

Oxidative Stress Assessment at Kronos Science Laboratory

by

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In recent years, research evidence has been accumulating that damage to cell components (proteins, cell membrane lipids, and the DNA of both chromosomes and mitochondria) by oxygen free radicals (also known as "reactive oxygen species" or ROS) is an important mechanism of aging. Oxidative damage seems also to play a role in various diseases, such as arthritis, diabetes, and arteriosclerosis. ROS are generated during the process of energy production in the cell's miniature "furnaces," the mitochondria. While most reactive oxygen produced by the mitochondria is used to oxidize fuel and generate high-energy phosphate compounds, which can be used by the cell for energy requiring processes, some ROS "escape" and can react chemically with various cell components. ROS include ozone, superoxide, peroxide, and the hydroxyl radical.

Cells have a number of mechanisms for protecting themselves against oxidative damage. For example, certain enzymes, such as superoxide dismutase (SOD), catalase, and thioredoxin reduce ROS and other radicals to harmless or less reactive forms. There are also oxygen scavenger compounds, such as glutathione and the ORAC antioxidants listed below, which "sacrifice themselves" by being oxidized in place of more critical molecules. Finally, various repair and disposal systems clean up the damage caused by any ROS that get past these defenses. Oxidative stress is the term used to describe the rate at which oxidative damage is occurring in living organisms and the organisms' ability to protect against such damage.

The Oxygen Radical Absorption (ORAC) assay is a method developed to measure the total antioxidant capacity of a sample. This assay has been used by scientists and physicians to assess Oxidative Stress because protection of the whole body is believed to be the result of all the different antioxidants that exist in a serum sample. The concentration of any one of these antioxidants may be small, but collectively they represent an important antioxidant component of the serum. In addition all the various antioxidants in the serum are known to interact with each other so that the net antioxidant protection may be substantially greater than the sum of the individual components.

The theoretical advantage of the ORAC assay is that it measures simultaneously the total antioxidant capacity of the serum sample including all the various synergistic effects that may exist in vivo. Thus, the ORAC value of a serum sample is thought to represent a global measure of antioxidant protection.

The major antioxidants in the serum and their approximate percent contribution to total serum antioxidant activity (ORAC value) are as follows:

Albumin:	(34%)
Uric acid:	(27%)
Vitamin C:	(6%)
Vitamin E:	(4%)
Bilirubin:	(3%)
Carotenoids:	(2%)
Total lipids:	(15%)
Polyphenols and Bioflavonoids:	(9%)

The disadvantage of the ORAC assay is that it is difficult to determine what antioxidant components in the serum sample are responsible for any changes observed in an individual's ORAC value. Since total antioxidant capacity is being measured, the assay is not very sensitive to changes in single antioxidants in the serum. Furthermore, the ORAC assay provides no measure of the actual oxidative damage that occurs to vital biomolecules (DNA, proteins, and lipids), despite the presence of these protective antioxidants in the serum. Finally, there is no compelling evidence that serum ORAC reflects anything meaningful about the overall oxidative stress status of other bodily fluids and tissues. Indeed, the ORAC may be completely misleading as to the total amount of oxidative damage a patient is suffering. Therefore, the ORAC assay is no longer offered at Kronos Science Laboratory (KSL).

At KSL, we attempt to assess oxidative stress status by measuring various endogenous and exogenous "protection factors" (antioxidants) in serum as listed in Table 1, plus indirect and direct "damage factors." Accurate measurement of oxidative stress needs a novel methodology. KSL has developed a multi-parameter biomarker set for oxidative stress assessment. We utilize the state of the art technology-LC/MS/MS method to measure DNA/RNA, lipid, and protein oxidation products. The methods have been recognized as the gold standard methodology in this field. Direct damage factors include oxidized lipid molecules (isoprostanes: 8-iso-prostaglandin F_{2a} (iPF₂ α -III); 2,3-dinor-iPF₂ α -III; iPF₂ α -VI; and 8,12-iso-iPF₂ α -VI); two important DNA damage adducts (8-hydroxy-2'-deoxyguanosine and 5-hydroxymethyl-2'-deoxyuridine); an RNA damage product (8-oxo-guanosine); and several protein oxidation products (di-tyrosine, nitro-tyrosine, and Cl-tyrosine.) measured using LC-MS/MS (in serum and urine). Taken together, these assays give a reasonably complete assessment of an individual's oxidative stress status. In fact, the every important component of oxidative damage that we know about is measured directly. Repeated measurement of these oxidative damage products over time should provide a good estimate of and individual's overall level of oxidative stress.

Until recently, none of the assays normally used to assess oxidative stress had been shown to discriminate patients having pathological conditions associated with increased oxidative stress from normal, healthy persons. Without such clinical validation, it is impossible to reliably evaluate interventions designed to decrease the rates of oxidative damage because interpretation of the assay results was uncertain. As described above, KSL has developed and analytically validated a number of assays designed to assess oxidative stress. In collaboration with the Kronos Longevity Research Institute and others scientists around the world, KSL is now in the process of validating these assays in various high-risk human populations (such as smokers, Alzheimer's, cancer, cardiovascular disease, diabetes, and etc) to confirm their clinical utility in assessing oxidative stress.

Once clinically validated, these assays may then be used to evaluate promising interventions. The first assays to have been validated in this manner are the DNA damage and isoprostane assays. Therefore, these assays, together with our new di-tyrosine assay have been substituted for the ORAC assay as part of our standard panel for the assessment of oxidative stress. We believe that this strategy for measuring oxidative damage products in the urine, along with measuring many specific antioxidants in the serum, provides the most complete and scientifically sound assessment of oxidative stress status available for use in a clinical environment.

Table 1 Protection Factors (Antioxidant)

Albumin
Uric acid
Glutathione (GSH/GSSG)
Glutathione peroxidase
Vitamin C
Vitamin E: Tocopherols (α , β , δ)
Vitamin A:
Retinol
Retinyl palmitate
Carotenoids :
Lutein
Cryptoxanthin
Lycopene
Zeaxanthin
Carotenes (α , β)
CoQ-10
Bilirubin
Ceruloplasmin
Antioxidant Trace metals