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Isoprostanes: Biomarkers for Oxidative Stress

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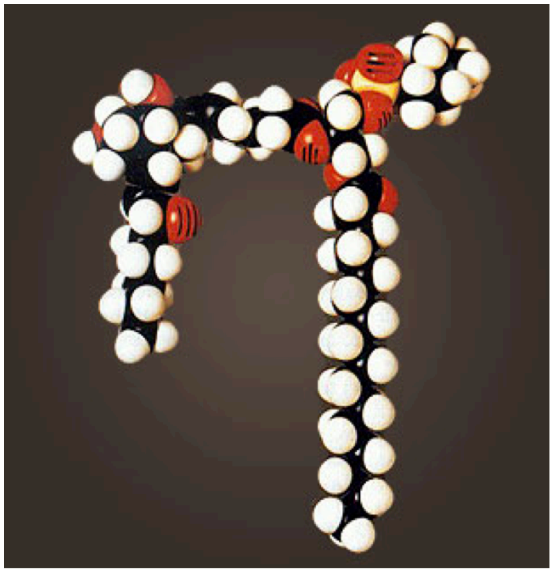
What are isoprostanes? Isoprostanes are a group of prostaglandin-like compounds that are produced by the reaction of free radicals with arachidonic acid, including arachidonic esters in phospholipids. Initially reported by Roberts and Morrow as major products of radical mediated lipid oxidation *in vivo* (1-3), they are now widely recognized as reliable biomarkers of oxidative stress (4,5). Isoprostane formation from arachidonyl moieties in phospholipids significantly disrupts biological membranes (6). Hence Isoprostanes are rapidly cleaved, released, metabolized and excreted.



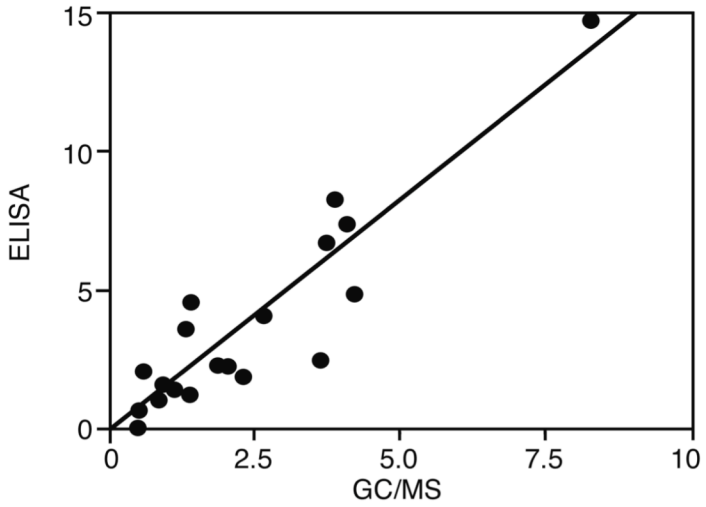
Space-filling model of a phosphatidylcholine molecule containing an F_2–Isoprostane. Image from the Jack Roberts Laboratory at Vanderbilt University.

Isoprostanes as biomarkers for Oxidative Stress: Production of isoprostanes is well-documented to increase in direct proportion to the level of oxidative stress (2-5). Measurement of the concentration of a specific isoprostane (15F2t-IsoP, previously called 8-iso-PGF2a) in blood or urine is now a well-established method for the diagnostic assessment of oxidative stress (4,7). The selection of the 15F2t-IsoP isomer was historically based on the availability of an authentic standard, but extensive studies have documented its utility as a representative isoprostane.

Methods for Analysis of Isoprostanes: The initial, elegant studies of 15F2t-IsoP were conducted by Roberts and Morrow using GC/MS (1,2) which is still employed by a number of academic laboratories. Additional methods for measuring isoprostane levels have subsequently been developed, including LC/MS (8,9), RIA (10,11) and ELISA (12-14). Each has unique benefits and challenges.

Instrumental methods: It is generally agreed that the most accurate method is LC/MS/MS with the inclusion of a deuterated control to monitor loss during sample preparation (8,9). LC/MS/MS offers excellent separation of individual isoprostanes isomers. The 15F2t-IsoP peak measured in the original GC/MS method is now known to be comprised of four isoprostanes isomers (14). However, since all of these are primarily derived from the same radical-mediated pathway, this still provides a reliable method for assessment of oxidative stress. The drawback of these methods is the high cost of the equipment as well as extensive sample extraction and low throughput. Further, measuring a single isoprostane isomer may not be the best indicator of oxidative stress as many are rapidly metabolized and the rates of metabolism can vary significantly (see below).

Immunoassays for isoprostanes: Immunoassays, including RIA and ELISA are widely used alternatives for isoprostanes analysis. Antibody-based methods have been criticized based on the failure of some ELISA products to provide a good correlation with GC/MS (12,17,18), and the fact that ELISA values for isoprostanes are typically greater than those obtained by LC/MS or GC/MS, which has been shown to largely be due to polyclonal antibody recognition of related compounds. In the case of Oxford's ELISA, the related compounds are also derived from the isoprostane pathway (19,20), and the results obtained correlate well with GC/MS or LC/MS (18,19).



Correlation between results obtained using Oxford's isoprostane ELISA kit and results obtained using the standard GC/MS method, with the latter including two solid phase extractions and TLC.

Isoprostane Metabolism and the Assessment of Oxidative Stress: Although there is a strong scientific tendency to focus on individual metabolites of metabolic pathways, since radical-mediated isoprostane production gives rise to multiple isomers — with very rapidly, and probably different rates of enzymatic metabolism and then excretion — methods that measure the sum of multiple isomers should provide a more comprehensive assessment of oxidative stress compared to isolating and quantifying a single isomer (19, 21).

A critical factor for optimal assessment of oxidative stress by the measurement of isoprostanes(s) is the consideration of inter-individual variations in the rapid metabolism of these compounds that are generated in radical-mediated reactions but metabolized by multiple enzymatic pathways (19,21). We've found that an average of 50% of all isoprostane in urine is excreted as a glucuronide (18). Further complicating this picture as that the percentage varies from 20 to 80% among normal human subjects. Recent publications have had similar findings (19, 22). This is assumed to be due to variations in UGT expression. This calls into question the value of prior urinary isoprostane measurements, including those performed using GC/MS and LC/MS, in which the sample preparation protocols removed the glucuronides (23). Figure 3 shows the parallel increase in the isoprostanes levels in the same pooled urine sample upon glucuronidase treatment when analyzed by GC/MS, Oxford's extraction-free ELISA and RIA. **Oxford Biomedical Research is the only ELISA manufacturer that addresses this issue and provides glucuronidase so that a true isoprostane measurement can be obtained**.

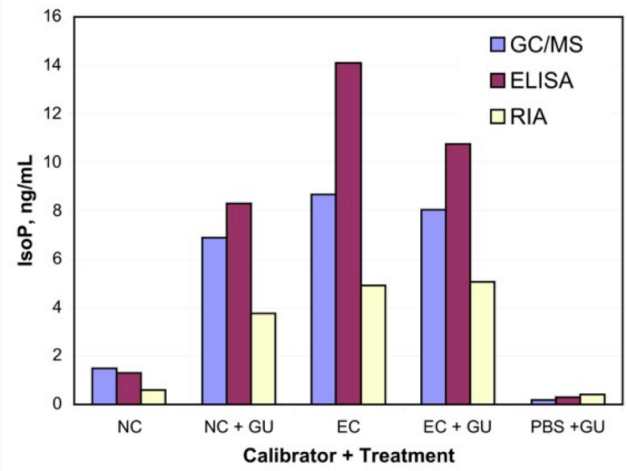


Figure 3. Analysis of normal human pooled urine (NC) and a sample spiked with a known concentration of 15F2t-IsoP (EC) by various methods.

Advantages of Oxford's Urinary Isoprostane ELISA

1. Immunoassay methods also lend themselves to high-throughput, and the assay sensitivity is well below the range of normal mammalian isoprostanes concentrations. Sample preparation methods (Sep-Pak or Immunoaffinity) can add time and expense to immunoassay methods. Oxford Biomedical Research developed a specially formulated buffer that allows users to skip solid phase extraction and simply dilute and run. This method has been validated and correlates to GC/MS measurements. Simple samples, such as urine, can be analyzed using Oxford's proprietary dilution buffer **without extraction** - vastly increasing the number of samples that can run per day.
2. **Cost:** The cost of GC/MS or LC/MS equipment is prohibitive for many labs and the sample prep time results in a cost per sample exceeding $75.00. Immunoaffinity columns drive up the cost of sample prep and introduce unacceptable error and cost approximately $43.00 per sample. Our urinary isoprostane assay does not require extraction or immunoaffinity making it the fastest and most economical method

available. With a per sample cost of just $7.50 this assay provides glucuronidase, is extraction free, and has the best correlation. The elimination of sample extraction or immunoaffinity steps drastically lowers the **cost** per assay as illustrated in the following table:

Total cost (based on analysis of 40 urine samples in duplicate on one plate for EIA)

Cost	GC/MS or LC/MS	Oxford Biomedical	Cayman
Instrumentation	≥ $100,000	$3,000 – 5,000 standard reader	$3,000 – 5,000 standard reader
Assay kit		$300	$270
Sample Preparation	$10 – 25	Not required	$37 (Immunoaffinity column)
Glucuronidase	Not provided	Included	Not provided
Solvents	Not included	DI Lab water	ACE compatible = $0.35
Technician time	hours	< 1 hr	hours
Total cost / sample†	>$75	$7.50	≥ $43.60

† Not including up front instrument costs and technician costs

3. **Accuracy:** More important than the cost is the accuracy and utility of the data. While ELISA in general detects more isoprostanes than GC/MS or LC/MS, competitor ELISA assays have been criticized for lack of a strong correlation to MS (15). While the immunoaffinity columns allows one to avoid solid phase extraction, it introduces as much as 20% variation. Coupled with poor correlation and missing glucuronidated isoprostane, this method can't give you a true measurement of isoprostanes (24). **Oxford Biomedical Research pioneered the development and validation of immunoassay kits for the quantitation of isoprostane and is the only ELISA that correlates to GC/MS**.

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