BRUNSWICK LABORATORIES
Clinical / Preclinical Testing & Research for Phytonutrients

+1-508-281-6660
blservices@brunswicklabs.com
200 Turnpike Road, Southborough, MA 01772, USA

Service Inquiry



## Brunswick Labs Beat

# From ORAC to ORAC 6.0, ORAC Has Come a Long Way: Historical Overview and Current Impact

*Posted by Jasenka Piljac Zegarac, PhD on Tue, Oct 25, 2016 @ 11:00 AM*

Find me on:




**In the past few decades, ROS-induced oxidative stress has been recognized as a contributing factor in the development of a range of chronic conditions and diseases.[1] Consequently, antioxidants, both endogenous and exogenous (ingested), capable of neutralizing or quenching oxygen radicals, quickly came into focus of the research community. In the wake of the enthusiasm created by naturally-derived antioxidants, a need emerged for a diagnostic technique capable of quantitatively measuring the scavenging activity against oxygen radicals. Hence, the original Oxygen Radical Absorbing Capacity (ORAC) assay was born. Let's take a brief look at the history behind the ORAC assay.**

In the early 1990s, a range of *in vitro* antioxidant capacity and radical scavenging assays were already available, each one with its own advantages and limitations. Some of the most commonly used antioxidant capacity tests included the Folin-Ciocalteu (FC) total phenol assay, the Trolox Equivalent Antioxidant Capacity (TEAC) assay and the Ferric Reducing Antioxidant Power (FRAP) assay. The mentioned assays are based on the following general electron-transfer mechanism:[2]

Probe (oxidant) + electron (antioxidant-derived) ® reduced probe + oxidized antioxidant where the antioxidant donates an electron to the probe, the oxidant, causing a color change which can be monitored colorimetrically. The FC assay was originally developed for analysis of proteins, but was later adapted by Singleton and co-workers to measure a sample's overall reducing capacity at basic pH.[3] The FC reagent does not only react with phenolic compounds, rather, it may be reduced by other nonphenolic compounds present in a sample, such as vitamin C or Cu(I). Due to its convenience, affordability, simplicity and reproducibility, the FC assay quickly gained popularity.

The TEAC assay was first reported by Miller and Rice-Evans in 1993, and later improved by Re and coworkers.[4] It measures the ability of antioxidants to quench the ABTS×$^{-}$ radical (colored) to ABTS$^{2-}$ (colorless) via electron donation at neutral pH. Although this assay is also simple and convenient, it does not reflect the differences in reaction rates between antioxidants and oxidants, or the differences in their chemical structures.[2] For example, one-electron antioxidants like glutathione (1.28 TEAC) and two-electron antioxidants like a-tocopherol (0.97 TEAC) often produce similar TEAC values.[2]

The FRAP assay was first introduced by Benzie and Strain in mid-1990s to measure the reducing ability of plasma.[5] It is based on the reaction between a ferric salt, used as an oxidant, and antioxidants under acidic conditions. One of the problems with the FRAP assay is that it does not accurately measure the reducing capacity of antioxidants with slow kinetics, since the reaction time is limited to only 4 minutes.[2]

The ORAC assay was originally developed by Cutler and Cao[6] in an attempt to quantify, *in vitro*, the interaction between antioxidants and the peroxyl radical - the most abundant free radical in the human body. The assay measures a fluorescent signal given off by a probe that decreases in the presence of an ROS source. The probe competes with an antioxidant for peroxyl radicals generated from thermal decomposition of AAPH (2,2'-azobis-(2-amidinopropane dihydrochloride)). Antioxidants inhibit the free radical damage to the fluorescent probe, which manifests as preservation of the fluorescent signal. The degree of protection offered by antioxidants is quantitatively compared with that obtained for Trolox to derive a Trolox Equivalency value, aka, the ORAC value.

Since its introduction in 1993, the ORAC assay has undergone several major improvements. While the first ORAC assay only quantifies antioxidant capacity of a test sample against the peroxyl radical, the improved version of the assay introduced by Brunswick Labs, ORAC 5.0, quantifies a sample's scavenging potential against five primary oxygen species - peroxyl radical, hydroxyl radical, peroxynitrite, singlet oxygen and superoxide anion - allowing for a more comprehensive evaluation of a sample's oxidant-scavenging capacity. Because antioxidant enzymes do not scavenge ROO·, HO·, $O_2$, and ONOO-, assays that measure enzymatic activity cannot quantify radical scavenging of all oxygen radicals. The ORAC 5.0 assay addresses this shortcoming of enzymatic assays.

In 2014, Brunswick Labs added hypochlorite to the ORAC radical panel, replacing ORAC 5.0 with ORAC 6.0 assay. "The motivation behind the expansion to a six-radical test", explains Dr. Jin Ji, CTO and Executive Vice President of Brunswick Labs, "was to provide a full analysis of antioxidant capacity based on all physiologically relevant radicals." Hypochlorite is a powerful oxidant produced during activation of monocytes and neutrophils. By targeting plasma proteins, it can cause alteration of amino acid side chains, cleavage of backbones, and cross-linking of protein molecules.[7] Dr. Ji further noted that hypochlorite-modified proteins have been found to be related to atherosclerosis and cancer, therefore, assessing antioxidant capacity against hypochlorite is important in any total antioxidant capacity assessment.

Since its inception, the ORAC assay has gained wide popularity in the food and dietary supplement industry for promotion of a product's added value. Unfortunately, oversimplified interpretation of assay results, as well as misrepresentation of the ORAC values by some product marketers led to a messy ORAC label competition in the market. Consequently, in 2012, the USDA Nutrient Data Laboratory removed the USDA ORAC Database for Selected Foods from their website. In a statement issued in

response to USDA's action, Dr. Ronald Prior, former USDA scientist who pioneered antioxidant research, emphasized that the ORAC assay is an *in vitro* method ''subject to the disadvantages that are inherent in any *in vitro* method.'' Dr. Prior also noted that, while ORAC can be extremely useful for comparison of the relative total quantities of antioxidants in a sample, this does not necessarily imply that analyzed compounds have antioxidant effects *in vivo*. ORAC assays serve as an *in vitro* screening tool, whereas *in vivo* investigations give the final confirmation on whether analyzed samples have *in vivo* effects.

In a recent review article, Dr. Prior *et al* established a relationship between dietary antioxidants, oxidative stress and health, and, for the first time, offered a practical context for ORAC assay results.[8] The article presented an overview of several clinical and epidemiology studies which demonstrated that consumption of antioxidant-rich fruits and vegetables results in an increase in plasma antioxidant capacity. In addition, it highlighted several other studies associating increased dietary intake of total antioxidant capacity with reduced risk for gastric cancer, endometrial cancer, ischemic stroke, hypertension, and pulmonary function. Based on earlier study results, the authors estimated the average dietary ORAC intake for a person consuming 5 servings of fruits and vegetables per day to be 4360 ORAC units, and 8600 ORAC units for those subjects consuming 10 servings of fruits and vegetables. The authors concluded the article by noting that ORAC intakes of at least 12,000 units or more are needed for reduced risk for studied diseases, which translates to consumption of about 7 to 10 servings of fruits and vegetables high in ORAC.

In light of this extensive literature overview, the potential utility of the ORAC assay to the research community remains current and relevant. In conjunction with bioavailability data and oxidative stress biomarker assays, the ORAC assay is an invaluable tool in early assessment of potential biological activity of a sample that provides guidance for natural product development.[2]

**References**


1. Dalle-Donne I, Rossi R, Giustarini D, et al. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta*. 2003;329:23-38.
2. Huang D, Ou B, Prior RL. The Chemistry behind Antioxidant Capacity Assays. *J Agric Food Chem.* 2005;53:1841-1856.
3. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999;299:152-178.
4. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med*. 1999;26:1231-1237.
5. Benzie IFF, Strain JJ. The ferric reducing ability of plasma as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239:70-76.
6. Cutler GH, Alessio HM, Cutler RG. Oxygen-radical absorbency capacity assay for antioxidants. *Free Radical Biol Med*. 1993;14(3):303-311.
7. Hawkins CL, Davies MJ. Hypochlorite-induced oxidation of proteins in plasma: formation of chloramines and nitrogen-centred radicals and their role in protein fragmentation. *Biochem J*. 1999;340:539-548.
8. Prior RL, Wu X. Diet Antioxidant Capacity: Relationships to Oxidative Stress and Health. *Am J Biomed Sci.* 2013;5(2):126-139.


*Author Jasenka Piljac Zegarac is a scientist and freelance writer. She holds a PhD in biology and a BS degree in biochemistry, and contributes on a regular basis to several health and science blogs. Her research articles have gathered more than 1200 citations. She may be contacted for assistance with a variety of science and medical writing projects.*

**Tags: ORAC, total antioxidant analysis, TEAC, DPPH, FRAP, antioxidant test, antioxidant assay, total phenols, folin ciocalteau, antioxidant analysis, orac 6.0, oxidative stress analysis, total phenolics**

---

Email*

Website

Comment*

Subscribe to follow-up comments for this post


Type the text

Privacy & Terms

Submit Comment