

THE READER RESPONSE

READER FEATURE PRODUCT

Q Our laboratory is currently running the AspirinWorks® Test and several of the physicians who are ordering the test have asked for information on how to interpret the test results. Do you have any guidelines that we can supply to our physicians?

A The following algorithm has been suggested for interpreting test results based on the assay cutoff of 1500 pg/mg 11-dehydro thromboxane B₂. The patient's history, lifestyle and other risk factors should be considered when interpreting these results. It is important to take into account an individual's medications and nutritional or dietary supplements when determining if a patient is demonstrating an aspirin effect, as certain agents such as alcohol, green tea extract, chocolate, omega-3 fatty acids, ibuprofen, and COX-2 inhibitors may reduce the amount of thromboxane production in certain individuals.

AspirinWorks® Test Kit

For *In Vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Sample matrix -	Human urine
Sample dilution -	1:5
Microwell coating -	Polyclonal anti-mouse antibody
Antigen -	Purified mouse mAb to 11dhTxB ₂
AP-tracer -	Purified 11dxTxB ₂ -conjugated alkaline phosphatase
Chromogenic substrate -	pNPP
Stopping solution -	EDTA
Assay incubations	
Sample/tracer/antigen -	120 min @ 18 - 26°C
Substrate -	30 min @ 18-26°C
Wavelength -	405 nm
Assay calibration -	Multi-point curve prepared from Reference Solution included in kit
Detection range -	300-4000 pg/mL
Product number -	12136 in North America 12136I International

Happy Holidays!

All of us at Corgenix send our best wishes to you and your families for the holidays and the coming year. We all recognize that our customers are our most valuable asset. We thank you for your business, and look forward to working with you in the New Year.

Our **holiday schedule** begins at the end of the business day on Friday, December 19 and extends until 8:00 am on Monday, January 5, 2009. We will be operating on a limited schedule during this time. Please check your inventory by the 18th to be sure you have an adequate supply of kits during the holidays. All standing orders due by the end of the year or early in January will ship by December 18th.

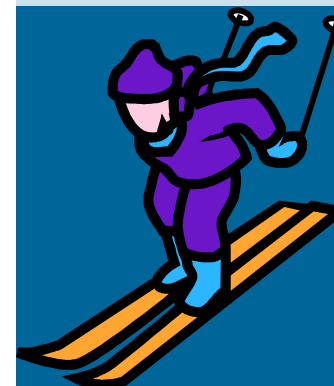
If you require technical assistance or need an emergency shipment during the holidays, please Call 800-729-5661 or 303-457-4345, press option 4, and leave a message with your name and telephone number. A Customer Service Representative will return your call.

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11-dehydro Thromboxane B₂: Drug Assessment Marker or Emerging Risk Factor?

Atherothrombosis of the coronary, cerebrovascular and peripheral arterial circulation is the world's leading cause of morbidity and mortality. One of the key facets of atherothrombosis is the activation and subsequent aggregation of platelets resulting in clot formation. Anti-platelet therapies have been employed in the form of thienopyridines and glycoprotein IIb/IIIa receptor blockers in an attempt to treat this disorder. However, the standard treatment and first line of defense for prevention is the utilization of aspirin (acetylsalicylic acid, or ASA) to inhibit the cyclooxygenase (COX) pathway and subsequent production of thromboxane.

Aspirin's antithrombotic effects have been recognized for many years and it is widely prescribed as an aid in the prevention of cardiovascular disease. In a recent study, low-dose aspirin reduced cardiovascular events by as much as 25% in patients with arterial vascular disease.¹ Similarly, in high-risk vascular patients, aspirin therapy resulted in a 34% reduction in non-fatal myocardial infarction, a 25% decrease in non-fatal stroke, and an 18% decrease in all-cause mortality.²

Aspirin functions by irreversibly acetylating the platelet cyclooxygenase-1 (COX-1) enzyme, thus inactivating it for the life of the platelet. Aspirin also inhibits COX-2, a second cyclooxygenase isoform induced by inflammatory stimuli, but to a much lesser extent than COX-1. Low-dose aspirin blocks more than 95% of platelet COX-1 activity, which results in a decrease in the production of thromboxane A₂ (TxA₂). Direct measurement of a response to aspirin, then, should involve the analysis of circulating levels of TxA₂. Unfortunately, TxA₂ has a very short half-life in the blood making it a difficult analyte to measure.

Thromboxane A₂ is produced from arachidonic acid by many cells and causes irreversible platelet aggregation and vascular and bronchial smooth muscle contraction. TxA₂ is rapidly hydrolyzed non-enzymatically to form TXB₂. Although it is possible to estimate TxA₂ levels by measuring serum or plasma TXB₂, most of the TXB₂ measured is due to *ex vivo* platelet activation during sample testing, or intra-renal production. Measurement errors are compounded by the fact that normal concentrations of circulating TXB₂ are extremely low (1-2 pg/ml), and highly transient (t_{1/2} = 5-7 minutes).

Thromboxane B₂ (TxB₂) in the plasma is converted enzymatically into a number of metabolites including 11-dehydrothromboxane B₂ (11dhTxB₂) and 11-dehydro 2,3-dinor thromboxane B₂ (11dh2,3DTxB₂), which are cleared by the kidney and excreted in the urine. 11dhTxB₂ is the most abundant urinary metabolite of TxB₂, has a relatively long circulating half-life (45 minutes), and is a very stable molecule in urine. Unlike 11dhTxB₂, the circulating half-life of 11-dehydro-2,3 dinor thromboxane (11dh2,3DTxB₂) is short (15 minutes) and plasma concentrations are low. To accurately measure 11dh2,3DTxB₂ levels, it would be necessary to purify and concentrate plasma samples.

Thus, quantitation of urinary 11dhTxB₂ may provide the optimum measure of TxA₂ production by platelets and of *in vivo* platelet activation.

The utility of urinary 11dhTxB₂ as a measure of thromboxane production was confirmed in studies of thromboxane B₂ metabolism.³⁻⁵ In these experiments, urinary levels of 11dhTxB₂ provided a more accurate indication of *in vivo* thromboxane metabolism than TxB₂ measured in the blood, the latter method being confounded by technical difficulties encountered in the blood collection

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December 2008
Volume 18, Number 4

process itself. Thus, quantitation of urinary 11dhTxB₂ may provide the optimum measure of TxA₂ production by platelets and of *in vivo* platelet activation. The AspirinWorks® Test by Corgenix is an FDA-cleared assay for measuring urinary 11dhTxB₂ levels in apparently healthy individuals on preventive aspirin therapy.

It is now well documented in the literature that aspirin does not inhibit platelet function to the same degree in all individuals. This phenomenon, termed aspirin “resistance,” is associated with an increased risk of cardiovascular events, which leads to the need to accurately identify individuals with a sub-optimal response to aspirin. Though the term “resistance” invokes the concept that aspirin is not achieving its pharmacological goal of binding to and inhibiting the COX-1 pathway, this is not normally the case. In fact in the ASPECT study by Paul Gurbel et al.⁶, a randomized crossover study of stable cardiovascular patients to three different doses of aspirin, all of the individuals responded to their aspirin doses when tested by arachadonic acid-induced light transmittance aggregometry, indicating that the aspirin was indeed blocking the COX-1

pathway. However, there was a distinct dose response between the 325 mg dose and the 81mg dose of aspirin when these same individuals were tested for thromboxane production by measuring urinary 11dhTxB₂. Of great significance was the variable individual response to different doses of aspirin (Table 1). These data support an earlier finding by Tran et al.⁷ suggesting that the COX-2 pathway serves as a bypass mechanism that continues to promote the production of thromboxane in the presence of aspirin. Indeed, the COX-2 pathway is up-regulated by inflammation that is encountered in cardiovascular disease.

		Aspirin Dose (30 days)		
Age	Gender	80 mg	162 mg	325 mg
56	M	331	333	334
59	M	1127	533	631
70	F	1124	1326	691
61	M	1307	1301	1455
Values reported in pg 11dhTxB ₂ /mg creatinine				

Table 1. Individual Response to Varying Aspirin Dose (Unpublished data from Gurbel et al. *Circulation* 2007;115:3156-64.)

An important study by Faraday et al.⁸ compared aspirin’s effectiveness in relation to atherosclerotic risk factors, as measured both by urinary 11dhTxB₂ levels and two different platelet aggregation methods. In this study, asymptomatic patients at an increased risk for coronary heart disease were given 81 mg ASA/day for two weeks, urine samples were obtained, and 11dhTxB₂ levels were determined. In parallel, blood samples were drawn and platelets were analyzed by aggregation methods. Patients were also characterized for the presence of other atherosclerotic risk factors in addition to their response to aspirin. The results showed that only high levels of urinary 11dhTxB₂ were associated with cardiovascular risk factors and predicted coronary heart disease risk. Platelet aggregation platforms failed to demonstrate this relationship, suggesting that the measurement of urinary 11dhTxB₂ levels may be an indicator of actual risk of atherosclerotic disease.

The original Heart Outcomes Prevention Study (HOPE Study) by Eikelboom et al.⁹ demonstrated that higher levels of urinary 11-dhTxB₂ reflected an increased risk of a second heart attack, stroke or most significantly cardiac death, in patients treated with aspirin who had previously suffered a cardiac or vascular event. Patients with urinary 11dhTxB₂ levels in the highest quartile had a risk factor for the composite outcome of myocardial infarction, stroke, or cardiovascular death that was 1.8 times higher than those in the lowest quartile. A pre-FDA cleared assay was used in this study to measure 11dhTxB₂ levels. Table 2 contains a summary of the odds ratio for each of the quartiles and 11dhTxB₂ levels as measured with the pre-FDA test, with equivalent 11dhTxB₂ values for the AspirinWorks test.

Quartile	Odds Ratio	11dhTxB ₂ levels (ng/mmol)	Equivalent AW values (pg/mg)
1st	1.0	< 15.1	< 399
2nd	1.3	15.1 - 21.8	399 - 576
3rd	1.4	21.8 - 33.8	577 - 892
4th	1.8	> 33.8	> 892

Table 2. Odds ratio for MI, Stroke, or CV death (Adapted from Eikelboom et al. *Circulation* 2003;105:1650-5)

These findings were further validated by the CHARISMA study investigators.¹⁰ In a prospective outcomes study of over 3000 high risk patients recently published in *Circulation*, urinary 11-dhTxB₂ was identified

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as an independent, modifiable predictor of risk for heart attack, stroke and cardiac death. Significantly higher urinary 11dhTxB₂ levels were seen in patients who developed stroke, MI or cardiovascular death (Cases) than in those who did not experience these outcomes during the study (Controls) (Table 3). Because 11dhTxB₂ is a

metabolite of thromboxane, it is no surprise that ingestion of aspirin will lower the level of this independent risk factor, as the target of aspirin therapy is to suppress thromboxane production. An interesting finding in this study was that statin therapy also lowered levels of the 11dhTxB₂ metabolite. This may be due to the anti-inflammatory effect of certain statins noted in recent studies. The notion that other medications may act in concert with aspirin to effectively and safely control thromboxane production may alleviate the concern of an increased bleeding risk associated with larger aspirin doses. Of greater interest was the fact that clopidogrel, when compared to placebo, did not reduce the hazard ratio for those individuals at greatest risk in the CHARISMA trial, as evidenced by high levels of the 11-dhTxB₂ metabolite. This would suggest that additional anti-platelet therapy is not necessarily the answer, which may lead to treatment of another facet of the disease and/or potentially place more emphasis on preventing the progression of the underlying disease. Additional clinical studies should help provide the answer to the optimum treatment of aspirin “resistant” individuals.

	Urinary 11dhTxB ₂ Concentrations		P-value
	Cases	Controls	
Pre-FDA Median (IQR)	72.7 (41, 135)	57.4 (40,90)	0.001
Log Mean	4.4 (0.96)	4.1 (0.73)	
Geometric Mean	84.1	63.1	
AspirinWorks® Equivalent results	1919 (1082, 3567)	1515 (1056, 2376)	

Table 3. CHARISMA Trial results. Upper Quartile: Adjusted OR 1.66; 95% CI: 1.06 to 2.61 (Modified from Eikelboom et al. *Circulation* 2008;118:1705-12)

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