

Oxidative Stress Analysis *Blood/Urine*

Overview For the past several decades, physicians and researchers alike have studied oxidative stress in the body. More recently, attention has been focused on the many disorders tied into free radical chemistry (such as diabetes) and its strong link to the aging process. While the biochemical significance of oxidative stress has been understood for some time, it has been more difficult to assess its impact upon individuals and then measure effectiveness of therapeutic intervention.

Genova Diagnostics has developed a sensitive assessment utilizing acetaminophen and salicylate challenges to evaluate oxidative stress status, antioxidant reserve and the interrelationship with hepatic detoxification. This test allows clinicians to develop individual therapies for patients and monitor treatment progress.

Oxidants and Antioxidants Dr. Jeffrey Bland has recently published a comprehensive review of oxidants and antioxidants in clinical medicine, portions of which have been excerpted in the following discussion. Bland's review discusses oxidative stress in much greater detail than can be done herein, and is strongly recommended for additional reading.¹

In a seminal paper published in 1957, Harman noted that free radicals increase with increasing metabolic activity and are related to alterations in biological oxidation/reduction reactions.² He suggested that aging and the degenerative diseases associated with it may be attributed to the deleterious side-effects of free radicals on cellular constituents and that antioxidants may play a very important role in helping to protect against free radical oxidative damage.

During the early 1970s, Irwin Fridovich posited that one of the most important oxidants in the cellular systems could be superoxide, which is created as a consequence of the univalent reduction of molecular oxygen to a free radical-like species.³ The late Linus Pauling was the first to propose that oxygen could be converted to superoxide by a variety of chemicals and physical methods and could be important in physiology. The birth of the field of free radical biology can be attributed in part to Pauling's pioneering work in inorganic chemistry, which occurred in the 1920s.⁴

More recently, Sies described the physiological state associated with increased production of reactive oxygen species (ROS) as a state of "oxidative stress."⁵ In 1985, he defined oxidative stress as a disturbance in the pro-oxidant/antioxidant balance in favor of the pro-oxidant state. In this situation the organism is under increased exposure to reactive oxygen species which participate in free radical-induced alterations of cellular components through exponential chain radical-carrying mechanisms.

Oxidative Stress Protection against the pathology induced by these oxidant species is provided by a broad class of protective agents termed antioxidants, represented by both the small molecules such as tocopherol and ascorbate, and enzymes such as superoxide dismutase and glutathione peroxidase.

What this test does:

Pinpoints the metabolic deficiencies that accelerate the aging process and lead to degenerative diseases.

Turn-around Time 7 days

Oxidative stress at the cellular level results from many factors, including exposure to alcohol, medications, trauma, cold, toxins or radiation (Figure 1). Oxidative stress also can be a consequence of liver exposure to xenobiotic substances which induce oxidative reactions through upregulation of the cytochrome P-450 mixed function oxidase system. This process can deplete specific cellular antioxidants such as glutathione, vitamin C or vitamin E.

Free Radicals Free radicals can be generated in a wide variety of normal physiological functions (Figure 2). In some cases they are protective in nature but they can be harmful if not processed rapidly. Free radicals and lipid peroxides have been found to be elevated in patients with rheumatoid arthritis and systemic lupus erythematosus, as well as patients with glomerular disorders.^{6,7} They play an important role in regulating hypertension through the degradation of prostacyclin and nitric oxide.⁸

Free radicals have been implicated in the etiology of diabetes and potentially in some of its long term complications through the destruction of pancreatic beta cells.⁹ Notably, patients with Type I diabetes and angiopathy were found to have substantially higher lipid peroxides than controls, leading to the suggestion that this might be involved in the development of atherosclerosis.

Reactive oxygen species have also been implicated in the development of tissue damage in ulcerative colitis, in breast cancer risk where lipid peroxides were found to be highest in women with mammographic dysplasia, and in a variety of liver diseases.¹⁰⁻¹²

All these processes have the potential to impact one another through the free radicals that they generate.

The free radical mechanisms of the human body might be viewed in analogy to an army that has the potential for great good, but must be kept well disciplined and well fed. When exhausted by repeated attacks and poorly fed, this army has the potential for vast damage.

Using Genova Diagnostics' Oxidative Stress Panel

The Oxidative Stress panel can be ordered by itself or in conjunction with the Detoxification Profile for a more complete picture of the body's detoxification function. The Oxidative Stress panel includes measurement of blood glutathione, lipid peroxides, Glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) and two derivatives of salicylate: catechol and 2, 3-dihydroxybenzoate (2,3-DHB).

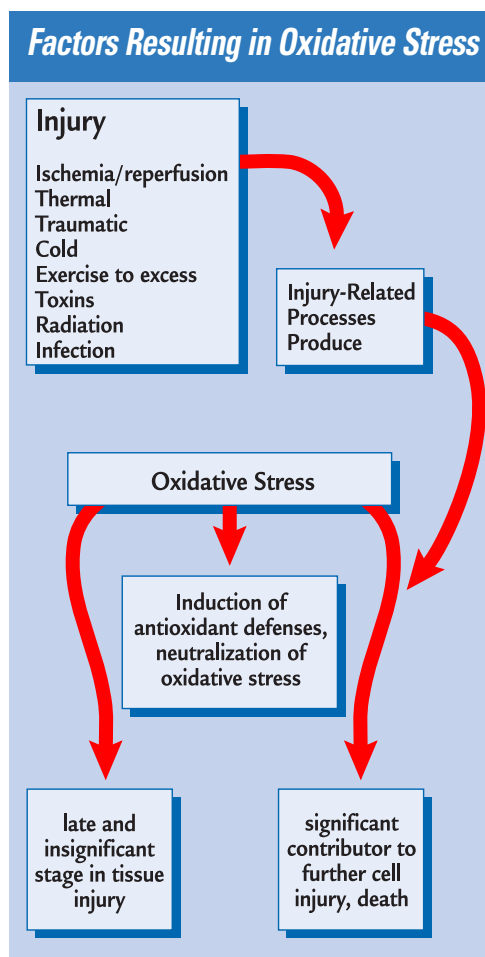


Figure 1

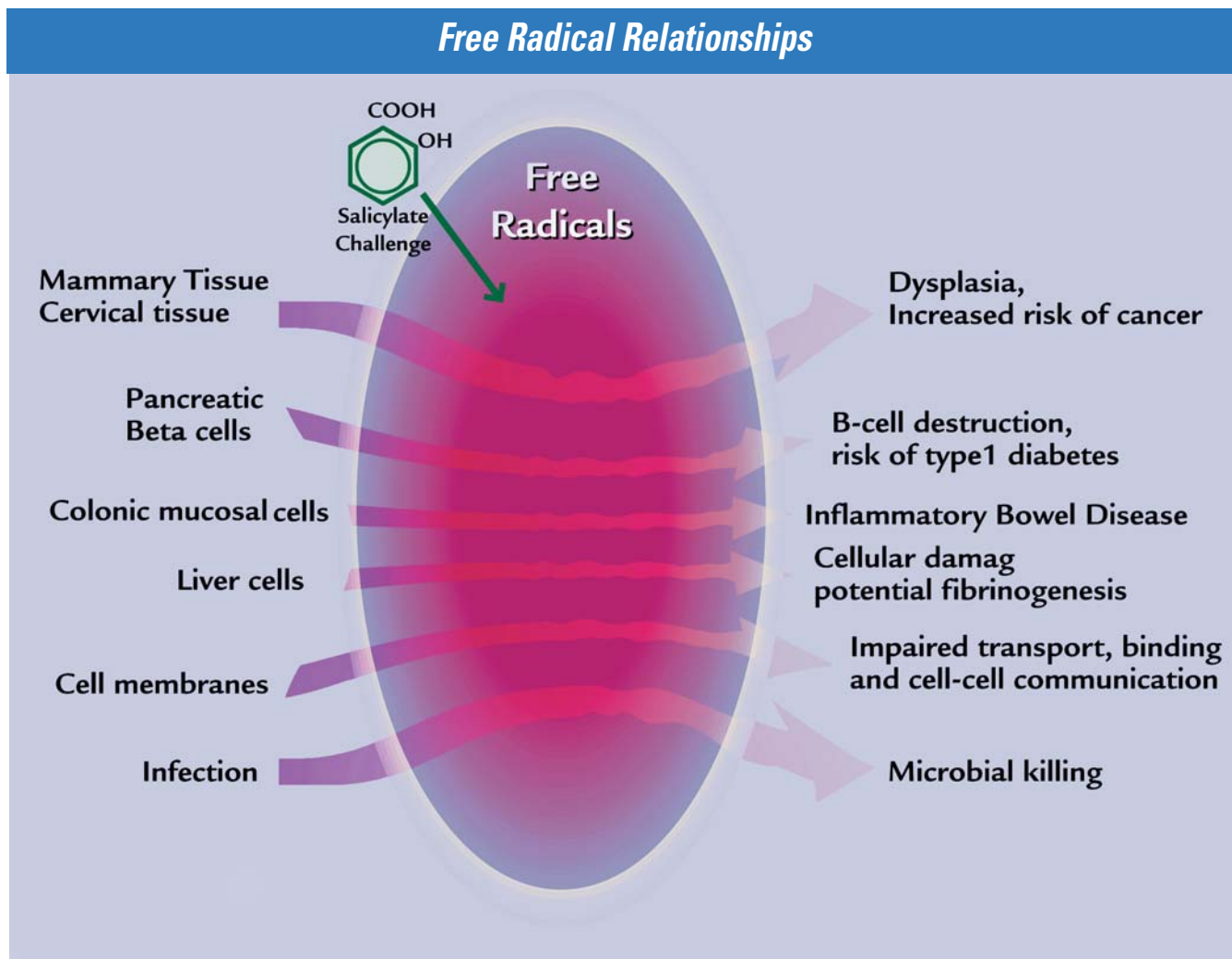


Figure 2

Glutathione

The measurement of whole blood Glutathione reflects the body's reserves of this critical molecule. Glutathione functions both as an antioxidant (in the form of glutathione peroxidase) and as a detoxifying agent for a vast array of xenobiotics. Glutathione, for example, plays a critical role in detoxifying an intermediate of acetaminophen metabolism and preventing acetaminophen toxicity. Glutathione, due to its cysteine content, is also a minor source of the organic sulfate used in detoxification reactions. Glutathione is measured in a fasting sample following an overnight acetaminophen challenge. This allows for assessment of functional reserve after detoxifying capacity has been tested.

Glutathione Peroxidase (GSH-Px)

Glutathione peroxidase is a selenium-dependant enzyme found primarily in the cytoplasm (70%) but also in the mitochondria (30%). Requiring four selenium atoms per active molecule, GSH-Px scavenges lipid peroxides throughout the membrane surfaces and quenches H₂O₂, converting it to water. Accumulations of oxidized lipids in mitochondrial and cell membranes have deleterious effects on function. Adequate levels of GSH-Px prevent this accumulation. The enzyme also participates in regeneration of the reduced (active) form of vitamin C. Studies indicate that low levels of GSH-Px activity are related to disease states.¹³⁻¹⁴ High levels of this enzyme have also

been associated with certain conditions such as Alzheimer's dementia and Beta-Thalassemia minor.¹⁵⁻¹⁶ This perhaps reflects a response to increased amount of oxidative stress.

By measuring glutathione, glutathione peroxidase, and the lipid peroxides, the clinician can evaluate the relationship involved in maintenance of oxidative stress protection. Perhaps most importantly, evidence exists that supplementation with selenium is able to increase the levels of glutathione peroxidase in patients.¹⁷

Superoxide Dismutase (SOD)

This important enzyme is found in both the cell cytosol and the mitochondria. The cytosol form is dependant upon zinc and copper co-factors while the mitochondrial form requires manganese. As the name denotes, the superoxide radical is the substrate upon which the enzyme works, converting it to the less reactive H₂O₂ four times faster than if the enzyme were not present.

As normal mitochondrial processes result in production of the superoxide radical, sufficient activity of Mn/SOD and GSH-Px protect this organelle from these damaging functional by-products. During periods of excessive muscular activity, this may be of particular importance. Investigations of SOD in clinical medicine include the observation that SOD is a sensitive marker for exposure to agricultural pesticides.¹⁸ SOD seems to be found at high levels in conditions such as systemic sclerosis, myositis, and malignant melanoma.¹⁹⁻²¹ SOD is decreased in patients with juvenile rheumatoid arthritis, affirming correlations of SOD and inflammation.²² SOD levels are also found to be low in patients with both early hyperglycemia AND impaired glucose tolerance.²³ SOD levels were found to be low in experimental zinc deficiency research. This provides physicians with clear therapeutic rationale for modifying zinc nutrition.²⁴

Lipid Peroxides and Hydroxyl Radical Markers

Lipid peroxides result from hydroxyl radical-attack on polyunsaturated fatty acids (PUFAs). Elevated levels of lipid peroxides are thus strongly suggestive of hydroxyl radical activity and reflect oxidative damage. The production of toxic radicals and metabolites is thought to be the main cause of much systemic damage. It has been suggested that hydroxyl radical attack upon membrane bound essential fatty acids (EFAs), leading to a loss of highly unsaturated EFAs, may have a direct relationship to EFA deficiencies, free radical damage and the aging process.²⁵

Catechol and 2,3-DHB are direct products of hydroxyl radical attack upon salicylic acid.^{26,27} The amount of 2,3-DHB appearing in the urine after an aspirin challenge appears to be a direct reflection of hydroxyl radical concentrations.²⁷

It is not surprising that 2,3-DHB has been reported to be a sensitive indicator of oxidative damage in diabetics.²⁸ The research by Ghiselli suggests that hydroxyl radicals are involved in the pathogenesis of late complications in diabetes.

Catechol is a minor metabolite of hydroxyl radical attack upon salicylic acid. Its generation involves the release of a molecule of carbon dioxide. Catechol is thought to correlate with 2,3-DHB as an additional indicator of oxidative stress.^{29,30}

Clinical Therapeutics for Oxidative Stress

In the 1970s, Pauling discussed the use of therapeutic doses of vitamin C to prevent and treat viral infections. Cathcart reported in numerous clinical instances therapeutic benefit of using "bowel tolerance" doses of vitamin C (the oral dose which will initiate diarrhea) for the treatment of many virus-related disorders.³¹ Although his theories have not been proven through detailed mechanistic studies, many clinicians have reported anecdotally that their patients have benefited from this therapeutic approach.

There is emerging recognition that individual antioxidants may not have as wide-ranging clinical benefits as the intake of balanced antioxidants which incorporate all the dietary redox-active substances people have consumed for millennia. This is due in part to the involvement by different antioxidants in the process of regenerating each other.

Oxidative Stress Analysis Application Guide

Specific antioxidants include ascorbate, carotenoids and tocopherols, but also extend into other phytonutrients such as phenols, flavonoids, and quinoids. There is growing acceptance among scientific and medical communities that enhanced antioxidant intake in the diet and specific application of antioxidants in certain states of oxidative stress may provide both preventive and therapeutic advantage.³²

For more information, refer to the Oxidative Stress Interpretive Guidelines available from Genova Diagnostics.

Related Tests Because oxidative stress attacks the body at the cellular level, virtually every part is affected by free radical damage. The relationship of the liver and its pivotal role in detoxification leads most practitioners to order the companion test, the Detoxification Profile. Among others, the **Amino Acids Analysis** looks at levels of cysteine and taurine. Low levels of cysteine (the rate-limiting factor for the synthesis of GSH) can result from impairments in methionine metabolism, made evident by the results of the analysis. Taurine is an important scavenger of the hypochlorite ion (OCI), which contributes to oxidative stress and inflammation.

Still other tests may be useful to the clinician. Recent toxic exposure and cumulative exposure over time can be assessed by Elemental Analysis of hair, urine, or blood. The test also examines levels of nutrient elements for excesses and deficiencies which can interfere with antioxidant activity in the body.

The key to effective antioxidant supplement is absorption, and suspected problems with digestion and absorption should be investigated with tests of gastrointestinal function. The **Comprehensive Digestive Stool Analysis (CDSA)** is the place to start, because its results and test commentary present a useful picture of total gut environment health. The Interpretive Guidelines for the CDSA will suggest additional digestive testing, such as **Intestinal Permeability Assessment** and **Bacterial Overgrowth of the Small Intestine**, if deemed necessary. Malabsorption of antioxidant and detoxifying nutrients and increased intestinal permeability to toxins can result in an overload to the liver's detoxication capacity.

Because nearly every modern diet is deficient in essential fatty acids, especially W-3 fatty acids, the **Essential and Metabolic Fatty Acids Analysis** can be instrumental in establishing a foundation for improved antioxidant utilization. Proper balance of fatty acids is necessary for effective cell communication, creating precursors for hormones, reducing inflammation throughout the body, and healthy neurological development. The interaction of metabolic mediators can be improved by restoring homeostasis of fatty acids.

Inflammation and nutrient insufficiencies, as well as disordered methionine metabolism, ultimately increase cardiac risk by elevating levels of the amino acid homocysteine and creating an environment where the C-reactive protein is called into action in response to injury and infection. Both metabolites are biochemical markers in the Comprehensive Cardiovascular Assessment, and their elevation has been associated with cardiovascular illness.

How do I order this test?

For Oxidative Stress Analysis kits or information, please call a Client Services representative at 800-522-4762 or order online at www.GDX.net.

References

1. Bland JS. Oxidants and antioxidants in clinical medicine: past present and future potential. *J Nutr Environ Med* 1995;5:255-280.
2. Harman D. Aging: a theory based on free radical and radiation chemistry. *Gerontol* 1956;298-300.
3. Fridovich I. *The Molecular Basis of Oxidative Damage by Leukocytes*. Boca Raton, FL: CRC Press, 1992.
4. Bland JS. Oxidants and antioxidants in clinical medicine: past present and future potential. *J Nutr Environ Med* 1995;5:255-280.
5. Sies H (ed.) *Oxidative Stress*. New York: Academic Press, 1985; 1-8.
6. Surya Prabha P, Das UN, Ramesh G, Vijar Jumar K, and Sravan Kumar G. Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus Prostaglandins, Leukotrienes and EFAs 1991; 43:251-55.
7. Das UN., Kumar KV, Prabha PS, Murthy BVR, and Neela P. Oxy-radicals, lipid peroxides and essential fatty acids in patients with glomerular disorders. Prostaglandins, Leukotrienes and EFAs 1993; 49: 603-7.
8. Surya Prabha P, Das UN, Koratkar R, Sangeetha Sagar P, and Ramesh G. Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. Prostaglandins, Leukotrienes and EFAs 1990;41:27-33.
9. Oberley LW. Free radicals and diabetes *Free Radical Biology & Med* 1988;5:113-24.
10. Sedghi S, Keshavarzian A, Klamut M, Eiznhamer D, and Zarlign E.J. Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 1994;89(12):2217-21.
11. Boyd NF and McGuire V. The possible role of lipid peroxidation in breast cancer risk. *Free Radical Biology & Med* 1991;10:185-90.
12. Britton RS and Bacon BR. Role of free radicals in liver diseases and hepatic fibrosis. *Hepato-Gastroenterol* 1994;41:343-8.
13. Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, et al. Serum selenium plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px) levels in asymptomatic versus symptomatic human immunodeficiency virus-1(HIV-1) infection. *Eur J Clin Nutri* 1997;51(4):266-72.
14. Ong-awyooh L, Ong-awyooh S, Tiensong K, Nilwarangkur S, et al. Reduced free radical scavengers and chronic renal failure. *J Med Assoc Thai* 1997;80(2):101-08.
15. Anneren G, Gardner A, Lundin T. Increase glutathione peroxidase activity in erythrocytes in patients with Alzheimer's disease/senile dementia of Alzheimer's type. *Acta Neurol Scand* 1986; 73(6):586-89.
16. Gerli GC, Beretta L, Bianchi M, Pellegatta A, Agostoni A, et al. Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in beta-thalassaemia (major and minor). *Scand J Haematol* 1980;25(1):87-92.
17. Hussein O, Rosenblat M, Refael G, Aviram M. Dietary selenium increases cellular glutathione peroxidase activity and reduces the enhanced susceptibility to lipid peroxidation of plasma and low density lipoproteins in kidney transplant recipients. *Transplantation* 1997;63(5):679-85.
18. Dowla HA, Panemangalore M, Byers ME. Comparative inhibition of enzymes of human erythrocytes and plasma in vitro by agricultural chemicals. *Arch Environ Contam Toxicol* 1996;31(1):107-14.
19. Morita A, Minami H, Sakakibara N, Sato K, Tsuji T. Elevated plasma superoxide dismutase activity in patients with systemic sclerosis. *J Dermatol Sci* 1996;11(3):196-201.
20. Mokuno K, Kiyosawa K, Honda H, Hirose Y, Murayama T, Yoneyama S, Kato K. Elevated serum levels of manganese superoxide dismutase in polymyositis and dermatomyositis. *Neurology* 1996; 46(5):1445-47.
21. Schadendorf D, Zuberbier T, Diehl S, Schadendorf C, Czarnetzki BM. Serum manganese superoxide dismutase is a new tumour marker for malignant melanoma. *Melanoma Res* 1995;5(5):351-53.
22. Sklodowska M, Gromadzinska J, Biernacka M, Wasowicz W, Wolkani P, Marszalek A. Vitamin E, thiobarbituric acid reactive substance concentrations, and superoxide dismutase activity in the blood of children with juvenile rheumatoid arthritis. *Clin Exp Rheumatol* 1996;14(4):433-39.
23. Vijayalingam S, Parthiban A, Shanmugasundaram KR, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabet Med* 1996;13(8):715-19.
24. Ruz M, Cavan KR, Bettger WJ, Fischer PW, Gibson RS. Indices of iron and copper status during experimentally induced, marginal zinc deficiency in humans. *Biol Trace Elem Res* 1992;34(2):197-212.
25. Horrobin DF. Is the main problem in free radical damage caused by radiation, oxygen and other toxins the loss of membrane essential fatty acids rather than the accumulation of toxic materials? *Med Hypoth* 1991;35:23-6.
26. Grootveld M and Halliwell B. 2,3 dihydroxybenzoic acid is a product of human aspirin metabolism. *Biochemical Pharmacology* 1988;37(2):271-80.
27. Grootveld M and Halliwell B. Aromatic hydroxylation as a potential measure of hydroxy-radical formation in vivo. *Biochem* 1986;237:499-504.
28. Ghiselli A, Laurenti O, De Mattia G, Maiani G, and Ferro-Luzzi A. Salicylate hydroxylation as an early marker of in vivo oxidative stress in diabetic patients. *Free Radical Biology & Med* 1992;13:621-6.
29. Halliwell B, Kaur H, and Ingelman-Sundberg M. Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note [letter]. *Free Radical Biology & Med* 1991;10:439-41.
30. Kettle AJ and Winterbourn CC. Superoxide-dependent hydroxylation by myeloperoxidase. *J Biological Chem* 1994;269(June 24):17146-51.
31. Cathcart RF 3d; Vitamin C: the nontoxic, nonrate-limited, antioxidant free radical scavenger. *Med Hypoth* 1985;18:61-77.
32. Marantz PR. Beta carotene, vitamin E, and lung cancer. *N Eng J Med* 1994;331:611.

Oxidative Stress Analysis Application Guide



63 Zillicoa Street
Asheville, NC 28801
800 522.4762
www.GDX.net

This information is for the sole use of a licensed health care practitioner and is for educational purposes only. It is not meant for use as diagnostic information. All claims submitted to Medicare/Medicaid for Genova Diagnostics laboratory services must be for tests that are medically necessary. "Medically necessary" is defined as a test or procedure that is reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member. Consequently, tests performed for screening purposes will not be reimbursed by the Medicare program.