

# ANTIOXIDANT ASSAY

## Plasma Antioxidant Capacity PAC

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An imbalance of oxidants and antioxidants within the human body, in which either oxidants are high or antioxidant protection is low, will lead to a state of "Oxidative Stress". This state of Oxidative Stress is associated with a variety of chronic degenerative diseases.

Measurement of the antioxidant status of biological fluids could be used as an early warning sign of possible disease onset if a simple reliable method were available for such a measurement. However, while several methods have been used to estimate this value, questionable sensitivity, unreliability, non-reproducible results and procedural difficulties hamper all. In addition, even for the best test, 8-isoprostanes, there is no significant elevation in the measured value until pathological disease is already evident. In other words, while it could be used to confirm that there is Oxidative Stress evident when a subject already has a clinically diagnosed disease; it is little value as a predictive tool before clinical symptoms are present.

In this new method the amount of isoprostanes in blood plasma is greatly amplified *ex-vivo*. As such, it is a much more sensitive measure of the amount of antioxidant protection present in blood. The method can therefore identify potential imbalances in antioxidant protection even before clinical symptoms exist.

The method consists of the following steps:

1. isolation of a sample of blood
2. centrifugation of the blood sample to obtain plasma
3. introduction of a source of free radicals which induces oxidation of the lipoproteins in the plasma
4. measurement of the markers of lipoprotein oxidation

A second variation in this method is to remove uric acid from the plasma sample prior to introduction of the free radical source. This is done enzymatically with the addition of uricase. Uric acid is a major water soluble antioxidant present in blood. Once uric acid is removed the antioxidant protection of other antioxidants

present in the blood (such as vitamins, phytochemicals, carotenoids etc.) can be measured. Taken together the method can measure the total antioxidant capacity of blood as well as the non-urate antioxidant capacity.

The method is extremely sensitive to the addition of antioxidants, vitamins, and phytochemicals present in blood and plasma as a result of oral ingestion of supplements. For example, in one experiment samples of blood were collected from volunteers after an overnight fast. After the fasting blood sample was taken, the subjects ingested 1200 mg of Vitamin C (from 2 tablets of USANA Poly C). Four hours later a second blood sample was taken. Isolated plasma from each sample was then mixed with a SIN-1 solution (a free radical source), incubated for 4 hours at 37 °C, and 8-isoprostanes were determined by standard protocols with an ELISA kit.

A second sample of plasma from above (both time 0 and 4 hours after ingesting vitamin C) was treated with uricase prior to treatment with SIN-1 solution as described above.

For comparison a FRAP (Ferric Reducing Antioxidant Power) assay, one of the most common assays of antioxidant capacity, was also run on the same plasma samples.

In a similar manor, on separate days the same subjects were given 1000 IU vitamin E, 600 mg green tea extract, 530 mg grape seed extract, and 600 mg olive fruit extract (Olivol™).

Summary data from these experiments is shown below:

|                | PAC assay (isoprostanes found in pg/mL) |             |           |             | FRAP assay (µM) |            |           |            |
|----------------|---|-------------|-----------|-------------|-----------------|------------|-----------|------------|
|                | Standard                                |             | Non-urate |             | Standard        |            | Non-urate |            |
|                | Baseline                                | 4 hr        | Baseline  | 4 hr        | Baseline        | 4 hr       | Baseline  | 4 hr       |
| Vitamin C      | 74                                      | 54          | 336       | 190         | 1011            | 1041       | 419       | 488        |
| % change       |   | <b>-27%</b> |           | <b>-44%</b> |                 | <b>3%</b>  |           | <b>16%</b> |
| Vitamin E      | 74                                      | 66          | 301       | 263         | 1064            | 1033       | 453       | 435        |
| % change       |   | <b>-11%</b> |           | <b>-13%</b> |                 | <b>-3%</b> |           | <b>-4%</b> |
| Grape Seed Ext | 72                                      | 68          | 313       | 277         | 999             | 973        | 442       | 446        |
| % change       |   | <b>-6%</b>  |           | <b>-12%</b> |                 | <b>-3%</b> |           | <b>1%</b>  |
| Green Tea Ext  | 74                                      | 63          | 302       | 277         | 1055            | 1058       | 422       | 447        |
| % change       |   | <b>-14%</b> |           | <b>-8%</b>  |                 | <b>0%</b>  |           | <b>6%</b>  |
| Olivol™        | 78                                      | 74          | 294       | 267         | 1055            | 1039       | 462       | 462        |
| % change       |   | <b>-5%</b>  |           | <b>-9%</b>  |                 | <b>-1%</b> |           | <b>0%</b>  |

In every case, there was a large measurable difference in the amount of isoprostanes formed in the initial fasting sample vs. the sample collected four hours after taking the antioxidant supplement. In each of these five cases, there were more isoprostanes formed in the fasting sample than in the sample taken after ingesting the antioxidants, showing that the ingested antioxidants in fact can

prevent oxidation from occurring. With the standard FRAP assay an increase in antioxidants should result in an increase FRAP value. However, only one of the five experiments (vitamin C) produced a result consistent with added antioxidants. Thus the new method is both more sensitive and predictable than existing methods.

In the above examples SIN-1 (a source of free radicals) is used as the initiator and isoprostanes were measured as a marker of lipid peroxidation. Other radical initiators and other markers of oxidation may also be used in this application.