ClevelandHeartLab®

Know your risk.

PRACTITIONER'S GUIDE

To Cardiovascular Testing & Treatment Options

Please Note: *All treatment plans provided within this guide are for educational purposes **only**. Patient-specific treatment plans should be provided and reviewed by the treating practitioner.*

About Cleveland HeartLab, Inc.

Cleveland HeartLab offers innovative, scientifically proven, and medically relevant biomarkers that are predictive of cardiovascular risk. We offer inflammatory and other advanced biomarker testing that practitioners from across the United States use in the management and prevention of heart disease. Our novel biomarker technologies are offered through our CAP-accredited and CLIA-certified clinical reference laboratory. We also run a research and development laboratory where next-generation cardiovascular disease biomarkers are being developed for use through our clinical laboratory.

Cleveland HeartLab is committed to *Innovation*:

We maintain a robust research and development program that partners with leading academic and medical institutions to bring unique biomarker technologies to market. Our first proprietary biomarker – Myeloperoxidase (MPO) – was the result of research and development at the Cleveland Clinic, and received 510(k) clearance from the FDA within 13 months of discovery. We have an expansive, global intellectual property portfolio that includes over 50 issued and pending patents. We consistently bring new, novel biomarkers to the market and maintain an active pipeline in development.

Cleveland HeartLab is committed to *Inflammation* Testing:

Cleveland HeartLab is an established, premier inflammation testing laboratory with the most experience in the field. Our scientifically-proven, peer-reviewed multi-marker approach of adding inflammation-specific tests to traditional lipid testing provides additional insight into an individual's cardiovascular risk. In addition, our approach measures risk across a spectrum, allowing for long-, mid-, and near-term assessment. This enables healthcare providers to identify 'hidden risk' and treat more aggressively when appropriate. Our testing is supported by over 100 peer-reviewed articles. Most recently, a seminal, peer-reviewed study published in the *Journal of Medical Economics* shows Cleveland HeartLab's inflammation testing could reduce the average heart attack and stroke rate by approximately 10%. Over five years, this reduction would avert $187 million in healthcare costs for cardiovascular disease – the number one killer of men and women in the U.S.

Cleveland HeartLab is committed to Clinical *Education*:

We are committed to educating healthcare providers and patients on the advancements in identifying cardiovascular risk. We organize signature CME-accredited events and local Medical Forums which attract hundreds of medical professionals from across the United States. Our clinical Education Team provides access to peer-to-peer support, the latest articles on the clinical utility of inflammatory biomarkers and individual case management support. We developed and provide a CME website for healthcare professionals (**chlcme.com**) and a website designed for patients (**knowyourrisk.com**).

References

1. Ridker PM et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med.* 2008; 359: 2195-2207.
2. Libby P et al. Inflammation in atherosclerosis: Transition from theory to practice. *Circ J*. 2010; 74: 213-220.

Contents

Inflammation and Heart Disease

Approximately 50% of patients experiencing a heart attack or stroke have *'normal'* cholesterol levels[1].

The Framingham study in 1948 initially established standard risk factors for heart disease, or atherosclerosis (hardening of the arteries), that include high blood pressure, age, family history, cholesterol, obesity, diabetes, an unhealthy diet and whether an individual smokes. However, evidence is continually accumulating that the Framingham risk analysis is limited and fails to accurately detect the presence of heart disease in individuals[2].

The risk of developing heart disease has traditionally been assessed by measurement of LDL-C (low-density lipoprotein cholesterol; the carrier of "bad" cholesterol) and HDL-C (high-density lipoprotein cholesterol; the carrier of "good" cholesterol). Recent studies demonstrate that about 50% of heart attacks and strokes occur in people with 'normal' cholesterol levels[1]. This suggests that many people at risk are presumed low-risk because they have "normal" or controlled cholesterol levels. Therefore, routine cholesterol tests may fail to fully identify people at risk for heart attack and stroke.

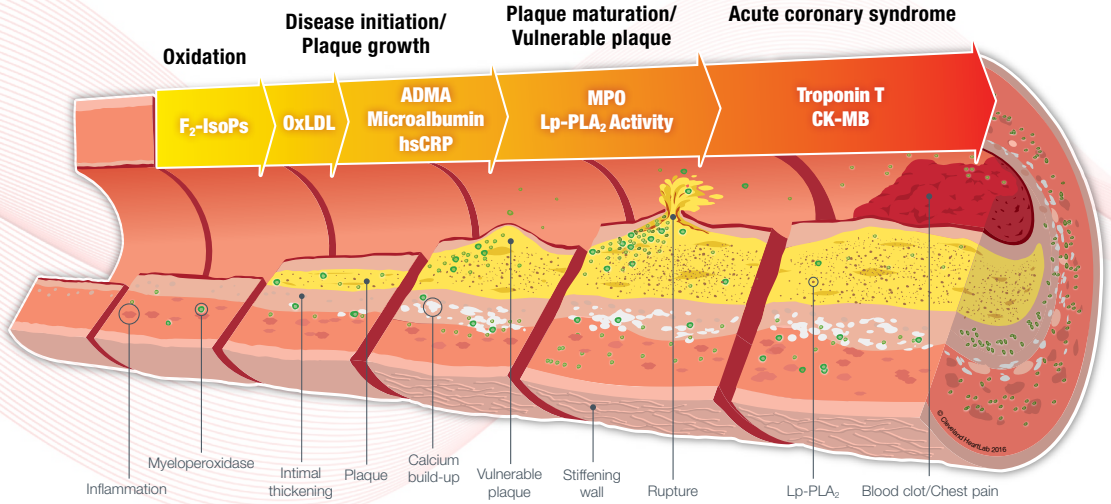
Although it is essential to assess your cholesterol levels, adverse cardiac events (such as heart attack, stroke or death) have been associated with inflammation[3], specifically vulnerable plaque related to increased white blood cell activation.

References

1. Ridker PM et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med.* 2008; 359: 2195-2207.
2. Johnson KM et al. Traditional clinical risk assessment tools do not accurately predict coronary atherosclerotic plaque burden: A CT angiography study. *Am J Roentgenol.* 2009; 192: 235-243.
3. Ross R. Atherosclerosis – An inflammatory disease. *N Engl J Med.* 1999; 340: 115-126.

Inflammation Testing

Cleveland HeartLab, Inc. offers inflammatory biomarker testing to help practitioners evaluate cardiovascular risk in patients. This group of tests covers a patient's biomarker profile which may result from lifestyle concerns (F_2-IsoPs, OxLDL) to the development of metabolic or cardiovascular disease (ADMA/SDMA, Microalbumin, hsCRP) and formation of vulnerable plaque and increased risk for an adverse event (Lp-PLA_2 Activity, MPO).



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Inflammation testing provided by Cleveland HeartLab, Inc. includes the following tests:

- **Myeloperoxidase**
- **Lp-PLA_2 Activity**
- **hsCRP**
- **Microalbumin**
- **ADMA/SDMA**
- **Oxidized LDL**
- **F_2-Isoprostanes**

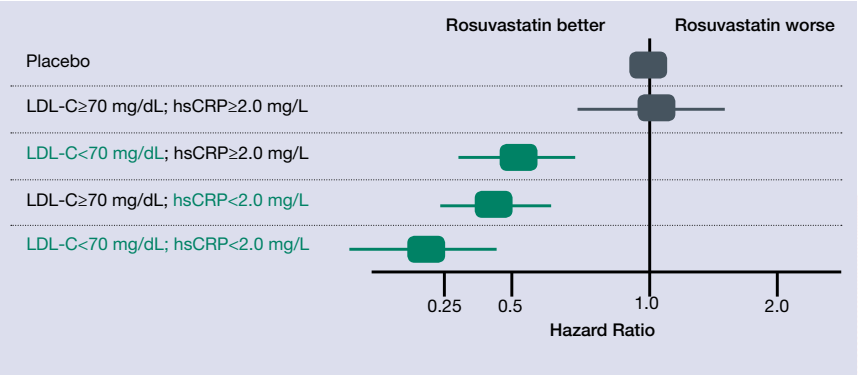
These tests are reviewed on the next pages, and can be ordered individually.

A Multimarker Approach Can Aid in Stratifying Cardiovascular Risk

The literature supports the concept that combining multiple markers increases our ability to risk-stratify patients for cardiovascular risk[1]. We have recently published the utility of combining inflammation tests to better define a patient's risk[2].

Where Inflammation Meets Lipids®

The JUPITER Trial was the first landmark trial to demonstrate increased identification and stratification of individuals at risk of adverse cardiovascular events using a multimarker approach[3]. Prior to the results of this publication, most research focused solely on lowering LDL-C with statin therapy to reduce cardiovascular risk[4]. The JUPITER Trial went beyond this traditional measurement to demonstrate that hsCRP, a marker of general- and cardiovascular-related inflammation within the body, can improve risk stratification of individuals who may benefit from rosuvastatin therapy. As shown in the figure here, individuals with low LDL-C, but high inflammation - as measured by hsCRP - had twice the risk of a cardiovascular event compared to individuals with low LDL-C and low hsCRP. These findings highlight the importance of a multimarker strategy in assessing cardiovascular risk, and measuring inflammation levels alongside traditional measurements such as LDL-C.



Incident cardiovascular events in the JUPITER Trial following initiation of rosuvastatin therapy. This figure was adapted from [ref 5].

References

1. Ikonomidis I et al. Multimarker approach in cardiovascular risk prediction. *Dis Markers*. 2009; 26: 273-285.
2. Penn MS and Klemes AB. Multimarker approach for identifying and documenting mitigation of cardiovascular risk. *Future Cardiol.* 2013; 9: 497-506.
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5. Libby P et al. Inflammation in atherosclerosis: From pathophysiology to practice. *J Am Coll Cardiol*. 2009; 54: 2129-2138.

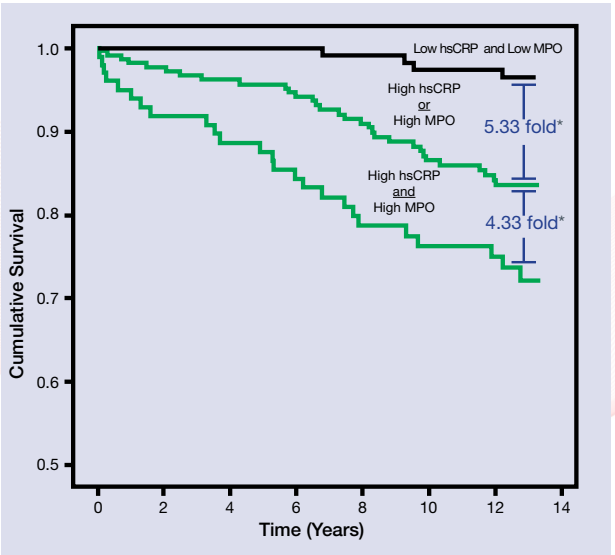
A Multimarker Approach Can Aid in Stratifying Cardiovascular Risk

A Deeper Dive Into Inflammation

In addition to the landmark JUPITER Trial, other impactful, peer-reviewed publications exist that demonstrate the utility of a multimarker approach to individualize cardiovascular risk assessment. Multimarker approaches, when carefully designed with their physiological relevance in mind, can have additive utility of identifying the acuity of the risk[1].

Systemic and Vascular Inflammation

In 2009, Heslop et al. published a study in the Journal of the American College of Cardiology which examined the clinical utility of hsCRP and MPO – two inflammatory biomarkers with different pathophysiologies[2]. Unlike hsCRP, free MPO within the bloodstream is a vascular-specific marker for vulnerable plaque formation. As shown in the figure here, individuals with elevated levels of both hsCRP (atheroma burden) and free MPO (active atheroma) in the bloodstream had approximately 4X increased risk of cardiovascular mortality compared to those with either hsCRP or MPO elevated.



Cardiovascular mortality according to elevations in hsCRP and MPO. This figure was adapted from [ref 2].

**Represents hazard ratio.*

References

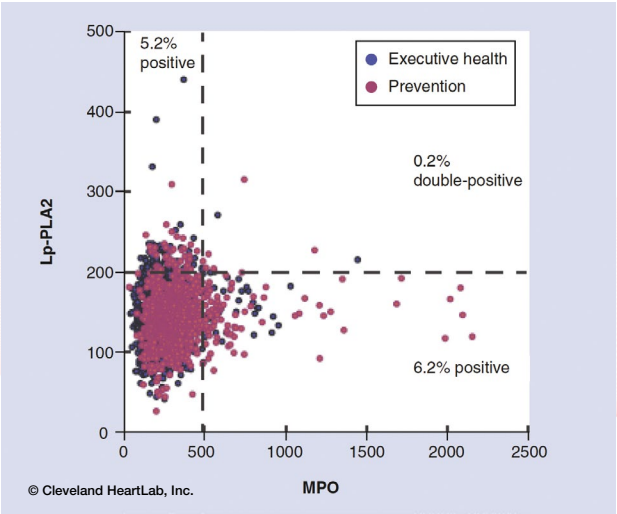
1. Penn MS and Klemes AB. Multimarker approach for identifying and documenting mitigation of cardiovascular risk. *Future Cardiol*. 2013; 9: 497-506.

2. Heslop CL et al. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J Am Coll Cardiol*. 2010; 55: 1102-1109.

A Multimarker Approach Can Aid in Stratifying Cardiovascular Risk

Vascular Inflammation - Two Sides to the Story

In 2013, Penn and Klemes published a study in Future Cardiology which examined the utility of a multimarker approach - when designed with the physiology of each marker in mind - to identify risk and ultimately acuity of risk[1]. In particular, this study highlighted the ability of Lp-PLA$_2$ and free MPO in the bloodstream to identify vulnerable plaque by measuring distinct physiologies. Lp-PLA$_2$ examines macrophage activation underneath the collagen cap within the artery wall while free MPO examines the white blood cell response in the bloodstream due to vulnerable plaque/erosions/fissures in the artery wall. As shown in the figure here, the combined use of both Lp-PLA$_2$ and MPO provides additional stratification of risk for plaque rupture beyond that of using each biomarker individually[1].



Risk based on vulnerable plaque risk markers in a stable clinical population of patients. For discussion, see [ref 1].

References

1. Penn MS and Klemes AB. Multimarker approach for identifying and documenting mitigation of cardiovascular risk. *Future Cardiol*. 2013; 9: 497-506.

Myeloperoxidase (MPO)

CPT Code 83876
Sample Type EDTA Plasma

Order Code C133
Tube Type Lavender Top

Description

MPO is a white blood cell-derived inflammatory enzyme and measures disease activity from the luminal aspect of the arterial wall.

Briefly, when the artery wall is damaged, or inflamed, MPO is released by invading white blood cells where it accumulates[1]. MPO mediates the vascular inflammation that propagates plaque formation[2] and activates protease cascades that are linked to plaque vulnerability[3]. White blood cell activation in the bloodstream, in response to luminal injury of the artery wall including fissures, erosions or a degrading collagen cap, leads to MPO release in the bloodstream. This combination of detrimental effects demonstrates that MPO is actively involved in the progression of atherosclerosis. The Cleveland HeartLab MPO test measures free MPO in the bloodstream.

Clinical Use

The MPO test may be performed on individuals with multiple risk factors for cardiovascular disease, or those with established disease.

Clinical Significance

- Elevated MPO levels predict the risk of heart disease in subgroups otherwise associated with low risk[4,5].
- Elevated MPO levels independently predict the risk of future cardiovascular events in patients presenting with an acute coronary syndrome[6,7].
- Individuals with elevated MPO levels are more than 2x as likely to experience cardiovascular mortality[8].
- MPO enhances cardiovascular risk prediction when used independently or alongside standard biomarker testing such as hsCRP[8].
- MPO levels are not likely to be elevated due to chronic infections or rheumatologic disorders due to the fact that free MPO in the blood is a specific marker of vascular inflammation and vulnerable plaque/erosions/fissures.
- The p-ANCA test (anti-MPO antibody test) is not the same as the MPO test performed by Cleveland HeartLab. The p-ANCA test primarily measures the amount of antibodies directed against the MPO protein.

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RELATIVE RISK

MPO
(pmol/L)

<470 Low
470-539 Moderate
≥540 High

Note: Numerous studies have documented that increasing MPO levels predict increasing risk for adverse events in various cohorts of individuals. Please visit www.clevelandheartlab.com for literature supporting the clinical utility of MPO testing.

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

✓ **Assess blood pressure.**
- If not at goal, consider initiating, or titrating, anti-hypertensive therapy.

NOTE: An elevated blood pressure may contribute to endothelial dysfunction and coronary disease formation.

✓ **Assess smoking habits.**

NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

✓ **Assess risk for pre-diabetes/diabetes.**
- If abnormal oral glucose tolerance test or insulin levels, consider insulin sensitizing therapy.

✓ **Assess the presence of coronary artery disease (CAD) with imaging techniques such as carotid intima media thickness testing (CIMT) or coronary artery calcium scoring.**
- If clinically appropriate, consider dual platelet inhibition.

✓ **Assess dental health (periodontal disease).**

NOTE: Poor dental health may cause significant inflammation and is associated with the presence of atherosclerosis[9].

✓ **Assess HDL-C levels.**
- If not at goal, consider niacin or omega-3 fatty acids.
- Assess CoQ10 levels as recent evidence suggests that low ApoA1 and/or HDL-C levels are associated with low CoQ10 levels[10].

✓ **Assess, if known to be present, the treatment of inflammatory conditions such as Crohn's disease, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).**

NOTE: Chronic inflammatory diseases may exhibit elevated MPO values due to increased vascular disease associated with these conditions. For example, RA is associated with a 5X increased risk for myocardial infarction[11].

✓ **Assess the presence of vasculitis.**

NOTE: MPO values may be elevated in individuals with vasculitis as it is characterized by increased vascular inflammation.

✓ **Assess the presence of bone marrow dyscrasias.**

NOTE: MPO values may be elevated in individuals with chronic lymphocytic leukemia or other leukemias that cause increased white blood cell destruction.

✓ **Assess level of exercise.**

NOTE: MPO values may be elevated in marathon runners[12] and extreme athletes may identify those with increased oxidative stress and basal levels of inflammation

If asymptomatic, with all of the above factors ruled out, an elevated MPO value may in fact be the patient's baseline. MPO levels should be monitored every 3-6 months.

References

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9. Buhlin K et al. Periodontitis is associated with angiographically verified coronary artery disease. *J Clin Periodontol.* 2011; 38: 1107-1014.
10. Toyama K et al. Rosuvastatin combined with regular exercise preserves coenzyme Q10 levels associated with a significant increase in high-density lipoprotein cholesterol in patients with coronary artery disease. *Atherosclerosis.* 2011; 217: 158-164.
11. Maradit-Kremers H et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: A population-based cohort study. *Arthritis Rheum.* 2005; 52: 402-211.
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Lp-PLA$_2$ Activity

CPT Code 83698
Sample Type Serum/EDTA Plasma

Order Code C570
Tube Type Tiger Top/Lavender Top

Description

Lp-PLA$_2$, or lipoprotein-associated phospholipase-A$_2$, measures disease activity within the artery wall below the collagen or calcified cap due to the activation of macrophages. Lp-PLA$_2$ is not an acute phase reactant. When disease is active in the artery, increased levels of Lp-PLA$_2$ are produced by macrophages and foam cells within the intima of the artery[1]. Lp-PLA$_2$ also interacts with oxidized LDL, which increases inflammation and enhances a proatherogenic state, as well as plaque vulnerability[2]. Research suggests that it plays a direct role in the atherosclerotic disease process[3].

Clinical Use

The Lp-PLA$_2$ Activity test may be performed on individuals at intermediate or high risk for developing coronary heart disease.

Clinical Significance

- Lp-PLA$_2$ accumulates within human atherosclerotic plaques and vulnerable lesions[4].
- Individuals with elevated Lp-PLA$_2$ Activity are nearly twice as likely to develop CHD at 7 years regardless of non-HDL cholesterol levels[5].
- Individuals with elevated Lp-PLA^2 Activity are twice as likely to experience a CHD event (MI, coronary revascularization or CHD-related death) at 5 years[6].

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RELATIVE RISK

Lp-PLA$_2$ Activity
(nmol/min/mL)

<75 Low
≥75 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated[7].

✓ **Assess omega-3 fatty acid levels.**
- Omega-3 fatty acid supplementation, along with statin therapy, may reduce Lp-PLA_2 levels[8,9].

✓ **Assess HDL-C levels.**
- If not at goal, consider niacin or fenofibrate therapy.
- Assess CoQ10 levels as recent evidence suggests that low ApoA1 and/or HDL-C levels are associated with low CoQ10 levels[10].

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or a history of cerebrovascular disease (i.e., TIA or stroke).

✓ **Assess dental health (periodontal disease).**
- Refer to dentist to identify gum disease.

✓ **Assess smoking habits.**
- If not at goal, consider initiating, or titrating, anti-hypertensive therapy.

NOTE: An elevated blood pressure may contribute to endothelial damage and coronary disease formation.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate[11].
- Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

References

1. Ferguson JF et al. Translational studies of lipoprotein-associated phospholipase A(2) in inflammation and atherosclerosis. *J Am Coll Cardio*. 2012; 59: 764-772.
2. Gonçalves I et al. Evidence supporting a key role of Lp-PLA_2-generated lysophosphatidylcholine in human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol*. 2012; 32: 1505-1512.
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High-Sensitivity C-Reactive Protein (hsCRP)

CPT Code 86141
Sample Type Serum/EDTA Plasma

Order Code C121
Tube Type Tiger Top/Lavender Top

Description

The hsCRP test is a highly sensitive quantification of CRP, an acute-phase protein released into the blood by the liver during inflammation, which has been associated with the presence of heart disease.

Clinical Use

The hsCRP test may be performed on individuals at intermediate risk (10-year risk of 10-20%) of developing CHD who are metabolically stable without inflammatory or infectious conditions.

Clinical Significance

- hsCRP is a well-documented clinical marker of general and cardiac-related inflammation.
- Apparently healthy individuals with elevated hsCRP values are up to 4X as likely to have coronary heart disease[1,2].
- Elevated hsCRP is associated with the risk of future adverse cardiovascular events (heart attack, stroke and death) in apparently healthy individuals[1,3] and in individuals with stable CAD[4].
- Reductions in both hsCRP and LDL-C are associated with reduction in the rate of atherosclerosis progression[5] and improved clinical outcomes[6].
- Introduction of statin therapy in patients with elevated hsCRP, even with normal lipid levels, significantly reduces risks for heart attack, stroke and death[7].

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RELATIVE RISK

hsCRP
(mg/L)

<1.0 Low
1.0-3.0 Moderate
>3.0 High

Note: Levels >10 mg/L warrant follow-up for consideration of a non-cardiovascular-related cause[8].

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess presence of acute (flu, cold, etc.) or chronic (bronchitis, chronic obstructive pulmonary disease, RA) illness.**

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or cerebrovascular disease (i.e., TIA or stroke).
- If the presence of vascular disease is confirmed by imaging studies, consider statin-based lipid-lowering therapy unless contraindicated.

✓ **Assess dental health (periodontal disease).**
- Refer to dentist to identify gum disease.

NOTE: Poor dental health may cause significant inflammation and is associated with the presence of atherosclerosis[8].

✓ **Assess blood pressure.**
- If not at goal, consider initiating, or titrating, anti-hypertensive therapy.

NOTE: An elevated blood pressure may contribute to endothelial dysfunction and coronary disease formation.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

References

1. Ridker PM et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997; 336: 973-979.
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Microalbumin

CPT Code 82043/82570
Sample Type Urine

Order Code C919
Tube Type Yellow Top

Description

Microalbumin is the quantification of small amounts of albumin, a serum protein, in urine that can be used to identify microvascular endothelial dysfunction. The presence of small amounts of albumin in the urine may suggest the presence of systemic endothelial dysfunction - an early indicator of heart disease. This test is more sensitive than a standard dipstick test routinely performed in an office setting.

Clinical Use

Microalbumin may be performed on individuals with type 1 or type 2 diabetes, hypertension, a family history of chronic kidney disease, those at intermediate (10-20%) risk for CVD or those with known vascular disease.

Clinical Significance

- **Renal Significance:** The American Diabetes Association has defined microalbuminuria as a microalbumin value of 30-300 mg/g creatinine[1]. A persistent microalbumin of >30 mg/g indicates a loss of kidney function and is used in the diagnosis of chronic kidney disease[2].
- **Cardiovascular Significance:** Increases in microalbumin excretion in the 'normal' range (<30 mg/g) are associated with increased risk for development of cardiovascular morbidity and mortality, as well as all-cause mortality[3-8].
- In particular, it was shown that healthy individuals (defined as non-hypertensive, non-diabetic, and without prevalent CVD) with low microalbumin levels had approximately 3x greater risk for developing cardiovascular disease[3]. These levels were gender-specific and noted to be ≥3.9 mg/g for men and ≥7.5 mg/g for women.
- A direct, linear relationship exists between microalbuminuria and the risk of heart attack, stroke and death[5].

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RELATIVE RISK[1]

Microalbumin
(mg/g creatinine)

Women	Men
<7.5 Low	<3.9 Low
≥7.5 High	≥3.9 Low

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess blood pressure.**
- If not at goal, consider initiating, or titrating, anti-hypertensive therapy.

NOTE: An elevated blood pressure may damage the endothelium in the kidney and contribute to disease. The presence of urinary microalbumin may suggest systemic endothelial dysfunction and the presence of CAD.
- Retest urinary microalbumin levels in 2-3 months.

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD. (i.e., myocardial infarction or revascularization) and/or a history of cerebrovascular disease (i.e., TIA or stroke).

✓ **Assess risk for pre-diabetes/diabetes.**
- If abnormal fasting glucose or oral glucose tolerance test, consider PPAR agonists, metformin or DPP-IV inhibitors if not contraindicated.

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ADMA/SDMA

CPT Code 82542
Sample Type Serum

Order Code C301
Tube Type Tiger Top

Description

One of the earliest manifestations of endothelial dysfunction is nitric oxide (NO) deficiency, which promotes atherosclerosis. ADMA (asymmetric dimethylarginine) and SDMA (symmetric dimethylarginine), its structural isomer, are metabolites of L-arginine, an amino acid that is catalyzed to L-citrulline and NO by nitric oxide synthase (NOS).

Both ADMA and SDMA have distinct pathophysiologies and manifestations. ADMA is a competitive inhibitor of NOS thereby reducing NO production and promoting endothelial dysfunction. SDMA also interferes with NO production, but does so indirectly by reducing the cellular availability of arginine. ADMA is primarily cleared through enzymatic degradation in the bloodstream and identifies subclinical cardiovascular disease. Conversely, SDMA is primarily excreted in the urine and identifies reduced renal function.

Clinical Use

ADMA/SDMA may be measured in individuals with multiple risk factors for the development of cardiovascular disease.

Clinical Significance

- Elevated ADMA levels are associated with the presence of hypertension[1], insulin resistance[1], and hyperlipidemia[2].
- Elevated ADMA levels are associated with subclinical atherosclerosis:
  - Elevated ADMA concentrations correlate with internal carotid artery bulb intimal media thickness[3], a hemodynamically unstable region vulnerable to nitric oxide deficiency[4] and plaque formation.
  - Elevated ADMA in young adults has been associated with increased CT coronary artery calcification[5].
- Individuals with established coronary artery disease and elevated ADMA levels have more than twice the risk for adverse events (MI, stroke) than those with normal ADMA levels[6].

Renal Significance:

- Elevated SDMA levels positively correlate with reduced renal function as measured by eGFR[7].

inflammation testing
from ClevelandHeartLab

RELATIVE RISK

ADMA
(ng/mL)

<100 Low
100-123 Moderate
>123 High

REFERENCE RANGE

SDMA
(ng/mL)

73-135 Low
>135 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess blood pressure.**
- If not at goal, consider initiating, or titrating, antihypertensive therapy.

NOTE: An elevated blood pressure may contribute to endothelial dysfunction and the development of coronary artery disease and subsequent renal disease.
- Consider L-Arginine supplementation to improve vasodilation and vascular tone.

NOTE: L-Arginine enhances the production of nitric oxide which has anti-inflammatory, anti-thrombotic, anti-hypertensive, and anti-oxidant effects.

✓ **Assess risk for pre-diabetes/diabetes.**
- If abnormal fasting glucose or oral glucose tolerance test, consider PPAR agonists, metformin or DPP-IV inhibitors if not contraindicated.

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e. myocardial infarction or revascularization) and/or cerebrovascular disease (i.e. TIA or stroke).

References

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Oxidized LDL (OxLDL)

CPT Code 83516
Sample Type Serum/EDTA Plasma

Order Code C335
Tube Type Tiger Top/Lavender Top

Description

OxLDL measures protein damage due to the oxidative modification of the ApoB subunit on LDL cholesterol. The oxidation of LDL cholesterol is one of the first steps in the development of atherosclerosis. Briefly, LDL-C enters the artery wall where it becomes oxidized. OxLDL is then recognized by scavenger receptors on macrophages which engulf OxLDL, resulting in foam cell formation, vascular inflammation and the initiation of atherosclerosis.

Clinical Use

The OxLDL test may be performed on individuals at risk of metabolic syndrome.

Clinical Significance

- Individuals with high levels of OxLDL are 3.5X times more likely to develop metabolic syndrome in the next 5 years[1].
- Increased OxLDL levels are associated with the presence of coronary artery disease[2-4].
- In healthy middle-aged men, high OxLDL levels are associated with a 4X greater risk of developing coronary heart disease[5].
- Levels of OxLDL increase in a step-wise fashion as the severity of CAD increases[6].

inflammation testing from ClevelandHeartLab

RELATIVE RISK

OxLDL
(U/L)

<60 Low
60-69 Moderate
≥70 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess insulin sensitivity.**
- Consider an OGTT since metabolic syndrome is associated with an insulin insensitive state. This is especially prudent if other markers such as hsCRP, Lp-PLA_2 and/or MPO are elevated.

References

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F_2-Isoprostanes (F_2-IsoPs)

CPT Code 82542/82570
Sample Type Urine

Order Code C261
Tube Type Yellow Top

Description

F_2-IsoPs are prostaglandin-like compounds formed from the free radical-mediated oxidation of arachidonic acid[1], and are the 'gold standard' for measuring oxidative stress in the body. F_2-IsoPs also have potent biological effects associated with inflammation and therefore may mediate chronic disease initiation and progression. Additionally, F_2-IsoPs may also act as potent vasoconstrictors[2] via thromboxane formation in the endothelium, and promote platelet activation resulting in thrombus formation[3].

Clinical Use

F_2-IsoPs may be performed on individuals at risk of future cardiovascular disease due to lifestyle risks, or those with a family history of cardiovascular disease.

Clinical Significance

- Elevated levels of urinary F_2-IsoPs are seen in conditions associated with increased risk for atherosclerosis[4] and certain forms of cancer[5,6].
- F_2-IsoPs are elevated in smokers[7] and with increased intake of red meat[8] and are decreased with exercise[9].
- Lower steady state levels are associated with cardiovascular fitness and reduced risk.

it™
inflammation testing
from ClevelandHeartLab

REFERENCE RANGE

F_2-Isoprostanes
(ng/mg creatinine)

<0.86 Low
≥0.86 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess smoking habits.**

NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts as appropriate.
- Consider improving cardiovascular conditioning. Individuals who are not conditioned may have increased oxidation, but this will reduce as conditioning improves.
- Consider optimal caloric intake as individuals who exercise a lot may not be taking in enough calories for their activity level. In short, they may be at risk for increased oxidation in their bodies due to lack of nutritional balance.

References

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Advanced Lipids and Heart Disease

Abnormal cholesterol levels are a well-known risk factor for CVD and are a part of routine clinical testing[1]. Current recommendations focus on LDL-C as a target of therapy for CVD risk reduction. The LDL-C result is typically calculated from a fasting specimen according to the Friedewald formula[2], which indirectly determines the amount of LDL-C based on the measurement of other lipoproteins (total cholesterol (TC), HDL-C, and very low-density lipoprotein cholesterol (VLDL-C) based on triglyceride (TG) results). These tests themselves are subject to measurement uncertainty. Therefore, calculated LDL-C results can vary as much as 15% in some patients[3-5].

Knowing your patient's lipid levels is a useful clinical and diagnostic tool, even with the aforementioned limitations. However, advanced lipid testing is currently available to provide a more powerful insight into your patient's true lipid risk.

References

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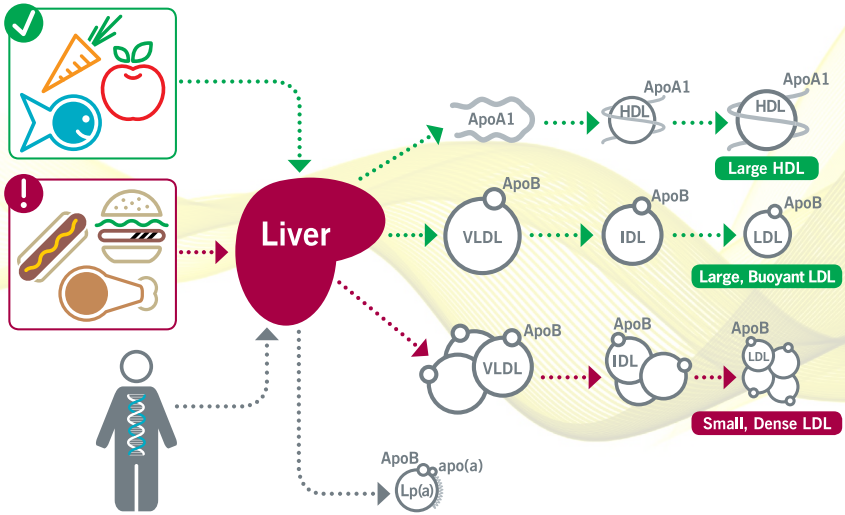
Advanced Lipid Testing

Cleveland HeartLab, Inc. offers advanced lipid testing to aid in determining cardiovascular risk in patients, alongside a Standard Lipid Panel which is commonly performed at least once a year in most medical practices. While a Standard Lipid Panel provides cholesterol and triglyceride measurements, other measurements readily available can address additional risk factors for disease including the number of atherogenic particles, the size of these particles and the inherent risk of developing CVD.



Advanced lipid testing provided by Cleveland HeartLab, Inc. includes the following tests:

- **Standard Lipid Panel (includes non-HDL cholesterol and automatic reflex to direct LDL when TGs >400 mg/dL)**
- **ApoB**
- **ApoA1**
- **ApoB/ApoA1 Ratio**
- **sdLDL**
- **Lp(a)**
- **HDL2b**

These tests are reviewed on the next pages, and can be ordered individually.

Standard Lipid Panel

CPT Code 80061
Sample Type Serum

Order Code C909
Tube Type Tiger Top

Description

The lipid panel is a well-established group of tests that provide general information used to identify patients at risk for CVD. A typical lipid panel includes total cholesterol, LDL-C, HDL-C, and TGs.

Clinical Use

The lipid panel may be used to identify individuals at risk of, or with, CVD or dyslipidemia. It also may be used to monitor an individual's progress on anti-lipid therapy.

Clinical Significance

- Numerous laboratory and clinical studies have demonstrated that lipid abnormalities are associated with cardiovascular disease risk[1].
- National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATPIII) guidelines recommend a complete lipid profile as the preferred initial test for determination of CVD risk[1].
- NCEP/ATPIII guidelines state that LDL-C is the primary target for cholesterol treatment[1] and therefore should be monitored routinely.
- Lipid panels are an easy way to monitor therapeutic response to various drugs including statins, niacin, and fibrates[2].

RELATIVE RISK

Total Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	
		WOMEN	MEN
<200 Low	<100 Low	≥50 Low	≥40 Low
200-239 Moderate	100-129 Moderate	<50 High	<40 High
≥240 High	≥130 High		

Triglycerides (mg/dL)	Non-HDL Cholesterol (mg/dL)
<150 Low	<130 Low
150-199 Moderate	130-159 Moderate
≥200 High	≥160 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.

✓ **Assess HDL-C levels.**
- If not at goal, consider niacin or fenofibrate therapy.
- Assess CoQ10 levels as recent evidence suggests that low ApoA1 and/or HDL-C levels are associated with low CoQ10 levels[3].

✓ **Assess risk for pre-diabetes/diabetes.**

✓ **Assess smoking habits.**

NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

✓ **Assess triglyceride levels.**
- If not at goal, first consider fasting status at time of blood draw, risk of pre-diabetes/diabetes, alcohol intake, thyroid status, renal function, smoking status or pregnancy.
- Once the aforementioned have been addressed, consider statins, niacin, fenofibrate, omega-3 fatty acid supplementation, PPAR agonist or combination therapy if not contraindicated.

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or if a history of cerebrovascular disease (i.e., TIA or stroke).

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

References

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Apolipoprotein B (ApoB) and A1 (ApoA1)

CPT Code 82172 (same for both biomarkers)
Sample Type Serum

Order Code C123 (ApoB), C122 (ApoA1)
Tube Type Tiger Top

Description

ApoB is the primary apolipoprotein found on the surface of LDL (the carrier of "bad" cholesterol), IDL (intermediate-density lipoprotein), VLDL and Lp(a) (lipoprotein (a))[1,2]. ApoB acts as a ligand for LDL receptors on various cells throughout the body thereby regulating cholesterol influx into tissues[3]. ApoA1 is the major apolipoprotein of HDL (the carrier of "good" cholesterol) and promotes cholesterol efflux from the artery wall to the liver for excretion[4].

Clinical Use

The ApoB test may be performed on individuals undergoing management for lipoprotein abnormalities, individuals with established coronary heart disease or diabetes, or individuals with two or more major risk factors for coronary heart disease. The ApoA1 test may be performed on individuals with hyperlipidemia, those with decreased levels of HDL-C, those at risk of cardiovascular disease or those with a family history of cardiovascular disease. Both may be used to monitor the efficacy of lifestyle and therapeutic interventions in individuals with established cardiovascular disease.

Clinical Significance

- Because the amount of LDL cholesterol per LDL particle varies within and between individuals, ApoB measurements provide a true indication of the number of atherogenic particles[5,6].
- ApoB and ApoA1 are measured directly, unlike LDL-C which is calculated from measurements of total cholesterol, TG, and HDL-C using the Friedewald formula (this is only accurate if TG values are ≤400 mg/dL).
- Elevated levels of ApoB may signify increased risk of fatal MI even when LDL levels are within normal range[7].
- Low levels of ApoA1 are associated with low levels of HDL-C[8] and reduced cholesterol clearance.
- The ApoB/ApoA1 ratio indicates 'cholesterol balance' and provides a strong, direct relationship for the risk of virtually all ischemic events[8].
- The ApoB/ApoA1 ratio was more strongly related to risk of MI than either smoking or diabetes, and individuals with a high ApoB/ApoA1 ratio have more than a 3X greater risk of having an MI[5].

RELATIVE RISK

ApoB/ApoA1 Ratio

WOMEN	MEN
<0.70 Low	<0.75 Low
0.70-0.80 Moderate	0.75-0.90 Moderate
>0.80 High	>0.90 High

ApoB (mg/dL)

<100 Low
100-120 Moderate
>120 High

ApoA1 (mg/dL)

WOMEN	MEN
>130 Low	>120 Low
≤130 High	≤120 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess LDL-C levels.**
- If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.
- Consider diet/exercise/weight reduction efforts if appropriate.

✓ **Assess HDL-C levels.**
- If not at goal, consider niacin or fenofibrate therapy.
- Assess CoQ10 levels as recent evidence suggests that low ApoA1 and/or HDL-C levels are associated with low CoQ10 levels[9].

✓ **Assess risk for pre-diabetes/diabetes.**

✓ **Assess smoking habits.**
NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

References

1 Chapman MJ et al. Further resolution of the low density lipoprotein spectrum in normal human plasma: Physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. *J Lipid Res*. 1988; 29: 442-458.

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Small-Dense LDL (sdLDL)

CPT Code 83701
Sample Type Serum

Order Code C281
Tube Type Tiger Top

Description

LDL, which carries "bad" cholesterol, exists either as large, more buoyant particles or as smaller, more dense particles (sdLDL)[1]. sdLDL is more easily oxidized, has a higher affinity for vessel walls, and remains in the circulation longer because it is less likely to be cleared by the liver, making it more atherogenic than larger LDL particles[2].

Clinical Use

The sdLDL test may be performed on individuals at risk of metabolic syndrome, those with established/progressing coronary artery disease, those with triglyceride levels between 70 and 140 mg/dL, as well as those with a diet high in trans-fat or carbohydrates.

Clinical Significance

- Increased levels of sdLDL are found in patients with metabolic syndrome[3] and are part of the "atherogenic lipoprotein profile", which includes increased levels of TG and reduced levels of HDL-C[1].
- An increased level of sdLDL is a strong predictive factor for CVD independent of total LDL levels[4].
- Increased sdLDL levels at baseline are associated with increased rates of CVD progression[5].
- The amount of cholesterol in sdLDL is associated with risk of ischemic heart disease, and is independent of lipid and non-lipid risk factors, as well as ApoB[6].

REFERENCE RANGE

Small-Dense LDL
(mg/dL)

≤40.0 Low
>40.0 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

- ✓ **Assess LDL-C levels.**
  - If not at goal, consider lipid-lowering therapy, ideally with a statin-based regimen if not contraindicated.
  - Consider diet/exercise/weight reduction efforts if appropriate.
- ✓ **Assess risk for pre-diabetes/diabetes.**
  - If abnormal fasting glucose or oral glucose tolerance test, consider PPAR agonists, metformin or DPP-IV inhibitors if not contraindicated.
- ✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
  - Consider aspirin therapy if not contraindicated.
  - Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or a history of cerebrovascular disease (i.e., TIA or stroke).
- ✓ **Assess smoking habits.**
  NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.
- ✓ **Consider statin, niacin, omega-3 fatty acid supplementation, or combination therapy if not contraindicated.**

References

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Lipoprotein (a) (Lp(a))

CPT Code 83695
Sample Type Serum

Order Code C124
Tube Type Tiger Top

Description

Lp(a) is a plasma lipoprotein consisting of a cholesterol-rich LDL particle attached to an additional apolipoprotein called apo(a). Lp(a) levels are genetically determined[1] and not affected by changes in lifestyle[2].

Clinical Use

The Lp(a) test may be performed on individuals with a family history of premature coronary heart disease, a genetic predisposition for hypercholesterolemia, established atherosclerosis but with a normal routine lipid profile, hyperlipidemia refractory to treatment, or a history of recurrent arterial stenosis.

Clinical Significance

- Lp(a) possesses potent atherogenic and thrombogenic properties[3-5].
- Elevated Lp(a) levels signify increased risk for coronary heart disease[6] and are associated with increased risk for myocardial infarction[7].
- Lp(a) levels are elevated in ischemic stroke patients and correlate well with carotid artery atherosclerosis[8].

RELATIVE RISK

Lp(a)
(mg/dL)

<30 Low
≥30 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess family history of heart disease.**

✓ **Assess Lp-PLA$_2$ and MPO levels.**
- If abnormal, treat individual biomarkers and retest in 3-6 months.

NOTE: Lp-PLA$_2$ and MPO may help to identify the presence of vulnerable plaque and increased risk of thrombosis in patients with early onset heart disease[9].

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or a history of cerebrovascular disease (i.e., TIA or stroke).

✓ **If abnormal, consider niacin therapy.**

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HDL2b

CPT Code 82664
Sample Type Serum

Order Code C324
Tube Type Tiger Top

Description

HDL cholesterol, like LDL cholesterol, can be divided into several subfractions, based on density, size and protein composition. The HDL2 subfraction (HDL2a, HDL2b) consists of larger, more buoyant particles while particles in the HDL3 subfraction (HDL3a, HDL3b, HDL3c) are smaller and denser. The largest and most buoyant HDL particle is HDL2b.

One primary function of HDL particles is to promote reverse cholesterol transport, or the movement of cholesterol from the tissues to the liver for excretion. HDL is first formed in the liver as the smaller HDL3 particles. Once released, HDL3 particles travel in the blood, where they receive cholesterol by various enzymatic events, eventually resulting in the formation of HDL2b particles. Assessment of HDL2b particles may provide a more powerful measure of cardiovascular risk than other HDL2 or HDL3 subfractions, individually or combined[1].

Clinical Use

The HDL2b test may be used for individuals at risk of diabetes or cardiovascular disease, those with cardiovascular disease or those with low total HDL levels or high triglyceride levels.

Clinical Significance

- Elevated total cholesterol and low HDL cholesterol levels, as well as high triglyceride levels, are associated with low HDL2b levels[1-4].
- Reduced HDL2b levels have been associated with insulin resistance[5].
- Women tend to have higher levels of HDL2b than men, and HDL2b levels tend to decrease as a person's BMI increases[6].
- HDL2b levels may be significantly increased by a combination of caloric restriction and high-intensity exercise[7].

RELATIVE RISK

HDL2b
(%)

WOMEN	MEN
>28 Low	>26 Low
18-28 Moderate	18-26 Moderate
<18 High	<18 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess HDL-C levels.**
- If HDL-C levels are not at goal, consider niacin or fenofibrate therapy. Fenofibrate therapy is indicated for use in patients with primary hypercholesterolemia or mixed dislipidemia to increase HDL-C levels.
- Assess CoQ10 levels as recent evidence suggests that low ApoA1 and/or HDL-C levels are associated with low CoQ10 levels[8].

✓ **Assess risk for pre-diabetes/diabetes.**

✓ **Assess smoking habits.**

NOTE: Smoking cessation is essential as individuals who smoke are at increased risk of heart disease and blood clots.

✓ **Assess triglyceride levels.**
- If triglyceride levels are not at goal, first consider fasting status at time of blood draw, risk of pre-diabetes/diabetes, alcohol intake, thyroid status, renal function, smoking status or pregnancy.
- If the aforementioned have been addressed and triglycerides remain high, consider statins, niacin, fenofibrate, omega-3 fatty acids, PPAR agonists or combination therapy if not contraindicated.

✓ **Assess the presence of CAD with imaging techniques such as CIMT or coronary artery calcium scoring.**
- Consider aspirin therapy if not contraindicated.
- Consider clopidogrel if history of CAD (i.e., myocardial infarction or revascularization) and/or a history of cerebrovascular disease (i.e., TIA or stroke).

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

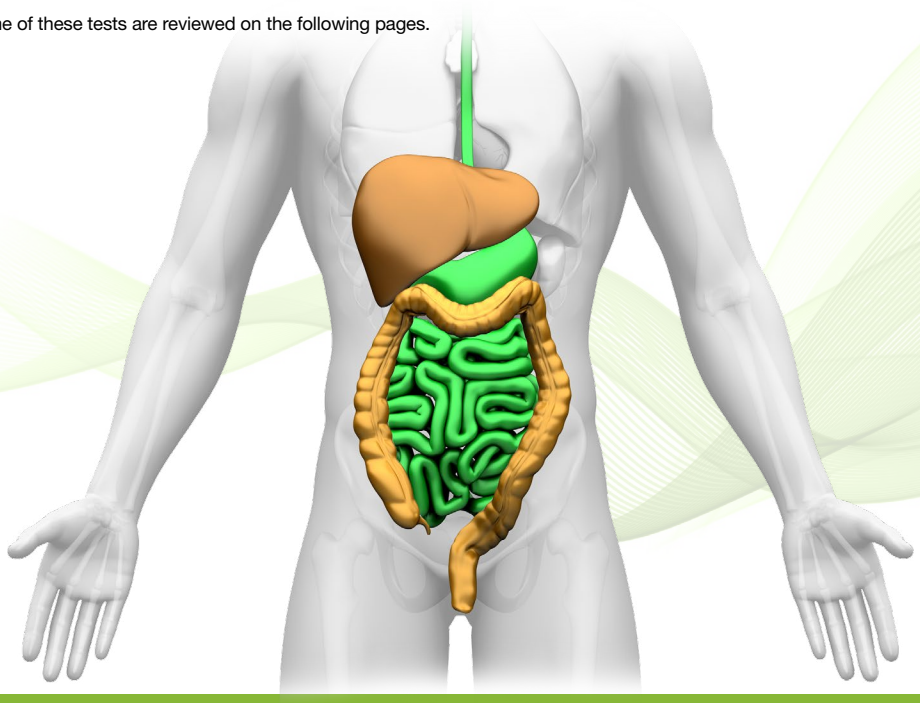
References

1. Johansson J et al. High density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. *Arterioscler Thromb*. 1991; 11: 174-182.
2. Yang Y eat al. Relationship between plasma lipid concentrations and HDL subclasses. *Clin Chim Acta*. 2005; 354: 49-58.
3. Tian L et al. Characteristics of high-density lipoprotein subclasses distribution for subjects with desirable total cholesterol levels. *Lipids in Health and Disease*. 2011; 10: 64-72.
4. Jia L et al. Alterations of high-density lipoprotein subclasses in hypercholesterolemia and combined hyperlipidemia. *Int J Cardiol.* 2007; 120: 331-337.
5. Tilly-Kiesi M et al. Hyperinsulinemia and insulin resistance are associated with multiple abnormalities of lipoprotein subclasses in glucose-tolerant relatives of NIDDM patients. *J Lipid Res*. 1996; 37: 1569-1578.
6. Williams PT et al. Associations of age, adiposity, alcohol intake, menstrual status, and estrogen therapy with high-density lipoprotein subclasses. *Arterioscler Thromb*. 1993; 13: 1654-1661.
7. Williams PT et al. Effects of low-fat diet, calorie restriction, and running on lipoprotein subfraction concentrations in moderately overweight men. *Metabolism*. 1994; 43: 655-663.
8. Toyama K et al. Rosuvastatin combined with regular exercise preserves coenzyme Q10 levels associated with a significant increase in high-density lipoprotein cholesterol in patients with coronary artery disease. *Atherosclerosis*. 2001; 217: 158-164.

Metabolic Testing

Cleveland HeartLab, Inc. also offers metabolic tests that are additive and complementary to our inflammatory and advanced lipid testing.

Some of these tests are reviewed on the following pages.



Metabolic tests include:

- **TMAO**
- **Adiponectin**

For a complete listing, see the test menu on our website.

Trimethylamine N-oxide (TMAO)

CPT Code 82542
Sample Type Serum

Order Code C524
Tube Type Tiger Top

Description

Gut microbes live symbiotically within the human digestive tract and play important roles in host defense, immunity, and nutrient processing and absorption. This diverse community is unique to each person and influenced by both acute and chronic dietary exposures to various food sources.

Nutrients such as phosphatidylcholine (also known as lecithin), choline, and L-carnitine are abundant in animal-derived products such as red meat, egg yolk and full-fat dairy products. When consumed, these nutrients are processed by gut bacteria resulting in the release of various metabolites including TMA (trimethylamine) into the blood. TMA is then transported to the liver where it is converted into TMAO (trimethylamine N-oxide) which has been shown to regulate various physiological processes involved in the development of atherosclerosis[1,2].

Clinical Use

TMAO may be measured in individuals with one or more risk factors for the development of cardiovascular disease and/or individuals whom may benefit from intensive dietary intervention.

Clinical Significance

- There is a dose-response relationship between TMAO and atherosclerotic burden in individuals undergoing elective diagnostic coronary angiography[1].
- In stable individuals undergoing elective cardiac evaluation, elevated TMAO levels are associated with increased risk of cardiovascular disease[1], or major adverse cardiovascular events (MACE: MI, stroke or death; at a 3 year follow-up)[3].
  - Plasma L-Carnitine (a dietary precursor to TMAO) is also associated with increased risk of cardiovascular disease and MACE, but only in individuals with simultaneously elevated TMAO levels[2].
  - In a 'low risk' subset of the aforementioned population, individuals with optimal LDL-C (<70mg/dL, or apoB <80mg/dL) but elevated TMAO, were just as likely to experience a major adverse cardiovascular event[3].

RELATIVE RISK

TMAO
(μM)

<6.2 Low
6.2-9.9 Moderate
≥10.0 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess dietary habits**
- Consider implementing a Mediterranean or plant-based diet.
  - A more diverse diet rich in vegetables can improve the health of the gut microbiota.
  - Consider limiting the intake of foods rich in TMA precursors such as red meat, eggs, and/or full-fat dairy products.

NOTE: Certain types of seafood contain high levels of TMAO particularly saltwater fish, sharks, rays, mollusks, and crustaceans. Arctic deep sea fishes are known to be rich in TMAO while surface fishes (trout) contain much less TMAO. These food sources may falsely elevate TMAO levels.

✓ **Assess supplementation**
- Consider probiotic/prebiotic supplementation to promote gut bacterial biodiversity.
- Consider discontinuing the use of lecithin or L-carnitine containing supplements in individuals with elevated TMAO levels.

Implement global risk reduction strategies

✓ **Assess LDL-C levels.**
- If elevated, consider LDL-lowering therapies.

✓ **Assess BMI.**
- If overweight/obese, consider weight management strategies.

References

1. Wang Z et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472: 57–63.
2. Koeth RA et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013; 19: 576-585.
3. Tang WH et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013; 368:1575-1584.

Adiponectin

CPT Code 83516
Sample Type Serum

Order Code C314
Tube Type Tiger Top

Description

Adiponectin is an abundant hormone released by adipocytes (or fat cells), commonly referred to as an adipokine. Adiponectin plays a large metabolic role in the body, participating in the regulation of glucose levels, insulin sensitivity and lipid catabolism. Adiponectin also helps support proper endothelial functioning and has multiple anti-inflammatory properties, including inhibiting the transformation of macrophages to foam cells, one of the first steps of atherosclerosis.

Unlike other adipokines, adiponectin levels are lower in obese individuals. As adipocytes become larger with weight gain, they release less adiponectin. Among healthy individuals, women typically have higher adiponectin levels than men, and adiponectin levels tend to decrease as a person ages.

Clinical Use

The adiponectin test may be used to assess future risk of metabolic syndrome or diabetes in individuals who make poor lifestyle choices.

Clinical Significance

- Individuals with low adiponectin levels have a 3X greater risk of developing metabolic syndrome[1].
- Men with two or more risk factors for metabolic syndrome and high adiponectin levels are half as likely to develop metabolic syndrome as men with low adiponectin levels[2].
- Individuals with the lowest levels of adiponectin are up to 9X as likely to develop type 2 diabetes[3].
- Individuals with low adiponectin levels have a 2X increase in the prevalence of CAD[4].

REFERENCE RANGE

Adiponectin
(µg/mL)

WOMEN	MEN
BMI <25 kg/m^2	
5-37	4-26
BMI 25-30 kg/m^2	
5-28	4-20
BMI >30 kg/m^2	
4-22	2-20

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess lifestyle habits.**

- Consider diet/exercise/weight reduction efforts if appropriate.

Note: The Look AHEAD Study demonstrated that intensive lifestyle interventions (resulting in moderate weight loss in obese diabetic individuals) resulted in higher HDL-C levels which were significantly associated with and possibly mediated by adiponectin levels[5].

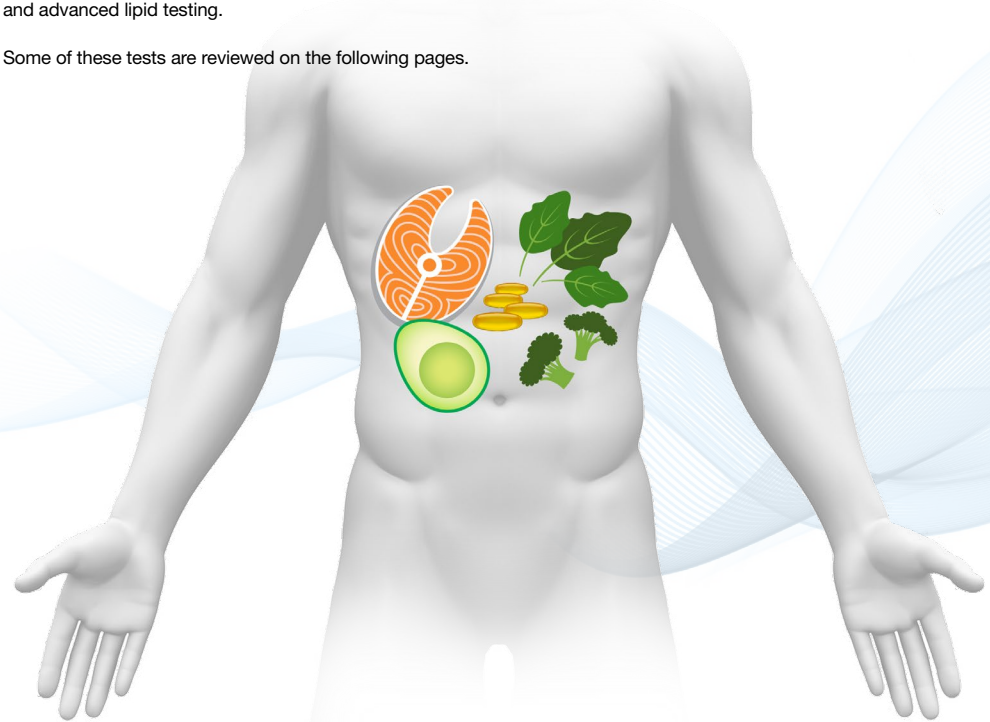
References

1. Chen SJ et al. Relationships between inflammation, adiponectin, and oxidative stress in metabolic syndrome. *PLoS ONE*. 2012; 7: e45693.
2. Kotooka N et al. Predictive value of high-molecular weight adiponectin in subjects with a higher risk of the development of metabolic syndrome: From a population based 5-year follow-up data. *Int J Cardiol*. 2012 Nov 26. pii: S0167-5273(12)01441-6. doi: 10.1016/j.ijcard.2012.10.066. [Epub ahead of print].
3. Daimon M et al. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese population. *Diabetes Care*. 2003; 26: 2015-2020.
4. Kumada M et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003; 23: 35-39.
5. Belalcazar LM et al. Adiponectin and mediation of HDL-cholesterol change with improved lifestyle: The Look AHEAD Study. *J Lipid Res*. 2012; 53: 2726-2733.

Vitamin and Supplement Testing

Cleveland HeartLab, Inc. also offers vitamin and supplement testing that are additive and complementary to our inflammatory and advanced lipid testing.

Some of these tests are reviewed on the following pages.



Vitamin and Supplement tests include:

- **OmegaCheck™**
- **CoQ10**
- **Vitamin D, 25 OH**
- **AspirinWorks®**

For a complete listing, see the test menu on our website.

OmegaCheck™

CPT Code 82542
Sample Type Whole Blood

Order Code C302
Tube Type Lavender Top

Description

Omega-3 and omega-6 fatty acids (FA) are polyunsaturated long chain FA required by the body for proper functioning, normal growth and the formation of neural synapses and cellular membranes. Omega-3 and -6 FA are considered “essential” and obtained primarily from dietary sources.

Three of the most important omega-3 FA are eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). Omega-3 FA are primarily obtained from oily fish. They have anti-oxidant[1], anti-inflammatory[2] and anti-thrombotic[3] effects, and can help to reduce triglyceride levels[4-6]. Two of the most important omega-6 FA are arachidonic acid (AA) and linoleic acid (LA). Omega-6 FA are obtained from animal sources and plant oils, and have pro-inflammatory[2,7] and pro-thrombotic[7] properties at high levels.

Clinical Use

OmegaCheck™ may be performed on individuals with hypercholesterolemia, hypertriglyceridemia, hypertension, and/or those at high metabolic or cardiovascular risk.

Clinical Significance

- Consumption of omega-3 FA reduces the occurrence of major acute cardiac events in healthy individuals or patients with cardiovascular risk factors or who have cardiovascular disease[8-14].
- Consumption of omega-3 FA leads to a reduction in triglyceride[4-6], non-HDL[6] and Lp-PLA_2 levels[6].
- A high intake of omega-6 FA precursors can interfere with the absorption of omega-3 FA[12].
- The mean omega-6:omega-3 ratio of the standard American diet is approximately 10:1[12], while a diet with a ratio of 4:1 or less may reduce total mortality up to 70% over 2 years[11].

RELATIVE RISK

OmegaCheck™
(% by weight)

≥5.5 Low
3.8-5.4 Moderate
≤3.7 High

The OmegaCheck™ was developed and validated at Cleveland HeartLab with the support of Nutrasource Diagnostics, Inc.

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess dietary intake of omega-3 and omega-6 fatty acids.**
- Dietary sources of omega-3 fatty acids include fatty fishes, such as salmon or sardines, nuts, and plant oils. Foods high in omega-6 fatty acids include poultry and eggs, plant oils, and nuts.

✓ **Consider omega-3 fatty acid supplementation.**
- If currently taking, consider adjusting dosage and retest in 1-2 months.

✓ **Assess lifestyle habits.**
- Consider diet/exercise/weight reduction efforts if appropriate.

References

1. Kesavulu MM et al. Effect of ω-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. *Diabetes Metab.* 2002; 28: 20-26.
2. James MJ et al. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr.* 2000; 71: 343s-348s.
3. Engstrom K et al. Effect of low-dose aspirin in combination with stable fish oil on whole blood production of eicosanoids. *Prostaglandins Leukot Essent Fatty Acids.* 2001; 64: 291-297.
4. Balk E et al. Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease. *Evid Rep Technol Assess* 2004: Mar(93): 1-6.
5. Musa-Veloso K et al. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid dose-dependently reduce fasting serum triglycerides. *Nutrition Reviews.* 2010; 68: 155-167.
6. Kastelein JJP et al. Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: The EpanoVa fOr Lowering Very high tyriglyceridEs (EVOLVE) trial. *J Clin Lipidol.* 2014; 8: 94-106.
7. Schmitz G. The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res.* 2008; 47: 147-155.
8. Saito Y et al. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: Sub-analysis of primary prevention cases from Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis.* 2008; 200: 135-140.
9. Marchioli R et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction. Time-course analysis of the results of the Gruppo-Italiano per lo Studio della Sopravvievenza nell'Infarto Miocardio (GISSI)-Prevenzione. *Circulation.* 2002: 105; 1897-1903.
10. Pottala JV et al. Blood eicosapentaenoic and docosahexaenoic acids predict all-cause mortality in patients with stable coronary heart disease: The Heart and Soul Study. *Circ Cardiovasc Qual Outcomes.* 2010; 3: 406-412.
11. de Lorgeril M et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet.* 1994; 343: 1454-1459.
12. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med.* 2009; 233: 674-688.
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14. Harris WS and von Schacky C. The Omega-3 Index: A new risk factor for death from coronary heart disease? *Prev Med.* 2004; 39: 212-220.

Coenzyme Q10 (CoQ10)

CPT Code 82542
Order Code C295
Sample Type Serum/EDTA Plasma
Tube Type Tiger Top/Lavender Top

Description

CoQ10 is a fat-soluble, vitamin-like substance present in most cells, primarily in the mitochondria. CoQ10 plays an integral role in the generation of cellular energy through aerobic cellular respiration. In addition, CoQ10 is a powerful antioxidant at physiologic concentrations.

Clinical Use

The CoQ10 test may be performed on individuals on statin therapy who may or may not be experiencing myalgia symptoms, hypercholesterolemic individuals, and asymptomatic individuals at risk for vascular disease who may have low ApoA1 and/or HDL levels.

Clinical Significance

- CoQ10 deficiency contributes to mitochondrial dysfunction and muscle dysfunction without myonecrosis[1].
- Statin use may inhibit the production of CoQ10 in a dose-dependent fashion by as much as 40% in hypercholesterolemic individuals[2].
- Exercise, in combination with statin therapy, can improve HDL levels and preserve CoQ10 levels[3].
- Low CoQ10 levels may be associated with low ApoA1 and/or HDL levels and poor outcomes[3,4], and may increase infarct size if/when an individual has an acute myocardial infarction[4] or stroke.

REFERENCE RANGE

CoQ10
(µg/mL)

0.36-1.59

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess dietary intake of CoQ10.**
- Dietary sources of CoQ10 include animal products such as beef, pork or chicken, and vegetables such as spinach, cauliflower and broccoli.

✓ **Consider CoQ10 supplementation.**
- If currently taking, consider adjusting dosage and retest in 1-2 months.

✓ **Assess ApoA1 and/or HDL levels.**
- If low, consider treatment with niacin or fenofibrate therapy.

References

1. Caso G et al. Effect of coenzyme Q10 on myopathic symptoms in patients treated with statins. *Am J Cardiol.* 2007; 99: 1409-1412.
2. Mabuchi H et al. Effects of CoQ10 supplementation on plasma lipoprotein lipid, CoQ10 and liver and muscle enzyme levels in hypercholesterolemic patients treated with atorvastatin: A randomized double-blind study. *Atherosclerosis*. 2007; 195: e182-e189.
3. Toyama K et al. Rosuvastatin combined with regular exercise preserves coenzyme Q10 levels associated with a significant increase in high-density lipoprotein cholesterol in patients with coronary artery disease. *Atherosclerosis*. 2011; 217: 158-164.
4. Dadabayev AR et al. Apolipoprotein A1 regulates coenzyme Q10 absorption, mitochondrial function, and infract size in a mouse model of myocardial infarction. *J Nutr.* 2014; 144: 1030-1036.

Vitamin D, 25 OH

CPT Code 82306
Sample Type Serum

Order Code C339
Tube Type Tiger Top

Description

Vitamin D is a fat-soluble vitamin naturally present in some foods, but the primary source is synthesis within the body after exposure to sunlight[1]. Vitamin D has various roles within the body, but primarily regulates the absorption of calcium in the gut maintaining adequate serum calcium and phosphate concentrations that contribute to mineralization of bone[2-4].

Clinical Use

The Vitamin D, 25-OH test may be used to determine the levels of Vitamin D in blood, particularly in individuals with bone weakness or malformation or those with impaired calcium metabolism. The test may also be used to monitor Vitamin D levels in individuals with conditions that impair fat absorption.

Clinical Significance

- Vitamin D deficiency is implicated in increased risk for CVD[5,6].
- Vitamin D deficiency is associated with an increased risk for hypertension, common cancers, autoimmune diseases and infectious diseases[7].
- Vitamin D is critical for the maintenance of healthy bones, and deficiency can cause osteoporosis, muscle weakness and muscle wasting[7,8,9].

RELATIVE RISK

Status of Vitamin D Sufficiency
Vitamin D, 25 OH
(ng/mL)

30.0-80.0 Sufficient
10.0-29.9 Insufficient 80.1-100.0 Excess
<10.0 Deficient >100.0 Potential Toxicity

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess dietary intake of vitamin D.**
- If not at goal, consider vitamin D-rich foods such as cheese, vitamin D-fortified milk, shiitake and button mushrooms (dried by sunlight), or supplementation with vitamin D3 (cholecalciferol) or vitamin D2 (ergocalciferol).

✓ **Consider an increase in direct sun exposure to 10-15 minutes a day.**

References

1. Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest.* 2006; 116: 2062-2072.
2. Holick MF. Vitamin D: Photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 6th ed. Washington, DC: American Society for Bone and Mineral Research, 2006: 129-137.
3. Bouillon R. Vitamin D: From photosynthesis, metabolism, and action to clinical applications. In: DeGroot LJ, Jameson JL, eds. Endocrinology. Philadelphia: W.B. Saunders, 2001: 1009-1028.
4. Dusso AS et al. Vitamin D. *Am J Physiol Renal Physiol.* 2005; 289: F8-F28.
5. Grandi NC et al. Vitamin D and cardiovascular disease: Systematic review and meta-analysis of prospective studies. *Prev Med*. 2010; 51: 228-233.
6. Wang TJ et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation.* 2008; 117: 503-511.
7. Holick MF and Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. *Am J Clin Nutr.* 2008; 87 (suppl): 1080S-1086S.
8. Bischoff-Ferrari HA et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower extremity function in both active and inactive persons aged > or = 60 y. *Am J Clin Nutr.* 2004; 80: 752-758.
9. Visser M et al. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): The Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab.* 2003; 88: 5766-5772.

AspirinWorks®

CPT Code 84431/82570
Sample Type Urine

Order Code C922
Tube Type Yellow Top and Cherry Red/Yellow Top

Description

AspirinWorks® is an enzyme-linked immunoassay (ELISA) to determine levels of 11-dehydrothromboxane B_2 (11-dhTXB$_2$) in urine which aids in the qualitative detection of aspirin effect in apparently healthy individuals post-ingestion.

Clinical Use

The AspirinWorks® test may be used to assess clotting risk in individuals on aspirin therapy.

Clinical Significance

- The effectiveness of aspirin therapy varies from individual to individual. Aspirin-insensitive individuals are twice as likely to have a cardiovascular event[1].
- High levels of 11-dhTXB$_2$ are associated with increased risk of heart attack and cardiac death in aspirin-treated patients[1].
- Hyperlipidemia and diabetes are associated with a diminished response to aspirin[2-4].
- 11-dhTXB$_2$ levels demonstrate a dose-related effect of aspirin treatment and have been shown to correlate with a Framingham Risk Score[5,6].

REFERENCE RANGE

Status of Aspirin Effect
11-dhTXB$_2$
(pg/mg creatinine)

≤1500 ASA Effect

>1500 Lack of ASA Effect

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

- ✓ **If not at goal, confirm that aspirin was ingested and/or patient compliance.**
- ✓ **Assess concomitant ibuprofen or NSAID use, LDL-C levels, and risk for pre-diabetes/diabetes.**
  - These can reduce the effectiveness of aspirin therapy.
- ✓ **If not at goal, and all of the aforementioned have been addressed, consider adjusting aspirin dosage and retest in 2-3 weeks.**
  - If not at goal following retest, consider clopidogrel therapy as the patient may be non-responsive to aspirin therapy.

References

1. Eikelboom JW et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation*. 2002; 105:1650-1655.
2. Friend M et al. Platelet responsiveness to aspirin in patients with hyperlipidaemia. *BMJ*. 2003; 326: 82-83.
3. Markuszewski L et al. Reduced blood platelet sensitivity to aspirin in coronary artery disease: Are dyslipidaemia and inflammatory states possible factors predisposing to sub-optimal platelet response to aspirin? *Basic Clin Pharmacol Toxicol*. 2006; 98: 503-509.
4. DiChiara J et al. The effect of aspirin dosing on platelet function in diabetic and non-diabetic patients: An analysis from the Aspirin-Induced Platelet Effect (ASPECT) study. *Diabetes*. 2007; 56: 3014-3019.
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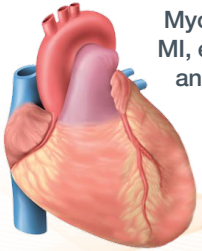
6. Faraday N et al. Relation between atherosclerosis risk factors and aspirin resistance in primary prevention population. *Am J Cardiol*. 2006; 98: 774-779.

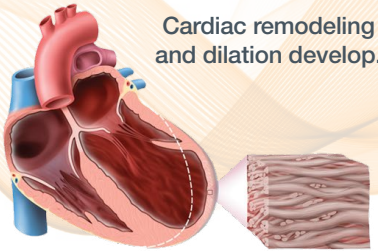
Other Advanced Cardiovascular Tests

Cleveland HeartLab, Inc. also offers other advanced cardiovascular testing that are additive and complementary to our inflammatory and advanced lipid testing.

Some of these tests are reviewed on the following pages.



Myocardial injury (hypertension, MI, etc.) triggers an inflammatory and wound-healing response.



Cardiac remodeling and dilation develop.

Other advanced cardiovascular tests include:

- **Galectin-3**
- **NT-proBNP**

For a complete listing, see the test menu on our website.

Galectin-3 (Gal-3)

CPT Code 82777
Order Code C315
Sample Type Serum/EDTA Plasma
Tube Type Tiger Top/Lavender Top

Description

Galectin-3 is one of the most widely studied galectins, a family of soluble B-galactoside-binding lectins that play a regulatory role in inflammation[1]. Galectin-3 affects the synthesis of matrix compounds, such as type I collagen[2]. When cardiac tissue is injured, macrophages infiltrate the tissue and secrete galectin-3, which promotes collagen synthesis and ultimately leads to cardiac fibrosis and adverse cardiac remodeling[3].

Clinical Use

The galectin-3 test may be used to identify individuals at risk of future chronic heart failure due to hypertension.

Clinical Significance

- Elevated levels of galectin-3 in hypertensive individuals may suggest increased inflammation, collagen deposition and fibrosis that can lead to adverse cardiac remodeling[3].
- Galectin-3 levels may be used to guide the selection of medications in hypertensive individuals, as ACE inhibitors and ARBs have been shown to more effectively reduce left ventricular mass[4].

RELATIVE RISK

Galectin-3
(ng/mL)

<17.9 Low
17.9-25.9 Moderate
≥26.0 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess blood pressure.**
- If abnormal, consider switching current hypertensive medication to an ACE inhibitor or ARB to prevent/reduce adverse cardiac remodeling[5] or titrate dose if currently taking either drug.

✓ **Assess the presence of conditions associated with organ fibrosis, cancer, human anti-mouse antibodies or rheumatoid factor, or high levels of gamma globulins (>2.5 g/dL) as these may contribute to abnormal galectin-3 results.**

References

1. Rubinstein N et al. The role of galectins in the initiation, amplification and resolution of the inflammatory response. *Tissue Antigens*. 2004; 64: 1-12.
2. Sharma UC et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004; 110: 3121-3128.
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4. Meredith PA et al. From hypertension to heart failure – are there better primary prevention strategies? *J Renin Angiotensin Aldosterone Syst*. 2006; 7: 64-73.
5. Dahlof B et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): A randomized trial against atenolol. *Lancet*. 2002; 359; 1004-1010.

N-Terminal proBNP (NT-proBNP)

CPT Code 83880
Sample Type Serum

Order Code C125
Tube Type Tiger Top

Description

During periods of increased volume or pressure overload, cardiomyocytes synthesize proBNP, a prohormone, which is cleaved to release BNP (an active hormone) and NT-proBNP (an inactive fragment) into the bloodstream.

Clinical Use

A NT-proBNP test may be used to assess myocardial stretch and support risk stratification of individuals with stable CAD or acute heart failure.

Clinical Significance

- NT-proBNP is co-secreted in quantities directly proportional to BNP, but has a much longer half-life[1,2].
- NT-proBNP levels positively correlate with New York Heart Association (NYHA) function class of dyspnea and echocardiographic findings[3].
- NT-proBNP levels fall in tandem with treatment of decompensated HF[4,5].
- In hospitalized patients with decompensated HF, changes in NT-proBNP levels were the strongest independent predictor of death or hospital readmission in the ensuing 6 months[5,6].

RELATIVE RISK

NT-proBNP
(pg/mL)

<75 yrs. old	≥75 yrs. old
≤125 Low	≤125 Low
	126-450 Moderate
>125 High	>450 High

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

✓ **Assess exercise regimen.**
NOTE: Younger individuals may have slightly elevated levels due to increased load on the heart with chronic exercise.

✓ **Assess cardiac function.**
- If abnormal, consider diuretics or nitrates to reduce preload, or ACE inhibitor or ARB therapy to improve cardiac function.

References

1. Pemberton CJ et al. Deconvolution analysis of cardiac natriuretic peptides during acute volume overload. *Hypertension*. 2000; 36: 355-359.
2. Kroll MH et al. Using single-compartment ratio model to calculate half-life, NT-proBNP as an example. *Clin Chim Acta*. 2007; 380: 197-202.
3. Song BG et al. Correlation between levels of N-terminal pro-B-type natriuretic peptide and degrees of heart failure. *Korean J Intern Med*. 2005; 20: 26-32.
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5. Bettencourt P. NT-proBNP and BNP: Biomarkers for heart failure management. *Eur J Heart Fail*. 2004; 6: 359-363.
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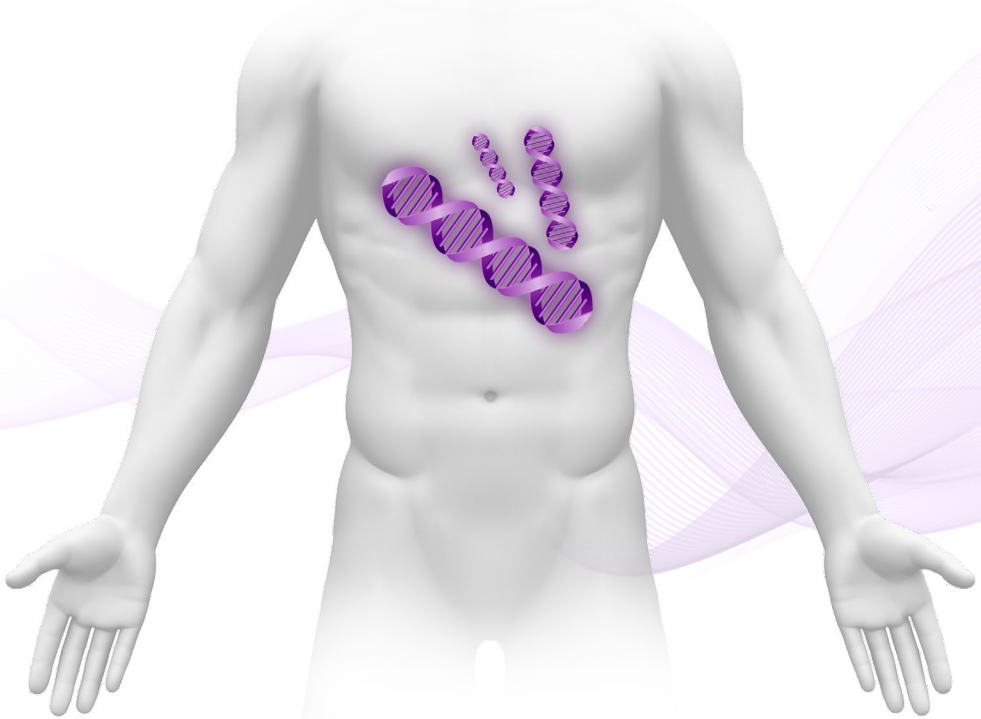
Genetic Tests

Cleveland HeartLab, Inc. also offers genetic tests which are reviewed on the following pages.



Genetic tests include:

- **CYP2C19**
- **Apolipoprotein E**
- **MTHFR**

For a complete listing, see the test menu on our website.

CYP2C19

CPT Code 81225
Sample Type EDTA Whole Blood

Order Code C603
Tube Type Lavender Top

Description

CYP2C19 is a member of the cytochrome P450 family of enzymes involved in the metabolism and bioactivation of drugs. In particular, CYP2C19 is integral for the generation of the active form of clopidogrel (Plavix®), which is prescribed in a prodrug form. This prodrug is converted by CYP2C19 to the active form in the liver. Several variants of CYP2C19 have been identified which have an impact on its ability to metabolize drugs.

Depending on their CYP2C19 genotype, an individual may be a poor metabolizer, an intermediate metabolizer, an extensive/normal metabolizer, or an ultra-rapid metabolizer. The frequency of the metabolizer classes differs by ethnic background, with 14-20% of Asians, 4% of African Americans and 2-4% of Caucasians being poor metabolizers[1-3].

Clinical Use

CYP2C19 testing may be performed on individuals who are candidates for or are currently taking clopidogrel (Plavix®), or those who have a family history of clopidogrel (Plavix®) inefficacy.

Clinical Significance

- In 2010, the FDA announced a boxed warning for clopidogrel (Plavix®) to alert patients and practitioners to the drug's inefficacy in individuals who cannot metabolize the drug to its active form[2].
- Poor metabolizers (loss of CYP2C19 activity) have 2X the risk of having a subsequent adverse cardiac event while receiving treatment with clopidogrel after a myocardial infarction[4].
- Ultra-rapid metabolizers (increased CYP2C19 activity) have a reduced risk of major adverse cardiac events while being treated with clopidogrel[5], but are at an increased risk of bleeding[6].

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

Phenotype	Genotype†	Interpretation	Treatment Consideration
Poor Metabolizer	**Two non-functional alleles (*2/*2 or *2/*3 or *3/*3)**	Poor metabolizers do not effectively convert the drug to an active metabolite and exhibit poor anti-platelet responsiveness.	Consider a higher dosage of clopidogrel (Plavix®) or an alternative therapy.
Intermediate Metabolizer	**One WT and one non-functional allele (WT/*2 or WT/*3)**	Intermediate metabolizers convert the drug to an active metabolite at a slower rate than a normal metabolizer and exhibit decreased responsiveness to the drug.	
Normal (Extensive) Metabolizer	**No mutations (WT/WT)**	Normal metabolizers effectively convert the drug to an active metabolite.	Consider a standard dosage of clopidogrel (Plavix®).
Ultra-Rapid Metabolizer	**One WT and one hyperactive allele or 2 hyperactive alleles (WT/*17 or *17/*17)**	Ultra-rapid metabolizers convert a higher percentage of the drug to an active metabolite, and have a greater therapeutic response to the drug compared to normal metabolizers. Ultra-rapid metabolizers may produce an adequate platelet response even when lower than normal doses of the drug are used, and are at increased risk of bleeding.	Consider a standard or reduced dosage of clopidogrel (Plavix®) and monitoring the patient for potential bleeding.
Unknown	**One non-functional allele and one hyperactive allele (*2/*17 or *3/*17)**	The metabolizer status is unknown for individuals with this genotype.	Consider an alternative therapy.

† *The Cleveland HeartLab assay identifies the non-functional alleles *2 and *3, and the ultra-rapid allele *17 of the CYP2C19 gene. The presence of less common alleles can not be ruled out by this test.*

References

1. Desta Z et al. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet*. 2002; 41: 913-958.
2. Product Information for Plavix (Sanofi/Aventis US). Label Information, approved Feb 2011. www.accessdata.fda.gov/drugsatfda_docs/label/2011/020839s052lbl.pdf. Accessed June 27, 2013.
3. Shuldiner AR et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009; 302: 849-857.
4. Mega JL et al. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med*. 2009; 360: 354-362.
5. Li Y et al. The gain-of-function variant allele CYP2C19*17: A double-edged sword between thrombosis and bleeding in clopidogrel-treated patients. *J Thromb Haemost*. 2012; 10: 199-206.
6. Sibbing D et al. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation*. 2010; 121: 512-518.

Apolipoprotein E

CPT Code 81401
Order Code C604
Sample Type EDTA Whole Blood
Tube Type Lavender Top

Description

ApoE is an apolipoprotein found in blood that, in association with lipids, forms lipoproteins including very low-density lipoproteins (VLDL). ApoE plays multiple roles in the regulation of lipid and lipoprotein levels in the blood[1]. ApoE serves as a ligand for members of the low-density lipoprotein (LDL) receptor family, and is involved in the removal of lipoproteins from the circulation for excretion in the liver. ApoE is also involved in the formation of chylomicrons and VLDL, and affects the activity of other proteins and enzymes that are involved in lipid metabolism, such as hepatic lipase and lipoprotein lipase[2].

Polymorphisms in the ApoE gene result in three separate alleles encoding three distinct protein isoforms: e2, e3, and e4. There are 6 possible genotypes: e2/e2, e2/e3, e2/e4, e3/e3, e4/e3, and e4/e4. The allelic frequencies differ between ethnic groups, but in general the e3/e3 genotype is the most common, while e2/e4 is the least common[1].

Clinical Use

ApoE testing may be performed on individuals with premature coronary heart disease (CHD) or individuals who have high total cholesterol and triglyceride levels, but are unresponsive to treatment with medication and lifestyle changes.

Clinical Significance

- An individual's ApoE genotype may affect their lipid levels. The e2/e2 genotype is associated with increased triglycerides and reduced total cholesterol, while the e4/e3 and e4/e4 genotypes are associated with increased total cholesterol, triglycerides and LDL cholesterol[3].
- ApoE genotypes have varying impact on risk of cardiovascular disease. Carriers of an e4 allele are at 42% higher risk for CHD[4].
- The ApoE genotype can affect an individual's response to lifestyle modifications. In those with the e2/e2 or e2/e3 genotype, extremely low fat diets can increase small dense LDL levels, and therefore these individuals should have moderate fat restriction[5]. Individuals with the e4/e3 or e4/e4 genotype, on the other hand, respond well to very low fat dietary restrictions[6].
- Responsiveness to treatment with statins is also affected by the ApoE genotype. Individuals with the e2/e2 or e2/e3 genotype respond well to statins[7], while statins are less effective in individuals with the e4/e3 or e4/e4 genotype[8].

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

Genotype	Population Frequency	Interpretation†	Treatment
*e2/e2	1%	Approximately 5% of the people with the ApoE *e2/e2 genotype develop type III hyperlipoproteinemia, which is a rare inherited disorder characterized by increased cholesterol and triglyceride levels, the presence of beta-VLDL, xanthomas, and premature vascular disease.	• Statin therapy[7] (▼LDL-C) • Moderate alcohol intake (▼LDL-C ▲HDL-C) • Moderate (35%) fat diet if elevated triglycerides[5]
*e2/e3	10%	This genotype is associated with lower LDL-C levels and lower risk of coronary heart disease compared to those with the *e3/e3 genotype.	
*e2/e4	2%	This genotype is associated with normal lipid metabolism and low cardiovascular disease risk. However, there is some association of this genotype with type III hyperlipoproteinemia.	• Normal dietary modifications
*e3/e3	62%	This genotype is associated with normal lipid metabolism and low cardiovascular disease risk.	
*e4/e3	20%	These genotypes are associated with a predisposition to elevated total cholesterol levels and slightly elevated LDL-C levels compared to those with the *e3/e3 genotype. Additionally, these genotypes are associated with an increased risk of metabolic syndrome and atherosclerosis along with a slightly higher risk of CHD when consuming a diet high in saturated fat.	• Statin therapy[8] (Limited ▼LDL-C) • Low alcohol intake • Very low fat diet (20%) if elevated LDL-C[6] (▼LDL-C ▼Triglycerides ▼sdLDL)
*e4/e4	5%		

† *Relative risk and interpretations reported for each genotype are associated with cardiovascular risk only. The interpretations should not be used to determine the relative risk of other diseases.*

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2. Siest G et al. Apolipoprotein E: Laboratory determinations and clinical interest. The Handbook of Lipoprotein Testing. 2nd ed. *AAAC Press*; 2000. p. 401-440.
3. Schaefer EJ et al. Effect of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 1994; 14: 1105-1113.
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MTHFR

CPT Code 81291
Sample Type EDTA Whole Blood

Order Code C605
Tube Type Lavender Top

Description

MTHFR (5,10-methylenetetrahydrofolate reductase) is an enzyme involved in the metabolism of folate. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulating form of folate[1]. In turn, 5-methyltetrahydrofolate is involved in the conversion of homocysteine to methionine. MTHFR has an important role in maintaining folate and methionine levels, as well as helping to keep circulating homocysteine levels low. MTHFR is also involved in the methylation pathway, which has multiple, wide-ranging roles in the body, including regulation of gene expression and enzymatic activities[1].

Multiple mutations have been identified within the MTHFR gene. One of the most common and best characterized mutations is the substitution of a T for a C at position 677[2]. There are three possible MTHFR genotypes at this position: the wild type CC, CT or TT. The frequency of the 3 genotypes differs between various populations, and the 677TT genotype is more common among Caucasians and Hispanics in the United States than African Americans. However, roughly 10% of the US population has the MTHFR 677TT genotype[1,3].

Other mutations are also found in the MTHFR gene. Another important mutation is at position 1298, where there is the substitution of a C for an A[1]. There are three possible genotypes at this position: the wild type AA, AC, or CC. Approximately 30% of the population has at least one C allele at position 1298.

Clinical Use

MTHFR testing may be performed on individuals with elevated homocysteine levels, those with a personal or family history of premature cardiovascular disease, and those who have family members with a known MTHFR mutation.

Clinical Significance

- Individuals with the 677CC genotype have:
  - Normal MTHFR enzyme activity
  - Normal levels of folate
  - Normal levels of homocysteine
  - Normal global DNA methylation levels
- Individuals with the 677CT genotype have:
  - Reduced MTHFR enzyme activity (~71% of normal)[4]
  - Normal levels of folate
  - Normal levels of homocysteine
  - Normal global DNA methylation levels
- Individuals with the 677TT genotype have:
  - Greatly reduced MTHFR enzyme activity (~34% of normal)[4]
  - Significantly lower levels of folate, regardless of folate intake[5]
  - Significantly higher levels of homocysteine at low circulating folate levels[5]
  - Significantly reduced global DNA methylation levels at low circulating folate levels[6]

Treatment Considerations

These treatment considerations are for educational purposes only. Specific treatment plans should be provided and reviewed by the treating practitioner.

Sequence		Interpretation	Interpretation/Treatment Consideration
677CC	1298AA	MTHFR enzyme activity is normal	Associated with normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis. Treat other risk factors as appropriate.
677CC	1298AC	MTHFR enzyme activity is slightly decreased	Associated with normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis. Treat other risk factors as appropriate.
677CC	1298CC	MTHFR enzyme activity is decreased	Associated with normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis. Treat other risk factors as appropriate.
677CT	1298AA	MTHFR enzyme activity is slightly decreased	Associated with normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis. Treat other risk factors as appropriate.
677CT	1298AC	MTHFR enzyme activity is slightly decreased	Associated with normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis. Treat other risk factors as appropriate.
677CT	1298CC	MTHFR enzyme activity is considerably decreased	Associated with elevated homocysteine levels as well as an increased risk of coronary artery disease and venous thrombosis, particularly in a setting of low folate status. Homocysteine levels should be assessed. Supplementation with folic acid and vitamins B6 and B12 may be beneficial.
677TT	1298AA	MTHFR enzyme activity is greatly decreased	Associated with elevated homocysteine levels as well as an increased risk of coronary artery disease and venous thrombosis, particularly in a setting of low folate status. Homocysteine levels should be assessed. Supplementation with folic acid and vitamins B6 and B12 may be beneficial.
677TT	1298AC	MTHFR enzyme activity is greatly decreased	Associated with elevated homocysteine levels as well as an increased risk of coronary artery disease and venous thrombosis, particularly in a setting of low folate status. Homocysteine levels should be assessed. Supplementation with folic acid and vitamins B6 and B12 may be beneficial.
677TT	1298CC	MTHFR enzyme activity is greatly decreased	Associated with elevated homocysteine levels as well as an increased risk of coronary artery disease and venous thrombosis, particularly in a setting of low folate status. Homocysteine levels should be assessed. Supplementation with folic acid and vitamins B6 and B12 may be beneficial.

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Contact

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Know your risk.

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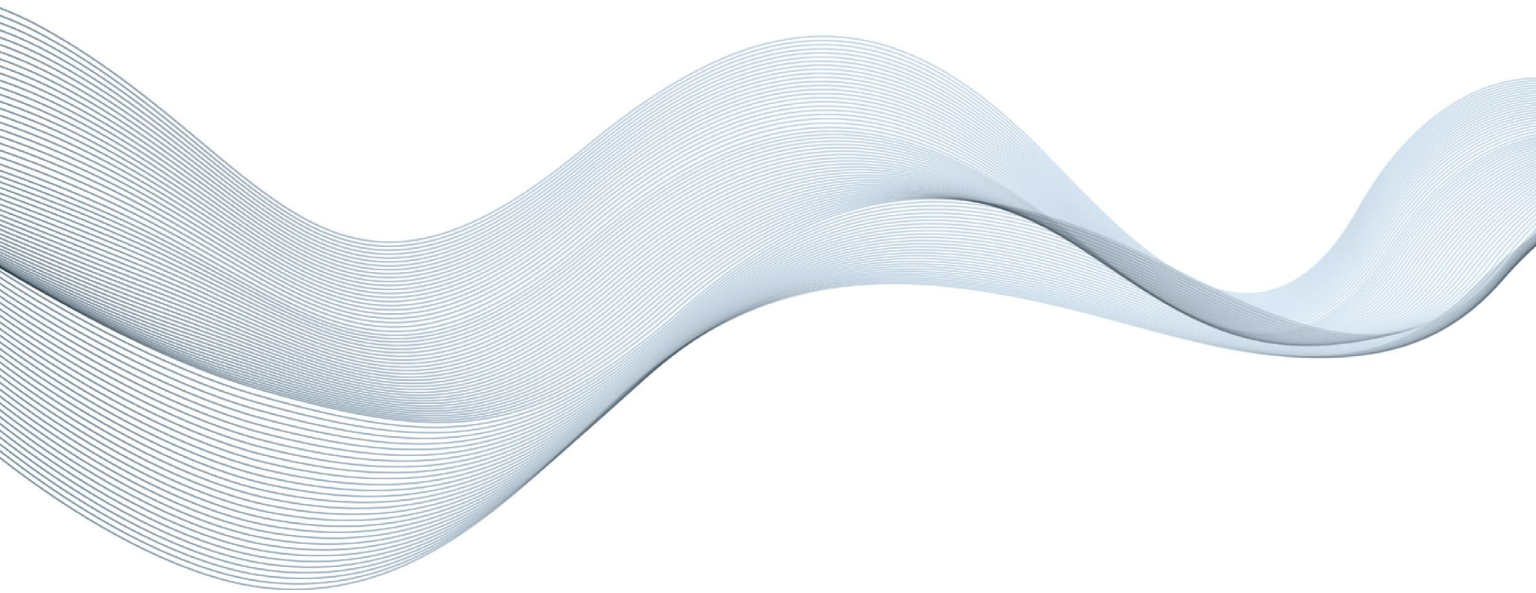

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