

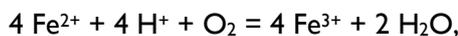
## ANTIOXIDANT PROFILE

January 2012

### Introduction

In this profile circulating levels of caeruloplasmin are assessed, along with the activity of four antioxidant enzymes. These measurements can be used as a guide to the preventative antioxidant activity of the blood, as well as the possible response to oxidative stress and to micronutrient deficiencies. In addition, vitamin E (alpha-tocopherol), alpha-carotene and beta-carotene are measured.

Most of the copper in plasma is present as the ferro-oxidase enzyme caeruloplasmin, which is a positive acute phase reactant and hence rises in concentration in the plasma during inflammation [1,2]. This process reflects the movement of copper from inside the cells into the extra-cellular fluid. The ferro-oxidase action of caeruloplasmin circulating in the serum is to catalyse the reaction:



thereby keeping available iron in the Fe(III) state, in which it can be incorporated into transferrin. This reaction also blocks the damaging effects of ferrous iron - Fe(II) - which can take part in Fenton reactions and cause propagation of reactive oxygen and reactive nitrogen species.

Every aerobic cell contains superoxide dismutase (SOD), which is required for the detoxification of the oxygen metabolite superoxide ( $\text{O}_2^{\cdot-}$ ). Superoxide "leaks" from the mitochondrial electron transport chain, primarily at complex I (NADH-coenzyme Q) and to a lesser extent at complex II (succinate-coenzyme Q) and complex III (coenzyme  $\text{QH}_2$ -cytochrome C reductase). The superoxide radical anion  $\text{O}_2^{\cdot-}$  plays a central role in the development of oxidative stress since other reactive oxygen species appear to be derived from it. Copper (with zinc and manganese) is an essential component of SOD [3,4,5].

The seleno-enzyme glutathione peroxidase (GSHPx) catalyses the reduction of hydrogen peroxide to water, with the simultaneous conversion of reduced glutathione to oxidised glutathione. The origin of this  $\text{H}_2\text{O}_2$  is primarily the lipid hydroperoxides released from membrane phospholipids by the action of phospholipase A2 during an inflammatory reaction. GSHPx thus de-toxifies this  $\text{H}_2\text{O}_2$  by reducing it to water. Any deficiency in this de-toxification cycle puts the cell at risk from the potentially mutagenic effects of lipid hydroperoxides. In the antioxidant profile both red cell glutathione peroxidase (GSHPx-1) and plasma glutathione peroxidase (GSHPx-3) are measured, giving an estimate of both intracellular and extracellular activities of the enzyme [6,7,8,9].

Paraoxonase (PON-1) is a calcium-dependant esterase that circulates in plasma bound to high-density lipoprotein (HDL). PON-1 was originally identified by its activity in the metabolism of organophosphates such as paraoxon and thus serves to neutralise anti-cholinesterase insecticides in the body. But studies have confirmed that PON-1 is also an oxidant-sensitive enzyme which inhibits the atherogenic oxidation of LDL. Low PON-1 activity has been associated with a number of risk factors for coronary heart disease, including diabetes, hypercholesterolaemia and smoking [10,11,12].

Vitamin E is the generic term that refers to all substances having the biological activity of d-alpha-tocopherol. Alpha-tocopherol itself has the highest scavenging activity against lipid peroxy radicals of all the nutritional antioxidants. The maintenance of its serum concentrations depends in part on the action of alpha-tocopherol transfer protein, a secretion of the hepatocytes, to preferentially take up alpha-tocopherol from the portal blood, while other forms of vitamin E are more rapidly metabolized and excreted. [13,14]

Alpha and beta-carotene are among the best absorbed dietary carotenoids and hence present in the human plasma at relatively high concentrations. Both alpha- and beta-carotene have a pro-vitamin A function in the human [15,16].

### **Indications**

Suspected oxidative stress states, including the effects of ageing, alcoholism, atherosclerosis, cancer, cataract, cystic fibrosis, diabetes, hepatitis, HIV infection, iron overload, pancreatitis, pre-eclampsia, pulmonary disease, rheumatoid arthritis, tooth and gum disease. Also suspected nutritional deficiencies, especially of copper and selenium.

### **Patient preparation:**

Patient should refrain from taking nutritional supplements for 24 hours before the collection of the blood samples.

### **Specimen requirements**

Two serum separator tubes (red top) and one lithium heparin tube (green top), filled with blood.

### **Interpretation of results**

The Biolab reference interval for caeruloplasmin is 18 – 34 mg/dL. Low levels of caeruloplasmin can result from nutritional copper deficiency and from Wilson's disease (which is characterised by copper toxicity, high levels of copper in the liver and urine, with very low levels of caeruloplasmin in the serum). High levels of caeruloplasmin are found in inflammation and can rise three-fold as part of the acute phase response to bacterial infection. Intake of oral contraceptives or post-menopausal oestrogen replacement therapy can result in a c.25% rise in serum caeruloplasmin.

The Biolab reference interval for red cell superoxide dismutase is 1102 - 1601 international units of activity per gram of haemoglobin. Low levels of red cell SOD suggest micronutrient deficiencies, either due to poor diet or as a later consequence of oxidative stress. In practice, the most common cause of poor SOD activity appears to be intracellular copper deficiency. Although it is primarily an intracellular enzyme, SOD has a general anti-inflammatory effect, protecting against excess superoxide radicals formed during leukocyte phagocytosis (killing of bacteria by white cells). This is probably why SOD deficiency can be associated with skin lesions in affected subjects. High levels of red cell SOD suggest an early response to oxidative stress, in which increased cellular demand induces synthesis of the enzyme.

The Biolab reference interval for red cell glutathione peroxidase (GSHPx-1) is 25 - 50 IU/gm Hb; for plasma glutathione peroxidase (GSHPx-3) it is 115 - 400 IU/L. Current concerns about possible selenium deficiency in the UK, together with concerns about modern agricultural methods, suggest that low levels of this enzyme may be found in a wide variety of subjects. Low activity of GSHPx may also be associated with an increase in the incidence of various cancers (especially prostate cancer in the male), and this therefore could be one mechanism for the association between antioxidant depletion and increased cancer risk. GSHPx measurement should also be considered in patients who are under oxidative stress from any cause; induction of the activity of this enzyme is one of the responses to a disturbance of the pro-oxidant/anti-oxidant balance.

The reference interval for serum paraoxonase (PON-1) is 150 – 1000 U/L. Low levels of paraoxonase can result from a poor diet or from smoking. Paraoxonase is synthesised in the liver and hence serum levels decrease in chronic liver disease. A high nutritional antioxidant intake will raise serum paraoxonase levels, as will exposure to organophosphates such as paraoxon, diazoxon, serin and soman. Since serum paraoxonase is associated with HDL activity, its measurement gives useful information about a subject's cardiovascular risk, as well as the ability to metabolise organophosphates and other xenobiotics.

The Biolab reference interval for plasma alpha-tocopherol 25 - 60 µmol/l. Low levels suggest dietary deficiency of vitamin E but may also reflect excessive utilisation of vitamin E in oxidative stress states. The Biolab reference interval for alpha- carotene is 0.30 – 1.50 µmol/L and for beta-carotene is 0.40 – 3.00 µmol/L. The measured serum levels reflect dietary consumption of these carotenoids.

**Turnaround time:** 5 working days.

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