Total Antioxidant Performance (TAP) assay

Plasma total antioxidant performance (TAP) was determined fluorometrically with a 1420-multilabel counter (Wallac Victor 2; Perkin-Elmer Life Sciences, Boston) as described by Aldini et al (1) with minor modifications. This method measures the rate of oxidation of 4,4-difluoro-5-(4-phenyl-1,2-butadienyl)-4-bora-3a,4a-diaza-s-indacence-3-undecanoic acid (BODIPY 581/191), a lipid-soluble fluorescent probe, and uses the lipid-soluble radical initiator 2,2'-azobis(4-methoxy-2,4-dimethylvalernitrile) (MeO-AMVN). Oxidation was determined by monitoring the appearance of green fluorescence of the oxidation product of BODIPY 581/591 (λ_{ex} = 500 nm, λ_{em} = 520 nm, slit= 10 nm). The results are expressed as TAP value (% protection), based on area under the curve (AUC) of the oxidation kinetic curve of each sample.

Conventional assays to determine antioxidant capacity, such as total radical trapping antioxidant parameter (TRAP) (2) and the oxygen radical absorbance capacity (ORAC)(3), primarily measure the antioxidant capacity in the aqueous compartment of plasma. Consequently, water-soluble antioxidants such as ascorbic acid, uric acid, and protein mainly influence these assays, whereas fat-soluble antioxidants such as tocopherols and carotenoids play only a minor role. However, using the TAP assay to induce radicals in the lipid compartment and to monitor lipid peroxidation makes it possible to avoid the sinking effect of protein and to elucidate antioxidant actions of fat-soluble antioxidants as well as water-soluble antioxidants that actively interact with the lipophilic antioxidants. Therefore, the TAP assay reflects the 'true' total antioxidant network between water- and fat-soluble antioxidants in plasma.

1. Aldini G, Yeum KJ, Russell RM, Krinsky NI. A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. Free Radic Biol Med 2001;31:1043-1050.
2. Valkonen M, Kuusi T. Spectrophotometric assay for total peroxyl radical-trapping antioxidant potential in human serum. J Lipid Res 1997;38:823-33.
3. Cao G, Prior RL. Measurement of oxygen radical absorbance capacity in biological samples. Methods Enzymol 1999;299:50-62.