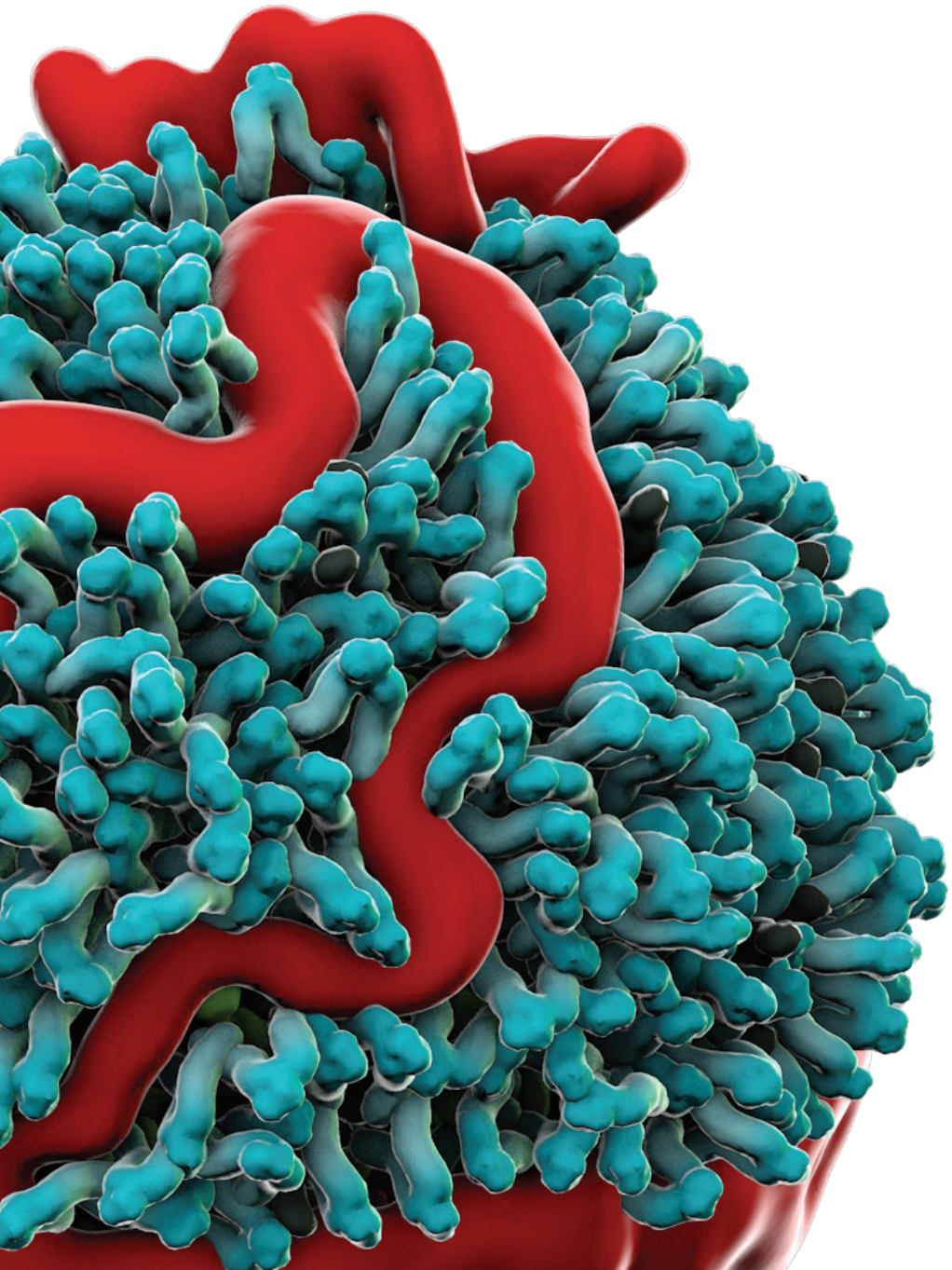
BOSTON HEART CHOLESTEROL BALANCE®

PERSONALIZED TREATMENT OPTIONS BASED ON INDIVIDUAL CHOLESTEROL PRODUCTION AND ABSORPTION



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BOSTON HEART CHOLESTEROL BALANCE®

WHAT IS THE CHOLESTEROL BALANCE TEST?

IMPROVE-IT data highlight cholesterol absorption as an important pathway for the treatment of CVD patients.†

The exclusive Boston Heart Cholesterol Balance® test directly measures the major cholesterol production and absorption markers associated with circulating total cholesterol (TC) by measuring lathosterol, desmosterol, beta-sitosterol, campesterol and cholestanol.

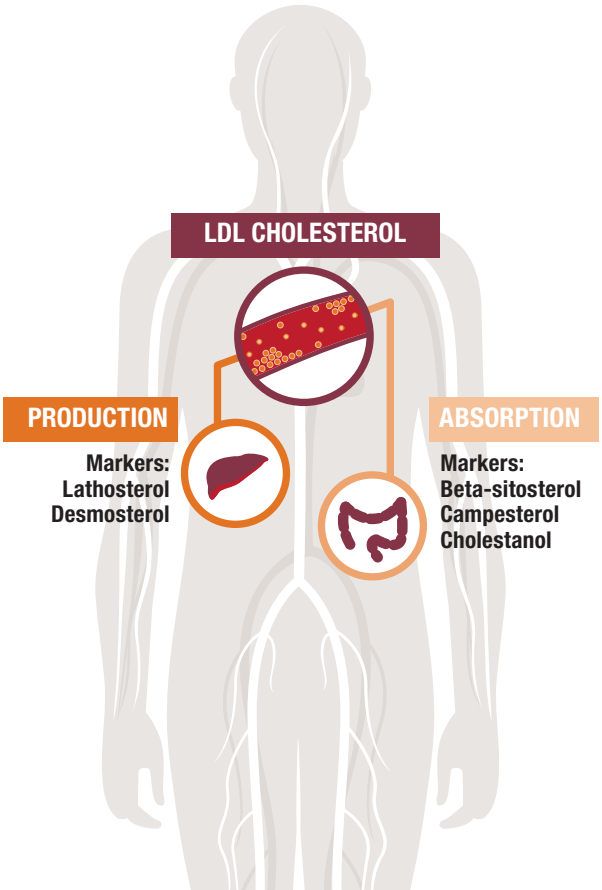
Most (80%) of in vivo cholesterol production goes through a major production pathway, measured by the precursor lathosterol, while the remaining (20%) goes through desmosterol.[1] Additionally, greater than 90% of beta-sitosterol and campesterol (plant sterols) are absorbed into intestinal cells making them excellent markers of cholesterol absorption. These markers of cholesterol production and absorption can be directly measured in plasma or serum and have been shown to be predictors of low-density lipoprotein cholesterol (LDL-C) lowering response to statins and ezetimibe. A third absorption marker, cholestanol, is also measured and serves as a marker of the conversion of cholesterol to the bile acid chenodoxycholate.

Cholesterol production and absorption marker values are always reported out in relative terms as µmol x 100/mmol of TC. If values are very high, absolute concentrations in mg/L are also reported. A visual representation of the Cholesterol Balance Score (the ratio of cholesterol production to cholesterol absorption) facilitates assessment of the patient's cholesterol balance status.

Compared to standard lipid testing and advanced lipid testing by other laboratories, the Cholesterol Balance test provides a more complete characterization of cardiovascular disease (CVD) and helps better guide treatment options to most effectively manage patients to their LDL-C goals.

MEASURES KEY PRODUCTION AND ABSORPTION MARKERS TO HELP DETERMINE EFFECTIVE THERAPY

- Determines whether the patient currently is an optimal, borderline or high producer or absorber of cholesterol—helps explain the causes of a patient's disease.
- Enables providers to prescribe the most effective strategy for lowering LDL cholesterol—dietary changes and statin monotherapy or a combination with a cholesterol absorption inhibitor.
- Tailors therapy based on patient results. Studies show patients with the highest cholesterol production get the greatest benefit from statin therapy in terms of LDL-C lowering and heart disease risk reduction, while those with elevated cholesterol absorption get the least.[2]
- Provides physiologic rationale for patients who are unresponsive to statin or ezetimibe therapy.



BOSTON HEART CHOLESTEROL BALANCE

TREATMENT ALGORITHM FOR CHOLESTEROL BALANCE ABNORMALITIES

1 Determine the patient's LDL-C goal per 2004 National Cholesterol Education Program guidelines.[3]

- <70 mg/dL in patients with established CVD and/or diabetes
- <100 mg/dL in other high risk patients
- <130 mg/dL in moderate or low risk patients

2 Assess if LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C) levels are at goal.

3 Assess for secondary causes of high LDL-C levels.

LIFESTYLE	CONDITIONS	MEDICATIONS
· High dietary intake of saturated fat and cholesterol · High intake of trans fatty acids · Physical inactivity	· Obesity · Hypothyroidism* · Obstructive liver disease · Nephrotic syndrome · Anorexia nervosa	· Cyclosporin · Glucocorticoids · Progestins · Androgenic steroids

**Condition must be optimally controlled to successfully lower LDL-C.*

4 Assess for family history of elevated TC or LDL-C levels.

- Collect information about premature (defined as <60 years of age) coronary heart disease in family members, especially parents and siblings.
- Collect information about lipid abnormalities in family members including parents, siblings and offspring as well as any history of cholesterol deposits on the tendons. There are three primary lipid disorders to look for: (1) familial combined hyperlipidemia, (2) familial hypercholesterolemia and (3) phytosterolemia.

	FAMILIAL COMBINED HYPERLIPIDEMIA[4,5]	FAMILIAL HYPERCHOLESTEROLEMIA[5]	PHYTOSTEROLEMIA[6]
Frequency of disorder in families with premature heart disease	· ~15% · Most common cause of elevated LDL-C	· 1%	· 1%
Lipid, lipoprotein, and cholesterol production and absorption markers commonly seen in disorder	· Elevations in either LDL-C or triglycerides (TGs) or both, usually with low high-density lipoprotein cholesterol (HDL-C) and significant increases in apolipoprotein B (apoB), small dense LDL-C (sdLDL-C) and lathosterol	· Very elevated LDL-C (usually >250 mg/dL) with borderline lathosterol, beta-sitosterol or campesterol	· LDL-C usually elevated, with very high beta-sitosterol and campesterol
Pathophysiology	· Overproduction of apoB-100 containing lipoproteins, i.e., VLDL and LDL	· Associated with defects in the LDL receptor, apoB, or proprotein convertase subtilin/kexin type 9 (PCSK9), causing a decreased ability to break down LDL	· Disorder caused by defects in the ATP binding cassette transporters G5 and G8 · Results in retention of phytosterols in the intestinal cell and increased absorption of phytosterols and cholesterol with enhanced delivery into the bloodstream
Risk for premature heart disease	· At increased risk	· At markedly increased risk	· At increased risk
Presence of xanthomas	· Do not usually develop tendon xanthomas	· Often have tendon xanthomas	· May have tendon xanthomas
Treatment	· Often require weight loss and statin therapy to normalize LDL-C	· Often require combination of statin and ezetimibe to normalize LDL-C · Sometimes require resin therapy as well	· Minimal reduction in LDL-C with diet low in animal fat and cholesterol or statin therapy · Marked reduction in LDL-C, beta-sitosterol and campesterol with ezetimibe · May need to add resin therapy

5 Interpret Cholesterol Balance test results.

Clinical implications of elevated LDL-C and cholesterol production and absorption markers

PARAMETER	CLINICAL SIGNIFICANCE	BOSTON HEART OPTIMAL GOAL
Elevated LDL-C[3]	· LDL-C levels (>160 mg/dL) are a significant risk factor for CVD · The more LDL-C is decreased by therapy, the greater is the decrease in CVD risk	· With established CVD <70 mg/dL · Without established CVD <100 mg/dL
Elevated Lathosterol[5] Normalized lathosterol level is used to categorize cholesterol production status	· Elevated levels are associated with increased cholesterol *production* · Elevated levels are often seen in insulin resistance, obesity and familial combined hyperlipidemia	· <90 µmol x 100/mmol of TC
Elevated Desmosterol[7]	· Elevated levels are associated with increased cholesterol *production or decreased conversion of desmosterol to cholesterol* · Moderate elevations have been associated with a significant increased risk of cognitive decline with aging · Very high levels are associated with desmosterolosis a disorder caused by a mutation in the 3β-hydroxysterol Δ^{24}-reductase gene – Mutations result in a significant lack of conversion of desmosterol to cholesterol and may be associated with significant neurologic disease	· <70 µmol x 100/mmol of TC
Elevated Beta-Sitosterol and Campesterol[6,8,9] The higher value of normalized beta-sitosterol or campesterol is used to categorize absorption status	· Elevated levels are associated with increased cholesterol *absorption* and have been shown to be an independent risk factor for CVD in population studies · Very high levels are associated with phytosterolemia, a disorder caused by defects in the ATP binding cassette transporters G5 and G8 – This disorder results in retention of phytosterols in the intestinal cell and increased absorption of phytosterols and cholesterol with enhanced delivery into the bloodstream	· Beta-sitosterol: <100 µmol x 100/mmol of TC · Campesterol: <150 µmol x 100/mmol of TC
Elevated Cholestanol[10,11]	· High levels have been shown to be an independent risk factor for CVD in population studies · Very high levels are associated with cerebrotendinous xanthomatosis, a disorder caused by a defect in the sterol 27-hydroxylase gene – Mutations result in a defect in converting cholesterol to chenodeoxycholic acid, a major bile acid – Associated with tendinous xanthomas, normal or elevated cholesterol levels and cholestanol deposits in tendons and the brain, which if untreated can lead to seizures and severe neurologic impairment	· Boston Heart does not report a normalized value · Boston Heart only reports the absolute concentration if it is very high at >15.0 mg/L, which will be reported in the report interpretation

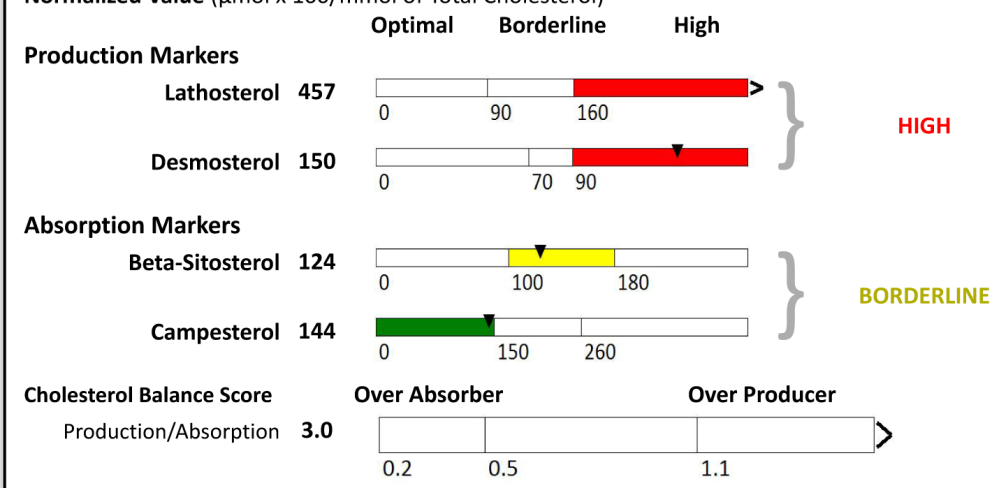
SAMPLE LABORATORY REPORT RESULTS

On the test report, values for lathosterol, desmosterol, beta-sitosterol and campesterol are presented and color-coded: optimal (in green); borderline (in yellow); and high (in red). If a marker's absolute value is very high it will be stated in the interpretation section. Refer to section entitled "Use of Very High Absolute Cholesterol Production and Absorption Markers" for reference ranges, clinical significance and treatment considerations.

OVER PRODUCER

Boston Heart Cholesterol Balance® Test[1]

Normalized Value (µmol x 100/mmol of Total Cholesterol)



Interpretation:

· HIGH cholesterol production and BORDERLINE absorption may be associated with elevated LDL-C levels and increased heart disease risk.

· Lathosterol level is very HIGH at 8.6 mg/L, consistent with markedly increased cholesterol production.

· Cholesterol Balance Score is very HIGH at 3.00, consistent with markedly increased cholesterol production.

Consideration:

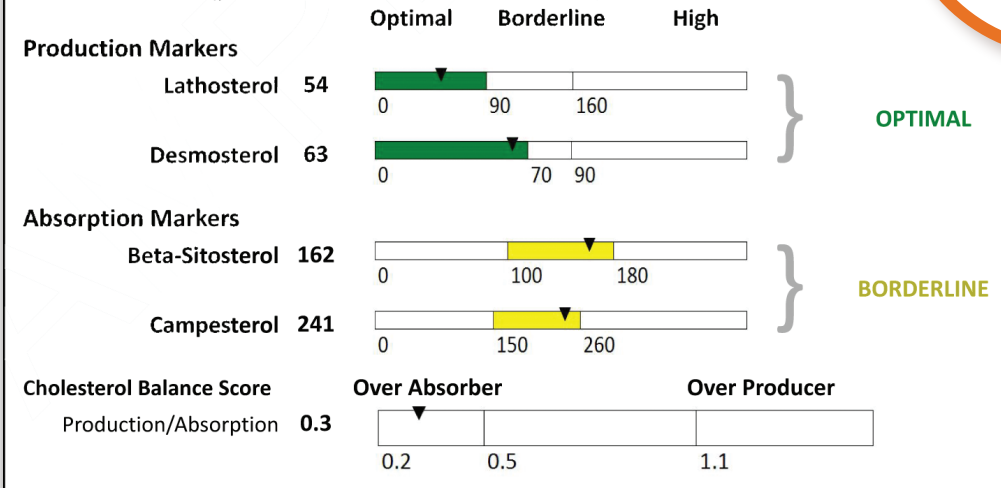
· Lifestyle modification and statin therapy if LDL-C lowering is indicated.

Know if your patient is likely to achieve optimal LDL-C lowering with a statin alone

OVER ABSORBER

Boston Heart Cholesterol Balance® Test[1]

Normalized Value (µmol x 100/mmol of Total Cholesterol)



Interpretation:

· OPTIMAL cholesterol production and BORDERLINE absorption may be associated with elevated LDL-C levels and increased heart disease risk.

Consideration:

· Lifestyle modification and ezetimibe therapy if LDL-C lowering is indicated.

USE OF TEST REPORT VALUES FOR NORMALIZED CHOLESTEROL PRODUCTION AND ABSORPTION MARKERS IN PATIENTS NOT AT LDL-C GOAL

Lathosterol, desmosterol, beta-sitosterol and campesterol values can be improved with appropriate therapy as indicated in the table below if the LDL-C values are not at goal. Dietary modification and weight loss (if indicated) are also beneficial considerations. Generally, patients with:

- Elevated cholesterol production markers (lathosterol and desmosterol) can be treated with a statin, which inhibits cholesterol production.[12]
- Elevated cholesterol absorption markers (beta-sitosterol and campesterol) can be treated with ezetimibe, or the combination of a statin and ezetimibe, if LDL-C lowering is needed. Ezetimibe blocks the absorption of cholesterol in the intestine.[6,8,9]
- Elevated cholesterol production and absorption markers can be treated with both a statin and ezetimibe.
- Markedly elevated cholestanol levels are best treated with chenodeoxycholic acid.[10,11]

PARAMETER/LAB VALUES	POTENTIAL DIAGNOSIS/CLINICAL SIGNIFICANCE All parameters may be associated with elevated LDL-C levels and increased heart disease risk	TREATMENT CONSIDERATION(S) If LDL-C lowering is needed in addition to dietary modification and weight loss (if indicated)
Borderline production with **borderline** absorption	· Borderline cholesterol production · Borderline absorption · Can be seen in patients with familial hypercholesterolemia who have defective LDL clearance	· **Statin** therapy would be best to decrease cholesterol production to optimal range · May add additional LDL-C lowering drugs such as ezetimibe or a bile acid sequestrant if unable to achieve LDL-C goal with statin
Borderline production with **high** absorption	· Borderline cholesterol production · High cholesterol absorption · Pattern seen in patients with phytosterolemia	· The combination of a **statin** and **ezetimibe** would be best to decrease both cholesterol production and absorption into the optimal range · May add additional LDL-C lowering drugs if unable to achieve LDL-C goal with ezetimibe and statin
Borderline production with **optimal** absorption	· Borderline cholesterol production · Optimal cholesterol absorption	· **Statin** therapy would be best to decrease cholesterol production into the optimal range
High production with **borderline** absorption	· High cholesterol production · Borderline cholesterol absorption · Pattern often seen in familial combined hyperlipidemia	· **Statin** therapy would be best to decrease cholesterol production into the optimal range · May add additional LDL-C lowering drugs if unable to achieve LDL-C goal with statin
High production with **high** absorption	· High cholesterol production · High cholesterol absorption	· The combination of a **statin** and **ezetimibe** would be best to decrease both cholesterol production and absorption into the optimal range
High production with **optimal** absorption	· High cholesterol production · Optimal cholesterol absorption	· **Statin** therapy would be best to decrease cholesterol production into the optimal range
Optimal production with **borderline** absorption	· Optimal cholesterol production · Borderline cholesterol absorption	· **Ezetimibe** therapy would be best to decrease cholesterol absorption into the optimal range
Optimal production with **high** absorption	· Optimal cholesterol production · High cholesterol absorption · Pattern often seen in patient on statin therapy when LDL-C is at goal	· **Ezetimibe** therapy would be best to decrease cholesterol absorption into the optimal range
Optimal production with **optimal** absorption	· Optimal cholesterol production · Optimal cholesterol absorption · Can be seen in patients with familial hypercholesterolemia who have defective LDL clearance	· **Statin, ezetimibe** and **colesevelam** therapy would be best to enhance LDL clearance

USE OF VERY HIGH TEST REPORT VALUES FOR ABSOLUTE CHOLESTEROL PRODUCTION AND ABSORPTION MARKERS

Absolute cholesterol production and absorption values will only be reported if they are very high and will be stated in the interpretation section.

STEROL AND REFERENCE RANGE	CLINICAL SIGNIFICANCE	TREATMENT CONSIDERATION(S) If LDL-C lowering is needed in addition to dietary modification and weight loss (if indicated)
Lathosterol >7.0 mg/L	· Markedly increased cholesterol production · May be associated with elevated LDL-C levels and increased heart disease risk	· **Statin** therapy
Desmosterol >5.0 mg/L	· Indicates either increased cholesterol production or decreased conversion of desmosterol to cholesterol · May be associated with elevated LDL-C levels and increased heart disease risk	· **Statin** therapy
Beta-sitosterol >7.0 mg/L	· Markedly increased cholesterol absorption consistent with phytosterolemia · May be associated with tendon xanthomas, elevated LDL-C levels and increased heart disease risk	· **Ezetimibe** therapy
Campesterol >10.0 mg/L	· Markedly increased cholesterol absorption consistent with phytosterolemia · May be associated with tendon xanthomas, elevated LDL-C levels and increased heart disease risk	· **Ezetimibe** therapy
Cholestanol >15.0 mg/L	· Decreased conversion of cholesterol to the bile acid chenodeoxycholate · May be associated with tendon xanthomas, neurologic disease and cerebrotendinous xanthomatosis	· **Chenodeoxycholate** therapy to prevent neurologic disease

USE OF TEST REPORT VALUE FOR THE CHOLESTEROL BALANCE SCORE

This production/absorption score is a marker of cholesterol balance. It reflects the relative amount of cholesterol production to cholesterol absorption and is calculated using weighted values of normalized lathosterol, desmosterol, beta-sitosterol and campesterol. The Cholesterol Balance Score should be considered in treatment decisions if further LDL-C lowering is indicated.

PARAMETER/LAB VALUES	POTENTIAL DIAGNOSIS/CLINICAL SIGNIFICANCE	TREATMENT CONSIDERATION(S) If LDL-C lowering is needed in addition to dietary modification and weight loss (if indicated)
Low Score: <0.5	· Over absorber · Markedly decreased relative cholesterol production · May be associated with elevated LDL-C levels and increased heart disease risk	· Addition of **ezetimibe** may be needed
Score: 0.5-1.1	· Neutral score · Refer to normalized production and absorption values	· Addition of **statin** with **ezetimibe** may be needed
High Score: >1.1	· Over producer · Increased relative cholesterol production · May be associated with elevated LDL-C levels and increased heart disease risk	· Addition of **statin** may be needed

†IMPROVE-IT DATA AND BOSTON HEART

IMPROVE-IT data highlight cholesterol absorption as an important pathway for the treatment of CVD patients. IMPROVE-IT is the first trial demonstrating incremental clinical benefit when adding a non-statin agent (ezetimibe) to statin therapy, and the results indicate that:

- Lowering LDL-C with ezetimibe reduces CVD events
- An average LDL-C of 53 mg/dL is even better than an LDL-C value of 70 mg/dL for CVD risk reduction
- Ezetimibe is well tolerated, safe, and does not increase the risk of cancer.

Boston Heart is the *only* company that offers a complete understanding of cholesterol production and absorption pathways through the proprietary Boston Heart Cholesterol Balance test. Our interpretations and treatment considerations are clinically accurate and help inform the right LDL-lowering treatment at the right dose at the right time.

Additionally, lifestyle modification is still the first line of defense in lowering the risk of heart disease. The Boston Heart Lifestyle Program is the first scientifically-designed, evidence-based approach to personalizing nutrition for heart health improvement and weight loss. The Program engages and supports patients, when warranted, to take actions that can impact modifiable risk factors using personalized eating and exercise strategies.

IMPROVE-IT also highlighted a well-known issue of patient compliance with statin therapy — 42% stopped taking either their statin or the statin/ezetimibe combination during the course of the trial. The Boston Heart Statin Induced Myopathy (SLCO1B1) Genotype test can assist you in choosing the right formulation of statin and appropriate dose by providing insight into the patient's genetic risk of statin induced myopathy.[13]

For more information about the Boston Heart Cholesterol Balance test, contact your sales representative or call 877.425.1252.

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