<sub>B</sub><sub>2</sub>, is a means of quantitating Tx<sub>A</sub><sub>2</sub> production *in vivo* and thus a direct way to analyze ASA's effect post ingestion.<sup>7,14-16</sup> The 11dhTx<sub>B</sub><sub>2</sub> test may determine if the ASA ingested by an individual is inhibiting platelet cyclooxygenase activity through the measurement of 11dhTx<sub>B</sub><sub>2</sub>. ASA is available under multiple brand names and/or in several different forms including Bayer Aspirin®.\*

**PRINCIPLE OF THE TEST**

The 11dhTx<sub>B</sub><sub>2</sub> Test Kit measures urinary 11dhTx<sub>B</sub><sub>2</sub> and is performed as a competitive ELISA. Diluted samples (Reference Solution, controls, and patient urine), purified 11dhTx<sub>B</sub><sub>2</sub> conjugated to alkaline phosphatase (AP), and purified mouse monoclonal antibody directed to 11dhTx<sub>B</sub><sub>2</sub> are combined and incubated in microwells coated with a polyclonal anti-mouse antibody. Incubation allows the endogenous 11dhTx<sub>B</sub><sub>2</sub> present in the samples to compete with the purified AP-conjugated 11dhTx<sub>B</sub><sub>2</sub> for binding to the mouse monoclonal anti-11dhTx<sub>B</sub><sub>2</sub> 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 11dhTx<sub>B</sub><sub>2</sub>. After the removal of unbound complexes by washing, the bound AP-11dhTx<sub>B</sub><sub>2</sub> conjugate is assayed by the addition of para-nitrophenylphosphate (pNPP) chromogenic substrate. Color develops in the wells at an intensity inversely proportional to the sample urine concentration of 11dhTx<sub>B</sub><sub>2</sub>, and is read at 405nm. Results (pg/mL) are calculated against a reference curve prepared from the Reference Solution provided in the kit.

Final results are reported as pg 11dhTx<sub>B</sub><sub>2</sub> per mg creatinine to normalize results for urine concentration.

**REAGENTS**

Store at 2–8 °C. Do Not Freeze.

Each 11dhTx<sub>B</sub><sub>2</sub> Test Kit contains the following reagents

**(volumes may vary depending on kit size and configuration):**

- 12 x 8 stabilized antibody (goat) coated microwells, with frame.
- 60 mL Sample Diluent\* (green capped bottle).
- 1.75 mL 5000 pg/mL Reference Solution\* (11dhTx<sub>B</sub><sub>2</sub> in buffer), for preparation of the reference curve; refer to vial label for lot specific correction factor.
- 1 vial lyophilized Level 1 Control (human urine) to be reconstituted to 0.5 mL with purified water; see vial label for expected range.
- 1 vial lyophilized Level 2 Control (human urine) to be reconstituted to 0.5 mL with purified water; see vial label for expected range.

\*Aspirin is a registered trademark of Bayer AG in certain countries.

- 1 vial lyophilized Level 3 Control (human urine) to be reconstituted to 0.5 mL with purified water; see vial label for expected range.
- 10 mL AP-Tracer Solution, red solution (purified 11dhTx<sub>B</sub><sub>2</sub> conjugated to alkaline-phosphatase).
- 10 mL Antibody Solution (murine), blue solution (purified anti-11dhTx<sub>B</sub><sub>2</sub> antibody).
- 23 mL One-Component pNPP Substrate (para-nitrophenylphosphate, stabilized); ready to use.
- 15 mL Stopping Solution (0.1 M EDTA); ready to use (red cap).
- 2 x 50 mL Wash Concentrate TBS/Tween 20 (20X).
- 2 adhesive plate sealers.

**\* CAUTION: Contains sodium azide**

## **WARNINGS AND PRECAUTIONS**

### **For In Vitro Diagnostic Use**

1. Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.
5. pNPP substrate can cause irritation to the eyes. Absorption through the skin is possible. Use gloves when handling substrate and wash thoroughly after handling.
6. Certain components of this product contain sodium azide as a preservative (Sample Diluent and Reference Solution). Sodium azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing azide must be thoroughly flushed with copious amounts of water to prevent the build-up of explosive metal azides in the plumbing system.
7. Certain components are labeled with the following:

Irritating to eyes (R 36). Irritating to skin (R 38). Avoid contact with skin (S 24). Avoid contact with eyes (S 25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). Wear suitable protective clothing (S 36). If swallowed, seek medical advice immediately and show this container or label (S 46).

Irritant  Biological Risk .

## **SPECIMEN COLLECTION AND PREPARATION**

Human urine is the recommended sample matrix. Samples should be collected and a urinary preservative should be added within 24 hours if not tested immediately. Recommended preservatives include Chlorstat tablets (Bio-Medical Products Corp.), BD C&S Vacutainer tubes or BD UAP Vacutainer tubes (Becton, Dickenson and Company). If not tested immediately, samples should be stored at 2-8°C. If samples are to be stored for more than 72 hours, they should be frozen at ≤ -20°C. Fresh or thawed samples should be centrifuged at 1000xg for 15 minutes prior to running test. Each sample must be tested for both creatinine and 11dhTx<sub>B</sub><sub>2</sub> for accurate results.

## **INSTRUCTIONS FOR USE**

### **Materials Provided:**

11dhTx<sub>B</sub><sub>2</sub> Test Kit; see "Reagents" for a complete listing.

### **Materials Required but not Provided:**

- Reagent grade water to prepare TBS/Tween 20 wash solution and lyophilized components
- Graduated cylinders
- Precision pipettors capable of delivering between 50 µL and 1000 µL, with appropriate tips
- Miscellaneous glassware appropriate for small volume handling
- Flask or bottle, 1 liter
- Wash bottles, preferably with the tip partially cut back to provide a wide stream, or an automated or semi-automated washing system
- Disposable gloves
- Plate reading spectrophotometer capable of reading absorbance between 405 and 420 nm
- Multichannel pipettors capable of delivering to 8 wells simultaneously
- Rotary shaker capable of 300 - 600 rpm (Speeds of 300-600 rpm are acceptable, however 600 rpm is recommended)

## **Procedural Notes**

1. Obtain urinary creatinine values for each patient sample.
2. Bring urine samples and kit reagents to room temperature (18–26°C) and mix well before using; avoid foaming. Return all unused reagents to refrigerated storage as soon as possible. Remaining controls may be dispensed into single-use aliquots and frozen at -20°C or below for subsequent use.
3. Frozen samples should be thawed and centrifuged at 1000xg before testing.
4. All dilutions of the Reference Solution, Controls, and patient samples must be made just prior to use in the assay.
5. The plate reader should be programmed to air blank.
6. Good washing technique is critical for optimal performance of the assay. Adequate washing is best accomplished by directing a forceful stream of wash solution from a plastic squeeze bottle with a wide tip into the bottom of the microwells. An automated microtiter plate washing system can also be used.  
**IMPORTANT:** Failure to adequately remove residual TBS/Tween 20 can cause inconsistent color development of the Substrate Solution.
7. Use a multichannel pipettor capable of delivering to 8 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
8. Add reagents carefully to the side of the microwells to avoid well-to-well pipet tip contamination, or change tips with each row of reagent addition.
9. Carefully controlled timing of all steps is critical. All Reference Solution dilutions, Controls, and samples must be added as quickly and consistently as possible.
10. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
11. Addition of all samples and reagents should be performed at the same rate and in the same sequence with the samples, controls, and Reference Solution dilutions added first, followed by the tracer. The monoclonal antibody must be added last.
12. Incubation temperatures other than normal room temperature (18–26°C) may contribute to inaccurate results.
13. Avoid contamination of reagents when opening and removing aliquots from the primary vials.
14. Do not use kit components beyond expiration date.
15. Do not mix kit components from different kit lot numbers.

## **Reagent Preparation**

**Wash Solution (TBS/Tween 20):** Measure 50 mL of Wash Concentrate (20X TBS/Tween 20) and dilute to 1 liter with reagent grade water. Store unused TBS/Tween 20 solution in the refrigerator at 2–8°C . Discard if the solution shows signs of microbial contamination.

**Urine controls:** Reconstitute urine controls (Levels 1, 2, and 3) with 0.5 mL reagent grade water. Swirl gently and allow 10 minutes for reconstitution. Unused portions may be dispensed into single-use aliquots and frozen at ≤ -20°C for up to one year.

## **Assay Procedure**

1. Remove any microwell strips that will not be used from the frame. Store them with the desiccant packet in the resealable bag provided.
2. Prepare the reference curve. Label six tubes #1-6. Add 500 µL 11dhTx<sub>B<sub>2</sub></sub> 5000 pg/mL Reference Solution to tube #1. Add 250 µL Sample Diluent to tubes #2-6. Remove 250 µL from tube #1, transfer to tube #2 and mix well. Repeat this 1:2 serial dilution series through tube #6. The resulting prepared standards are 5000, 2500, 1250, 625, 312.5, and 156.25 pg/mL.
3. Prepare a 1:5 dilution of the controls and patient samples in Sample Diluent, e.g., 100 µL sample added to 400 µL Sample Diluent equals a 1:5 sample dilution.
4. Duplicate well determinations are recommended for Reference Solution and control samples. Mix all sample dilutions thoroughly, and add 100 µL of the dilutions (6 Reference Solution dilutions, patient samples and controls) to the appropriate microwells.
5. A maximum binding sample (B<sub>0</sub>) should be run (the B<sub>0</sub> contains antibody and tracer, but no competing analyte). Add 100 µL Sample Diluent to duplicate wells designated for the B<sub>0</sub>.
6. An assay blank should also be run. Leave duplicate assay blank wells empty.
7. Add 50 µL AP-Tracer Solution (red) to each of the 6 Reference Solution wells, patient sample wells, control wells and the B<sub>0</sub> wells. Leave the assay blank wells empty.

8. Add 50  $\mu$ L Antibody Solution (blue) to each of the 6 Reference Solution wells, patient sample wells, control wells, and  $B_0$  wells. Leave the assay blank wells empty.

Microwells should contain the following reagent volumes:

<u>Reference Curve</u>	<u>AP-Tracer Solution</u>	<u>Antibody Solution</u>
100 $\mu$ L 5000 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L 2500 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L 1250 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L 625 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L 312.5 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L 156.25 pg/mL	50 $\mu$ L	50 $\mu$ L
100 $\mu$ L Sample Diluent ( $B_0$ )	50 $\mu$ L	50 $\mu$ L

<u>Samples/Controls</u>	<u>AP-Tracer Solution</u>	<u>Antibody Solution</u>
100 $\mu$ L	50 $\mu$ L	50 $\mu$ L

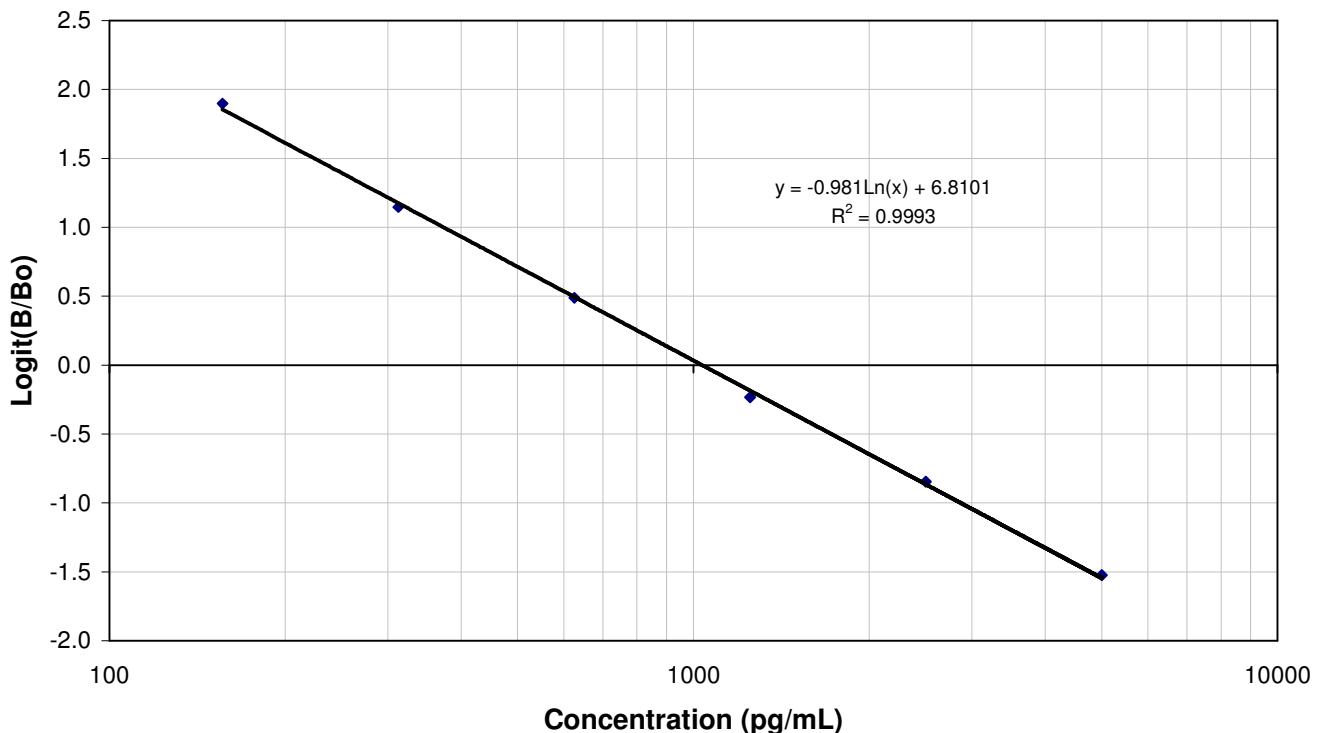
9. Cover plate with adhesive plate sealer provided and incubate 2 hours at room temperature (18-26°C) on a rotary shaker at 300-600 rpm. After the incubation is complete, grip the microplate frame firmly on the top and bottom to retain microwell modules and carefully invert the microwells to empty the sample fluid. Do not allow samples to contaminate other microwells.
10. Wash 5 times with room temperature wash solution. Each well should be completely filled with TBS/Tween 20 per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. Blot on absorbent paper to remove residual wash fluid. Do not allow wells to dry out between steps.
11. Add 200  $\mu$ L pNPP Substrate to each well including  $B_0$  and assay blank wells, cover with a fresh adhesive plate sealer, and incubate for 30 minutes at room temperature with rotary shaking. Add the substrate to the wells at a steady rate. A yellow color will develop in wells inversely proportional to the amount of 11dhTxB<sub>2</sub> present.
12. Add 100  $\mu$ L Stopping Solution to each well, including  $B_0$  and assay blank wells, to stop the enzyme reaction. Be sure to add Stopping Solution to the wells in the same order and at the same rate as the Substrate was added.
13. Air blank or zero the plate reader. Read the O.D. of each well between 405 and 420 nm. The O.D. values should be measured within 1 hour after the addition of Stopping Solution.

## Results

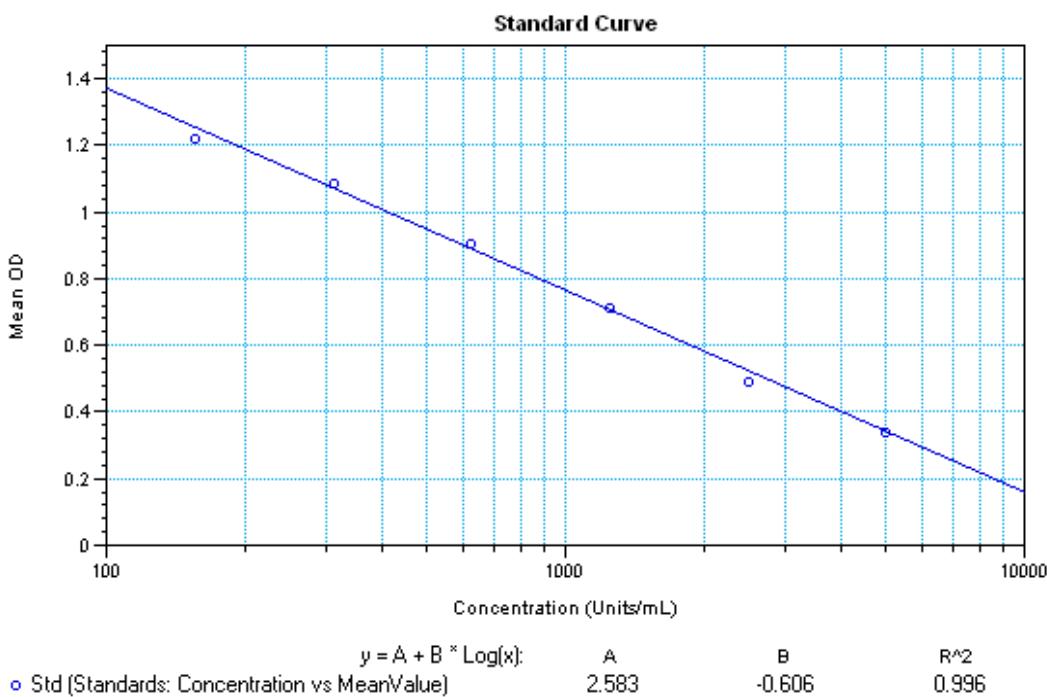
Assay results should be calculated by log-logit or semi-log analysis with linear regression (a “best fit” line) drawn through the reference points. Interpolate control and patient relative values from the reference curve, and multiply the relative values by the Correction Factor for the Reference Solution (see vial label). Normalize patient results by incorporating creatinine levels, i.e divide the 11dhTxB<sub>2</sub> result (in pg/mL) by the creatinine result for the patient sample (in mg/dL) and multiply by 100. The patient result may be reported as pg 11dhTxB<sub>2</sub>/mg creatinine. Ensure that all quality control parameters have been met (see Quality Control) before reporting test results.

An example of 11dhTxB<sub>2</sub> Reference Curves (log-logit and semi-log) are shown below. These reference curves are for the purposes of illustration only. A reference curve should be generated by the user for each assay performed.

**Log-Logit  
Reference Curve  
(Example Only - do not use)**



**Semi-Log Reference Curve  
(Example Only – do not use)**



## **Log-Logit Results Calculations**

Calculate the following for each concentration used in the reference curve and for each patient sample, based on the individual well OD:

$$P = \frac{(\text{Individual OD for that concentration} - \text{Mean OD for Blank wells})}{(\text{Mean OD for } B_0 - \text{Mean OD for Blank wells})}$$

Note that for each reference level and each patient sample one expects P to be between zero and one or do not proceed with calculations. Then calculate:

$$Y = \text{Log}(P/(1-P)) \text{ for each patient sample and for each reference level}$$

$$Y = \text{average from two individual well values} \quad (\text{if duplicates were performed})$$

Plot Y versus X=log (x) for each reference level where x is the actual concentration for that reference. Points should fall very close to a straight line. One can use log base 10 throughout or alternatively log base e (natural logarithm) but one should not mix the two log systems. Perform a linear regression and keep track of the slope ( $a_1$ ), intercept ( $a_0$ ), and  $R^2$  value. The latter should be greater than 0.95 to proceed.

Now you can proceed to evaluate your patient results by using the value of Y calculated for each patient, solving for X and then obtaining a value for your patient samples:

$$Y = a_1X + a_0 \quad \text{or} \quad X = (Y - a_0)/a_1$$

Patient relative value is:  $10^X$  if you used log base 10  
 $\exp(X)$  if you used the natural log

Determine the mean of duplicate X values (if performed)

X= average from two individual well values

## **Semi-Log Linear Regression Results Calculations**

Calculate the mean OD (blank subtracted) for the duplicates of each point of the reference curve and controls, and for patient samples (if duplicates were performed). Plot the mean OD obtained for each dilution of the reference curve (y-axis) against the corresponding value of the reference level on a log scale (x-axis). The reference curve can be plotted either automatically using a validated software program or manually using graph paper ("best fit" line). Determine control and patient sample values from the reference curve.

## **Patient Sample Results**

To calculate the level of 11dhTxB<sub>2</sub> in pg/mL, multiply the control and patient relative values by the Correction Factor for the Reference Solution (see vial label).

Normalize patient results by incorporating creatinine levels. Divide the 11dhTxB<sub>2</sub> result (in pg/mL) by the creatinine result for the patient sample (in mg/dL) and multiply by 100. The patient result may be reported as pg 11dhTxB<sub>2</sub>/mg creatinine.

Example:

- Patient relative value: 1000
- Reference Solution Correction Factor: 1.05
- Actual patient 11dhTxB<sub>2</sub> value:  $1000 \times 1.05 = 1050 \text{ pg/mL}$ .
- Patient creatinine value = 150 mg/dL
- Final reported value:  $(1050 \text{ pg/mL}/150 \text{ mg/dL}) \times 100 = 700 \text{ pg 11dhTxB}_2/\text{mg creatinine}$
- In this example, the result of 700 pg 11dhTxB<sub>2</sub>/mg creatinine is below the 1500 pg 11dhTxB<sub>2</sub>/mg creatinine cutoff point, suggesting that ASA effect is detected.

## Interpretation of Results

The sample result is based on the level of 11-Dehydro Thromboxane B<sub>2</sub> measured in the urine sample, normalized by the concentration of urine creatinine in the same sample, and reported in pg/mg quantities. Interpretation of results is based on the following assigned cutoffs:

- > 1500 pg/mg    Normalized Levels of 11-Dehydro Thromboxane B<sub>2</sub> indicate a lack of ASA effect  
≤ 1500 pg/mg    Normalized Levels of 11-Dehydro Thromboxane B<sub>2</sub> indicate an ASA effect

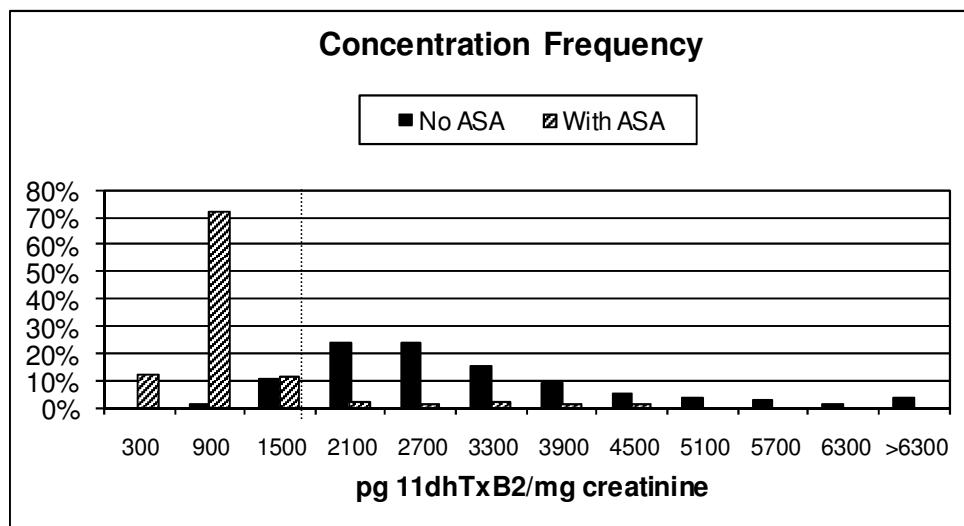
## QUALITY CONTROL

1. The mean O.D. of the B<sub>0</sub> (maximum binding) wells should be ≥ 0.600. Readings less than 0.600 may indicate possible reagent contamination or inadequate plate washing.
2. The mean O.D. of the assay blank should be ≤ 0.300. Readings greater than 0.300 may indicate possible reagent contamination or inadequate plate washing.
3. The 11dhTx<sub>B</sub><sub>2</sub> values obtained for the controls should be within the ranges indicated on the container labels.
4. Each laboratory should periodically determine their own normal range for the appropriate population of patients.
5. Samples with values outside 300 – 4000 pg/mL are outside the linear range of the reference curve and may be retested at an appropriate dilution. If a sample value is below 300 pg/mL, the sample may be retested using a fresh aliquot at a 1:2 dilution in Sample Diluent (250 µL sample + 250 µL Sample Diluent). If a sample value is above 4000 pg/mL, the sample may be retested using a fresh aliquot at a 1:10 dilution (50 µL sample + 450 µL Sample Diluent) or a 1:20 dilution (25 µL sample + 475 µL Sample Diluent). Since final results are based on a 1:5 dilution, be sure to adjust the calculations accordingly (i.e. for a 1:2 dilution multiply the final result by 0.4; for a 1:10 dilution multiply by 2).
6. O.D.s for the duplicates of the controls or patient samples (if performed) should be within 20% of the mean O.D. value for samples within the 300 – 4000 pg/mL range.
7. R<sup>2</sup> values for the Reference Curve should be ≥ 0.95.

## PERFORMANCE CHARACTERISTICS

### Expected Values:

Performance characteristics of the 11dhTx<sub>B</sub><sub>2</sub> Test Kit were evaluated in a study involving 166 apparently healthy adults before and/or after receiving controlled doses of ASA (201 samples from individuals on ASA and 204 samples from individuals not on ASA). 11-Dehydro Thromboxane B<sub>2</sub> 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 11dhTx<sub>B</sub><sub>2</sub> per mg urinary creatinine.



### **Clinical Performance:**

The clinical performance of the Coragenix 11dhTxB<sub>2</sub> test was also evaluated in these individuals. 11dhTxB<sub>2</sub> results are presented as positive or negative, based on a cutoff of 1500 pg 11-Dehydro Thromboxane B<sub>2</sub> per mg urinary creatinine. A table is presented for both 81 mg and 325 mg ASA doses.

		<b>ASA Ingestion</b>	
		Present	Absent
<b>11dhTxB<sub>2</sub> Result</b> <b>81 mg</b>	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%

		<b>ASA Ingestion</b>	
		Present	Absent
<b>11dhTxB<sub>2</sub> Result</b> <b>325 mg</b>	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%

### **Predicate Device Comparison**

The 11dhTxB<sub>2</sub> Test Kit was compared with the Accumetrics® VerifyNow™ Aspirin Assay using 173 urine samples from apparently healthy adults. The data is presented in the table below.

		<b>VerifyNow™ Result</b>	
		Positive (<550 ARU)	Negative (≥550 ARU)
<b>11dhTxB<sub>2</sub> Result</b>	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%

## Analytical Performance:

### Detection Range:

The detection range for 11-Dehydro Thromboxane B<sub>2</sub> in the 11dhTx B<sub>2</sub> Test Kit is 300 – 4000 pg/mL urine. For greatest accuracy, samples that generate values greater than 4000 pg/mL should be retested at an appropriate dilution. The analyte concentration reported should be normalized by dividing the measured 11dhTx B<sub>2</sub> by the concentration of creatinine as measured by a separate assay.

### Precision:

Three urine samples were run on 24 wells/plate over three plates/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/lot run on three lots of plates) on which to base the precision calculations shown in the table below.

Urine #	Mean 11-DehydroThromboxane B <sub>2</sub> 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%

### Interference:

Samples were tested for potential interference. The following materials had no significant effect on the measured concentration of 11-Dehydro Thromboxane B<sub>2</sub>:

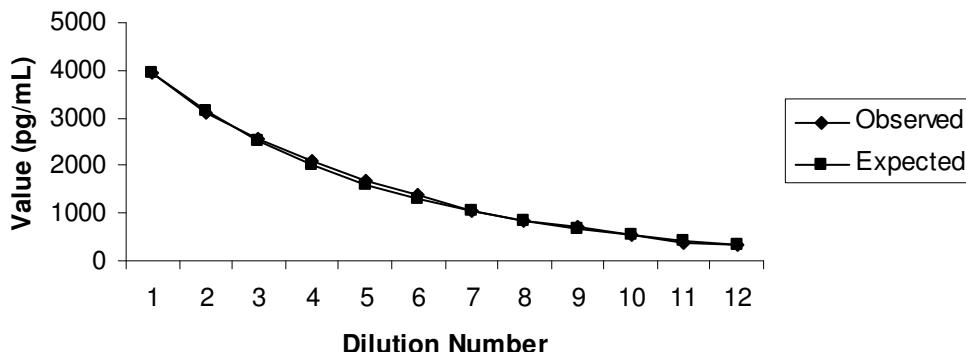
Compound	Concentration
Acetaminophen	200 mg/dL
Acetylsalicylic Acid	200 mg/dL
Ascorbic Acid	200 mg/dL
Caffeine	200 mg/dL
Gentisic Acid	200 mg/dL
Glucose	2000 mg/dL
Hemoglobin	1000 mg/dL
Protein	2000 mg/dL
Salicylic Acid	200 mg/dL

### Recovery:

Different samples containing a high value of 11dhTx B<sub>2</sub> were 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 11dhTx B<sub>2</sub> Test Kit. 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 as percentage. The table and graph below depict the results of one such urine tested. Other urine specimens tested showed similar results.

Dilution #	Observed (pg/mL)	Expected (pg/mL)	Obs/Exp (%)
1	3939	3939	100%
2	3095	3151	98%
3	2573	2521	102%
4	2082	2017	103%
5	1683	1613	104%
6	1391	1291	108%
7	1068	1033	103%
8	835	826	101%
9	694	661	105%
10	538	529	102%
11	399	423	94%
12	342	338	101%

## Recovery Example From Table



### Limit of Detection:

Based on 216 determinations using CSLI Document EP17-A (72 blank determinations and 144 positive determinations), the limit of detection for 11dhTxB<sub>2</sub> is 222 pg/mL, with a 95% probability of obtaining a positive response at this level and a 95% probability of obtaining a negative response on blank samples. A limit of blank of 151 pg/mL was used.

### **LIMITATIONS OF THE TEST**

11-Dehydro Thromboxane B<sub>2</sub> levels obtained with this assay may be used to assess the presence of an ASA effect in individuals. Each physician must interpret these results with regard to patient history, lifestyle, and other risk factors. It is necessary to take into account an individual's medications and nutritional and/or dietary supplements when determining if the patient is demonstrating an ASA effect as certain agents such as alcohol, green tea extract, chocolate, omega-3 fatty acids, ibuprofen, and COX-2 inhibitors may elicit an ASA-like effect and reduce the amount of thromboxane production in certain individuals.

Samples with excessive sediment, blood, or other insoluble material have not been evaluated and should be avoided for this assay.

Samples from individuals on oral anticoagulants, glycoprotein IIb/IIIa inhibitors, clopidogrel or heparin have not been studied with the 11dhTxB<sub>2</sub> Test Kit.

The 11dhTxB<sub>2</sub> Test Kit is not intended to measure the return of platelet function upon discontinuation of ASA ingestion.

It is not recommended to test individuals suffering from urinary tract infections, severe liver disease or end stage renal disease.

### **Warranty**

This product is warranted to perform as described in this package insert. Corgenix, Inc. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Corgenix, Inc. be liable for consequential damage.

**For Technical or Customer Service in the United States, phone 1-800-729-5661. Outside the United States, phone (303) 457-4345, fax (303) 457-4519, or contact a Corgenix authorized distributor.**

Some or all of the subject matter contained in the 11dhTxB<sub>2</sub> Package Insert is covered by a pending patent application.

## REFERENCES

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Manufacturer	Authorized Representative	In vitro diagnostic medical device	Batch Code	Use by/ Expiry Date	Temperature Limitation	Irritant	Biological Risk	Catalog Number	European Conformity	Consult Instructions for Use/ Package Insert
Hersteller	Bevoll-mächtigter	In-vitro-Diagnostikum	Chargennummer	Verfallsdatum	Temperatur-beschränkungen	Reizend	Biologisches Risiko	Katalognummer	CE-Konformitäts-kennzeichnung	Gebrauchsanweisung im Inneren der Verpackung beachten
Fabriqué par	Représentant agréé	Dispositif de diagnostic in vitro	Code de Lot	Utiliser jusqu' à/ Date de péremption	Limites de température	Irritant	Risque biologique	Numéro de catalogue	Conformité aux normes européennes	Consulter le mode d'emploi/ notice jointe au conditionnement
Fabricado por	Representante autorizado	Dispositivo médico para diagnóstico in vitro	Código de Lote	Usar antes de/ Fecha de caducidad	Limitación de temperatura	Irritante	Riesgo biológico	Número de catálogo	Conformidad europea	Consultar las instrucciones de uso/ prospecto del envase
Prodotta da	Rappresentante autorizzato	Dispositivo medico-diagnosticco in vitro	Codice del lotto	Scade il/ data di scadenza	Limite di temperatura	Irritante	Rischio biologico	Numero di catalogo	Conformità europea	Consultare le istruzioni per l'uso/ il foglietto illustrativo

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