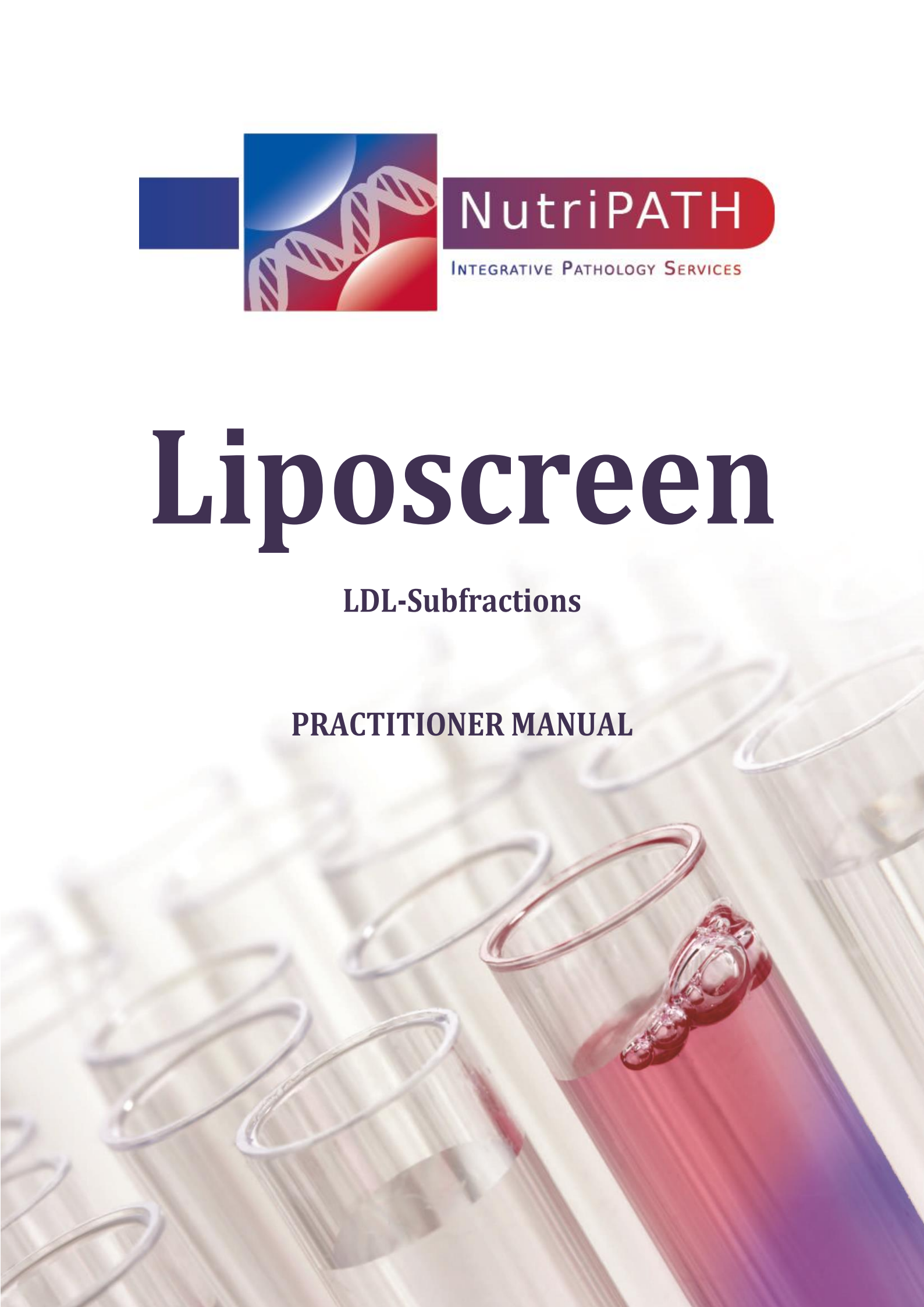
NutriPATH

INTEGRATIVE PATHOLOGY SERVICES

# Liposcreen

## LDL-Subfractions

## PRACTITIONER MANUAL



**NutriPATH Pty. Ltd.**

18a Harker Street, Burwood, VIC, 3125, Australia

Phone: 1300 688 522 (Australia)

+61 3 9880 2900 (International)

Fax: +61 3 9880 2999

Web: www.nutripath.com.au

v. 1.0

# Contents

# Introduction to LIPOSCREEN LDL-Subfractions

- It is estimated over 380,000 Australians have had a heart attack at some time in their lives.
- Each year around 55,000 Australians suffer a heart attack. This equates to one heart attack every 10 minutes.
- Heart attack claimed 9,811 lives in 2011, or on average 27 each day.
- CVD kills one Australian every 12 minutes.
- CVD affects one in six Australians or 3.7 million.
- 50% of people who are diagnosed with coronary artery disease (CAD) have normal lipid levels.
- Only 30% of all heart attacks can be explained on the basis of total cholesterol measurement.

### *Do high cholesterol levels automatically mean an increased heart attack risk?*

Approximately 50% of patients without heart attack have higher cholesterol levels and a considerable portion of heart attack patients have low cholesterol levels.

Individual differences exist in particular with respect to the LDL levels and in the size distribution of the LDL particles.

***The small LDL particles in particular have a very high atherogenic potential.*** Therefore, it is less important how much cholesterol a patient has, but which type of cholesterol is elevated and which size distribution the cholesterol particles have. These are the parameters the risk assessment and the therapy depend on.

Demographic studies have shown that the classical lipid profiles of patients with coronary artery diseases do not significantly differ from those of healthy persons. Nearly 50% of the persons who develop a cardiac disease have 'normal' cholesterol levels. LDL cholesterol, the lipid that is most frequently associated with cardiovascular diseases, is heterogeneous (different) and consists of up to seven subfractions.

Small dense LDL particles (subfractions) are associated with a three times higher risk of cardiovascular diseases versus large circulating LDL particles which are less atherogenic.

Traditional lipid profiles do not identify the risk of cardiovascular diseases that are caused by the presence of small dense LDL and IDL particles. Dangerous LDL particles may hide behind normal cholesterol levels and, conversely, elevated cholesterol levels may not inevitably have to be associated with a heart attack risk.

Patients supposedly in need of treatment (on account of high cholesterol levels) may turn out to be not at risk, whereas persons supposed to be healthy (on account of normal cholesterol levels) may turn out to be risk patients.

# The LIPOSCREEN LDL-Subfractions Test

Liposcreen is an in-vitro diagnostic test for separating and measuring cholesterol in lipoprotein fractions and lipoprotein subfractions.

It is a new procedure that determines the actual heart attack risk by means of a differentiated analysis of HDL and LDL subfractions.

It can:

- Identify and differentiates all cholesterol particles quantitatively by their size for the first time.
- Differentiate the highly atherogenic, small, dense LDL and IDL from the large, less atherogenic LDL and VLDL and the protective HDL.
- Determine IDL fractions.

Liposcreen separates and quantifies all lipoprotein subfractions including the large, less atherogenic LDL-1 and LDL-2 and the small, highly atherogenic LDL-3 to LDL-7.

Liposcreen measures the cholesterol level in mmol/L in every lipoprotein sub-fraction from VLDL to HDL. In all 14 parameters - total cholesterol, total LDL, HDL, VLDL, 3 IDL fractions, 7 LDL fractions.

The test also measures VLDL and IDL cholesterol linked with type III dyslipidaemia and associated hyperlipoproteinaemias.

## Benefits of the Liposcreen Profile

- Measures the amount of cholesterol in mmol/L in each lipoprotein fraction and sub-fraction from VLDL to HDL (14 parameters in total).
- Values outside the reference range flagged in red.
- Easy to interpret colour coded profile differentiates normal, Type A lipid profile from an abnormal, non-Type A profile.
- Identifies the highly atherogenic small dense LDL and IDL from the large, less atherogenic LDL and VLDL and the protective HDL.
- Clinical utility for screening, treatment decision and monitoring of lipid disorders associated with coronary artery disease (CAD) risk.

## Clinical benefit

Clinical benefit for screening, treatment decisions and monitoring of lipid disorders associated with risks of coronary artery diseases:

- Conventional lipid tests do not convey the CAD risk associated with the small dense LDL or IDL subfractions, and therefore offers determination of the patient's true atherogenic risk.
- These risks could be present even when other lipid risk factor (total cholesterol, LDL and HDL cholesterol and triglycerides) are normal.
- Carefully targeted therapy.

- Reducing possibly counter-productive drugs.
- Therapy control: particularly important since sometimes, in case of administration of cholesterol-lowering drugs, the total cholesterol decreases, but the atherogenic LDL particles accumulate. In this case, a normal LDL control would suggest a therapy success.

## Indications

The medical community recognises lipid testing as appropriate for evaluating atherosclerotic cardiovascular disease. Conditions in which lipid testing may be indicated include:

- Assessment of patients with atherosclerotic cardiovascular disease.
- Evaluation of primary dyslipidaemia.
- Any form of atherosclerotic disease, or any disease leading to the formation of atherosclerotic disease.
- Diagnostic evaluation of diseases associated with altered lipid metabolism, such as nephrotic syndrome, pancreatitis, hepatic disease, hypo and hyperthyroidism.
- Secondary dyslipidaemia, including diabetes mellitus, disorders of gastrointestinal absorption, chronic renal failure.
- Signs or symptoms of dyslipidaemias, such as skin lesions, as follow-up to the initial screen for coronary heart disease (total cholesterol + HDL cholesterol) when total cholesterol is determined to be high (>6.0 mmol/L) plus two or more coronary heart disease risk factors, or an HDL cholesterol < 0.9 mmol/L.

## Clinical Indications

Anyone with two or more of the following risk factors:

**Risk factors that cannot be changed:**

- Increasing age - 84% of people who die of CHD are 65 or older.
- Gender - Men (45 years or older) have a greater risk of heart attack than women (55 years or older).
- Heredity (including race) - Family history of early heart disease (father or brother affected before age 55; mother or sister affected before age 65).

**Risk factors that can be modified by lifestyle change or medications:**

- Tobacco smoke - Smokers and non-smokers exposed to cigarette smoke are at increased risk of heart disease.
- High blood cholesterol and triglycerides
- Total blood cholesterol (> 5.5 mmol/L)
- High LDL cholesterol (> 3.5 mmol/L)
- Low HDL cholesterol (< 1.2 mmol/L)
- Triglycerides (> 2.0 mmol/L)
- Diet - less meat, more fish, vegetables and fruit.
- High blood pressure (BP > 140/90 mmHg )
- Physical inactivity - inactive lifestyle is a risk factor for CHD
- Obesity / overweight (BMI of > 25-29.9)
- Diabetes mellitus

# Lipoprotein Subclasses and CAD Risk

Not all lipoprotein subclasses have the same atherogenic potential.

- Large buoyant LDL-1 and 2 are associated with average CAD risk.
- Presence of small dense LDL-3 through 7 are associated with 3 times greater risk for CAD independent of other risk factors.
- IDL levels above the normal reference range are also associated with increased CAD risk.
- Levels of small VLDL remnants above the normal reference range are also associated with increased CAD risk.
- Levels of HDL cholesterol below 1.0mmol/L are the equivalent of an additional risk factor while levels above 1.5mmol/L are equivalent to a negative risk factor.

# Influence in Diabetes and CVD

Whilst standard lipid profiles are reported as relatively normal, a high frequency of dyslipidaemia is revealed through Liposcreen assessment.

In particular, the observed adverse changes seen are:

- Increase in VLDL subclasses
- Increase in smaller VLDLs
- Shift towards smaller denser LDLs (subfractions)
- Decrease in cardioprotective large HDL
- Increase in non-cardioprotective small HDL
- More men than women had small LDLs
- Men also had higher levels of medium to small LDLs

IDL levels are generally increased in diabetes and generally high in subjects with diabetic nephropathy. IDL levels were historically difficult to measure and as such were not assessed. These are now readily assessed through Liposcreen assessment.

HDL is a heterogeneous substance comprising mainly of two major classes:

- HDL2 Large cardioprotective
- HDL3 Small non-cardioprotective

Elevations of the smaller, less protective HDL subfractions may result in misleading favourable interpretations of the standard lipid profiles. These changes in HDL subclass composition or function may contribute to accelerated vascular damage as seen in diabetes.

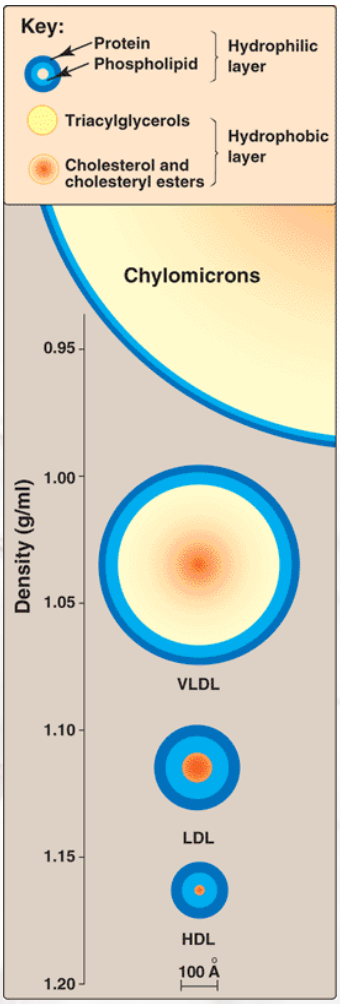
In diabetes there is loss of female cardioprotection when compared to the non-diabetic population. Glycaemia affects HDL subclasses in opposite directions. This is hidden in conventional lipid profiles.

# Lipoprotein Classes

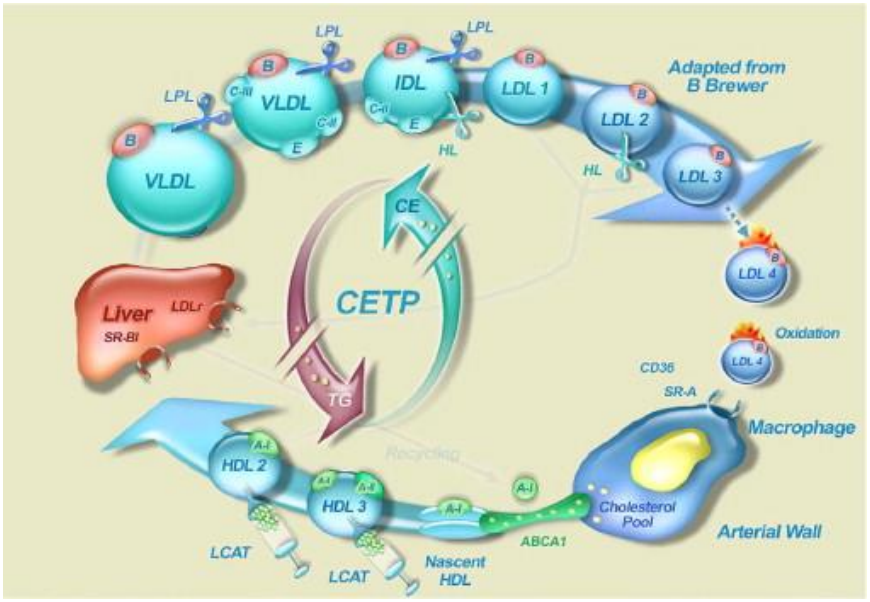
Lipoproteins have been classified into five major classes based on their physical and chemical properties and functionality. All lipoprotein classes contain cholesterol, triglycerides, phospholipids and protein (apoproteins) in varying proportions.

- Chylomicron – Continuum of particle sizes
- VLDL (very low density lipoprotein) – Continuum of particles of varying sizes including remnant particles
- IDL (intermediate density lipoprotein) – Two main subclasses (large and small)
- LDL (low density lipoprotein) - Up to seven subclasses (large buoyant LDL-1 and 2, and small dense LDL-3 through 7)
- HDL (high density lipoprotein) – Two major subclasses (large, HDL-2 and HDL-3)

## Classification of Lipoproteins



# Lipoprotein Metabolism




These lipoprotein classes are heterogeneous consisting of multiple subclasses within each class.

LDL subfraction testing conveys a level of CVD risk not possible with conventional lipid profiles. This risk could be present even with normal lipid levels.

### HDL

Traditionally, HDL has been separated into two major subclasses (HDL2 and HDL3). Depending on the separation method used, three or more subfractions have been reported namely - large HDL, HDL(L), intermediate HDL, HDL(I) and small HDL, HDL(S).

### Oxidised LDL cholesterol

It is mainly the so-called oxidised LDL that forms deposits on the vascular walls. The oxidation of plasma lipoproteins, in particular of LDL cholesterol, induced by oxygen radicals constitutes the main factor of arteriosclerosis.

Oxidised LDL can no longer bind to the LDL-receptors and cannot be cleaved, resulting in distinct cytotoxic effects and an increased transformation from monocytes into macrophages that can fix oxidised LDL by means of a special receptor (scavenger receptor).

Since this receptor is not inhibited by a high intracellular cholesterol level like the normal LDL receptor, cholesterol accumulates in the macrophages which then transform into so-called foam cells. The foam cells again promote connective tissue depositions that result in the formation of atherosclerotic plaques.

The recruitment of T-cells as well as the proliferation of smooth muscle cells provoke an inflammatory process at the vascular wall. This oxidative stress results in premature damaging and increased endothelial permeability.

The fibrous cap of the plaques rich in lipid is weakened by the inflammatory process: the risk of plaque rupture is increasing; thrombus can take place which eventually causes myocardial infarction.

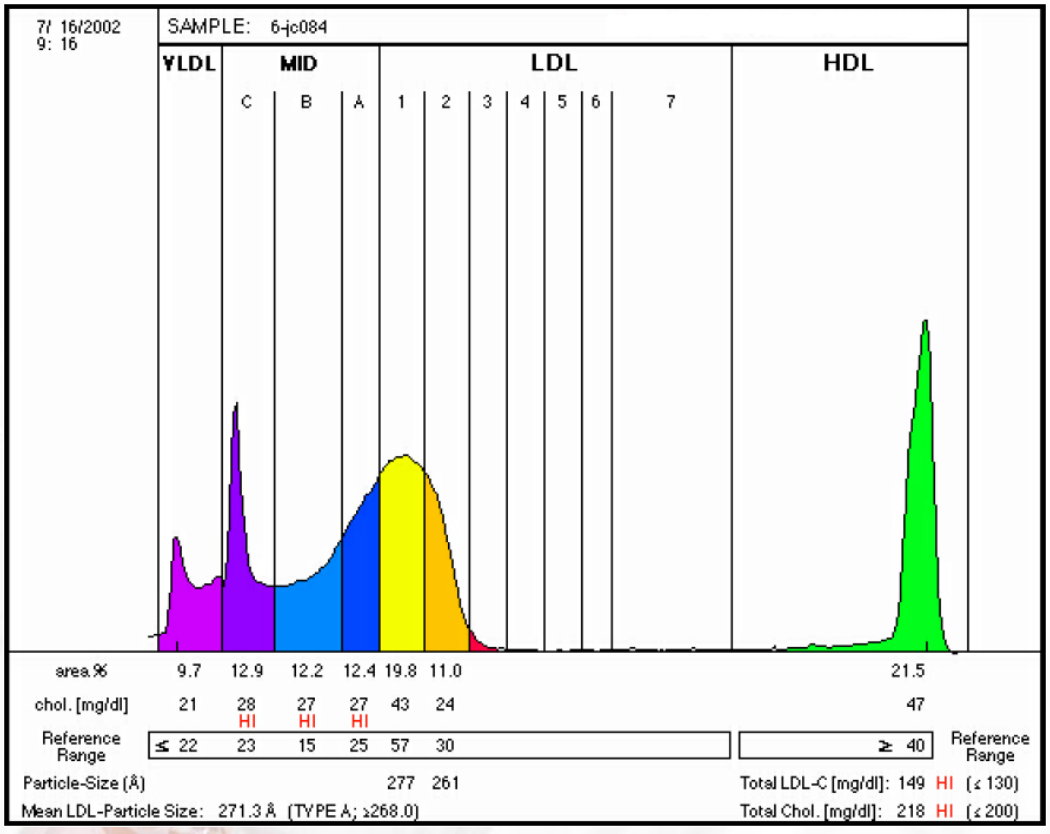
The analytical determination of oxidised LDL provides information about the progress of endothelial damage and what kind of therapeutic measure needs to be taken.

### Other Atherogenic Profiles

Type III dyslipidaemia is a lipid disorder caused by abnormally increased levels of intermediate density lipoprotein (IDL) and remnant lipoproteins.

This highly atherogenic profile can't be identified by other lipid tests and could be misdiagnosed.

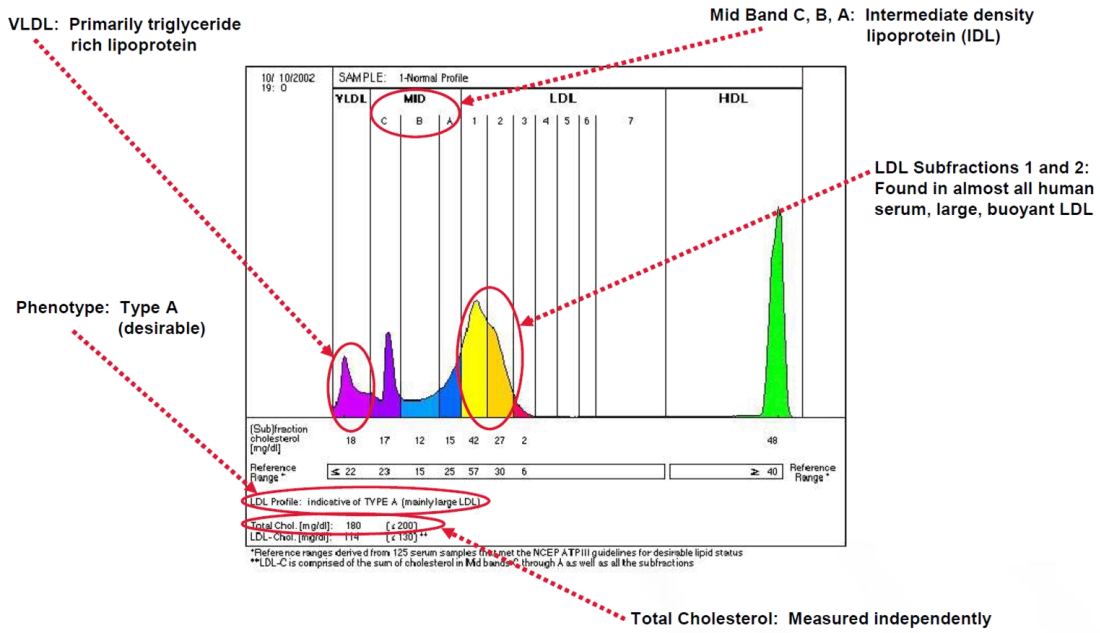
## Type III Dyslipidaemia



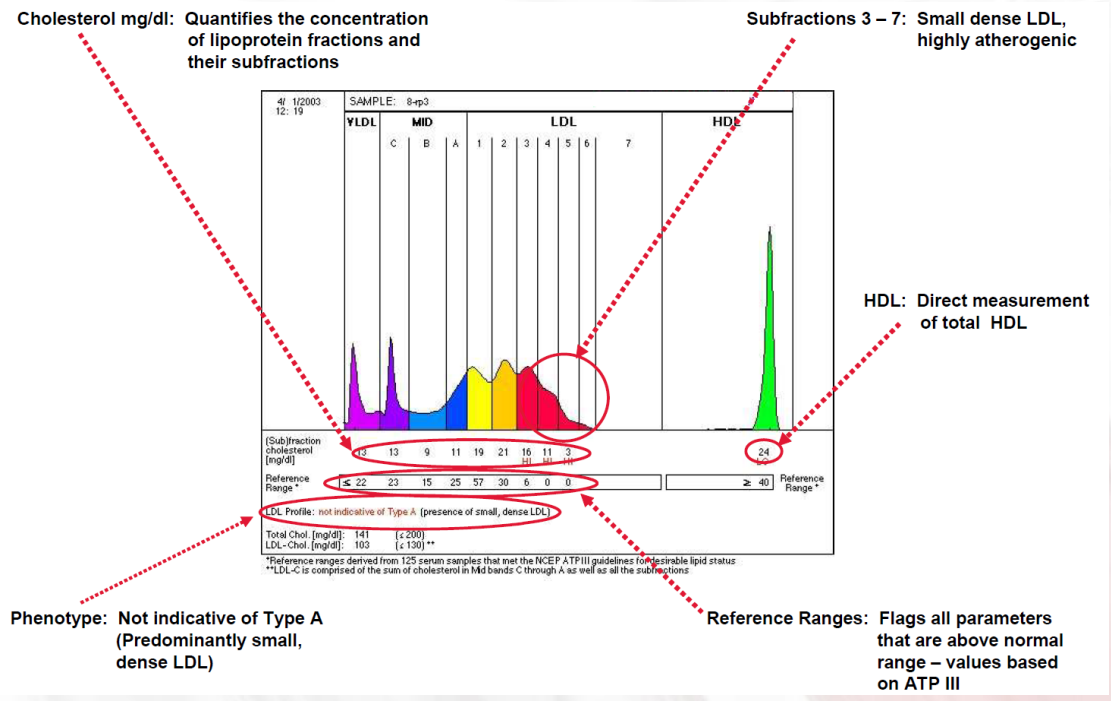

Reference Ranges: Flags all parameters that are above normal range in red text– values based on ATP III subfractions

# Profiles

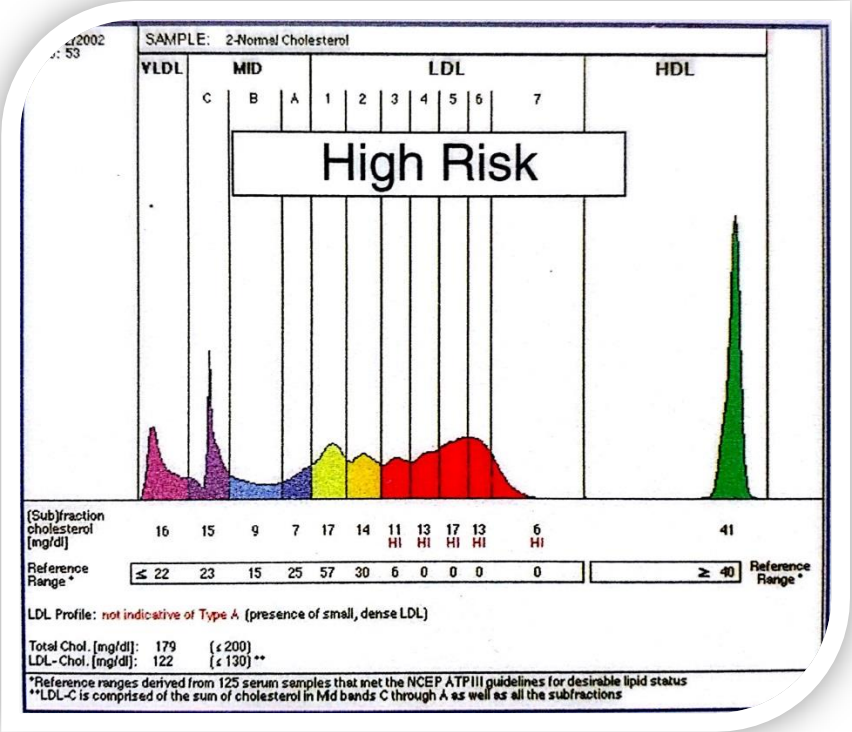
## NORMAL Type A Profile



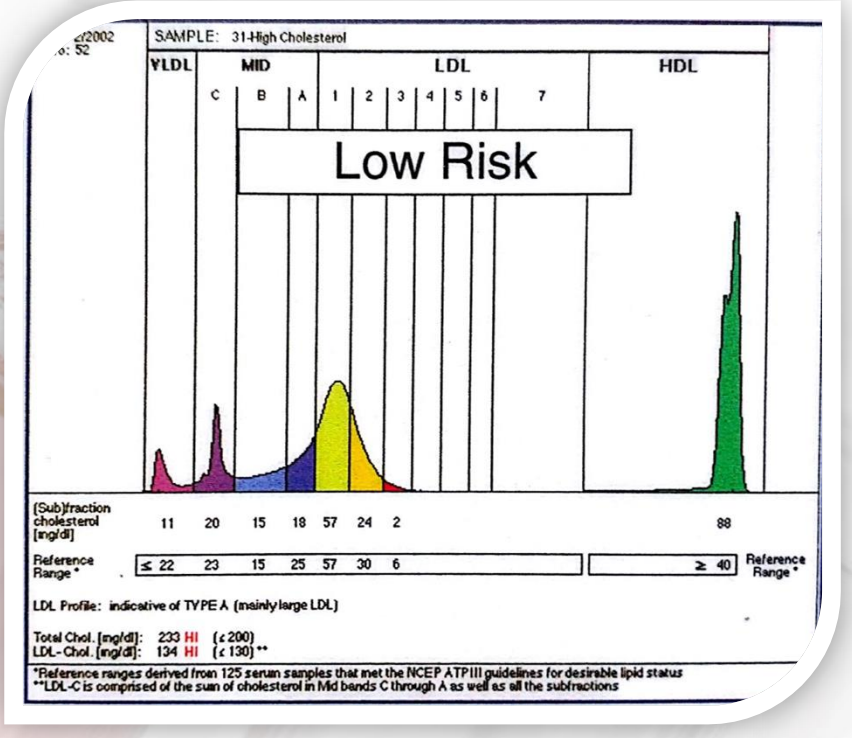

## ABNORMAL Non-Type A Profile



## High Risk despite Low Cholesterol (4.63mmol/L)




## Low Risk despite High Cholesterol (6.02mmol/L)



# Treatment Considerations

- Different lipoprotein subfractions respond differently to diet and drug therapy, therefore, Liposcreen can assist the health practitioner to decide on the appropriate therapy statin drugs reduce cholesterol levels in all lipoprotein subfractions.
- Niacin and fibrates shift the LDL particles from the small dense atherogenic to the large less atherogenic particles.
- Combination drugs may contain a statin and niacin or other drug that reduces cholesterol and causes a shift in particle size.

## Lipid Lowering Drugs

Six main categories of lipid lowering drugs: nicotinic acid, HMG-CoA reductase inhibitors (statins), bile acid sequestrants, fibric acid derivatives, combination drugs and cholesterol absorption inhibitors.

| Drug Class | Generic Name | Brand Name | Dose Range (mg/d) |
|---|---|---|---|
| Nicotinic acid (niacin) | Nicotinic acid | Niaspan | 500-2,000 |
| HMG-CoA reductase inhibitors (statins) | Atorvastatin<br>Simvastatin<br>Lovastatin<br>Pravastatin<br>Fluvastatin<br>Rosuvastatin | Lipitor<br>Zocor<br>Mevacor<br>Pravachol<br>Lescol<br>Crestor | 10-80<br>5-80<br>10-80<br>10-80<br>20-80<br>5-40 |
| Bile acid sequestrants | Cholestyramine<br><br>Colesevelam<br>Colestipol | Questran<br>Questran Light<br>WelChol<br>Colestid | 4-24 grams<br><br><br>2-16 grams in divided doses |
| Fibric acid derivatives | Gemfibrozil<br>Fenofibrate | Lopid<br>Tricor | <br>1,200 in divided dose<br>54-145 |
| Combination | Niacin (extended release)-lovastatin<br>Ezetimibe-simvastatin | Advicor<br>Vytorin | <br>500/20<br>10/10-10/80 |
| Cholesterol absorption inhibitors | Ezetimibe | Zetia | 10 |

Adapted from: Nurse Practitioner's Drug Handbook, 4th Ed. Philadelphia: Lippincott, Williams, & Wilkins, 2002.

## Dietary and Supplement Therapy

### Diet

- Eliminate refined carbohydrates
- Increase dietary fibre
- Eat more plant foods – fruit and vegetables
- Follow a low GI/low carbohydrate diet vs. low fat diet
- AVOID processed food, sugar, packaged food, flour, pasta, bread, milk, dairy and gluten.

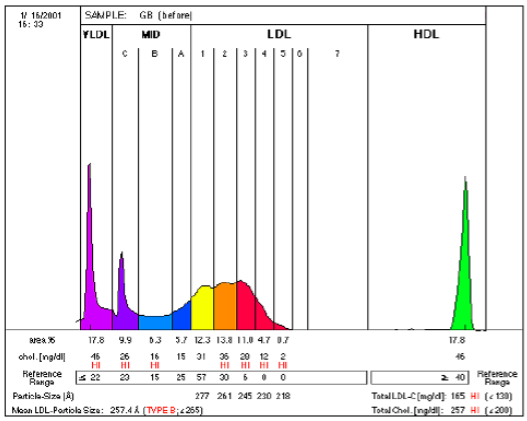
## Nutriceuticals and Herbals

- Policosanol
- Red Yeast Rice
- Plant sterols
- Soluble fibres - psyllium, guar gum, beta-glucan
- Omega 3 fish oils, Krill Oil
- Flaxseeds (ground only vs. oil)
- Green tea (EGCG)
- Rice bran oil (tocotrienols)
- Nuts - walnuts, almonds, macadamia
- Citrus - orange, grapefruit, pomegranate
- Blue green algae
- Monounsaturated fats e.g. olives, avocado
- Sesame: 40g/day
- Tocotrienols: gamma/delta 200mg with evening meal to increase absorption
- Niacin
- Pantethine (vs. pantothenic acid) 300mg t.i.d.
- Alpha lipoic acid
- Vitamin C
- CoQ10
- Chromium
- Probiotics and prebiotics
- Isoflavones and flavonoids
- Carotenoids
- Choleretics e.g. silymarin (milk thistle), globe artichoke (decreases cholesterol 18%), dandelion root
- Garlic
- Ginkgo
- Curcumin

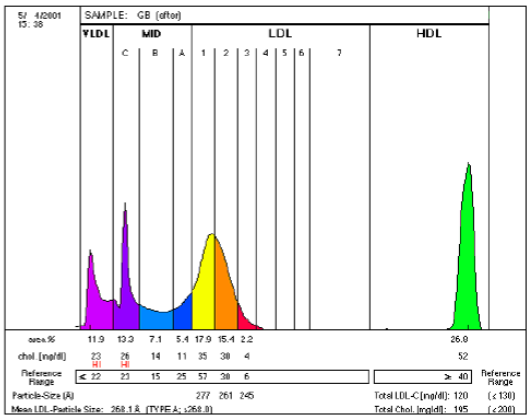
# Case Study

GB, a 65 year-old Caucasian male had his Liposcreen profile done in January. (Figure 1) The profile shows small, dense LDL particles (red portion of the profile) which indicates a lipid disorder.

After 3 months of niacin therapy combined with dietary changes and increased exercise the patient's profile vastly improved. With normal lipoprotein distribution (Figure 2) and only two cholesterol values that were slightly outside the normal reference range.




**GB's Baseline Liposcreen Profile**




**GB after 3 months of intervention**

# Related Tests

- Total Cholesterol, Triglycerides, HDL, LDL
- Lipoprotein (a)
- Apolipoprotein A-I
- Apolipoprotein B
- High sensitive C-Reactive Protein (hsCRP)
- Homocysteine
- Omega 3 Index

# Sample Collection Requirements

- Fasting sample of blood is collected in an EDTA plasma tube.
- Only fasting (12 hours) samples should be used.
- Freezing of the sample is not recommended.

# Frequently Asked Questions

**1. Why is LDL subfraction testing so important?**

The fact that 50% of people who suffer from coronary artery disease (CAD) have normal cholesterol levels indicates the need for a more specific diagnostic tool. The relationship between small dense LDL particles and an increased risk for CAD has been well established. Research indicates that individuals exhibiting a non-A pattern are at 3 times increased risk for CAD. Thus, by identifying non-A patterns using the Liposcreen system and treating them at an early stage, CAD may be greatly reduced.

**2. How can physicians utilize the results from the Liposcreen test?**

There is strong evidence which indicates a positive shift in the LDL subfraction profile in response to lipid lowering medications as well as non-drug therapies. Based on the patient's Liposcreen profile and assessment of certain complementary tests, a physician can prescribe a personal treatment plan.

# References

Duncan D. Morais J., Muniz N., Neyer G. Lipoprotein Subfraction Testing with the Lipoprint R System – Easy, Accurate and Comprehensive. Presented at CLAS, Northbrook, IL (May2004)

Després J-P., Lamarache B., et al., (1997). Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Circulation 95 :69

Abstract: A prospective study of 4,637 men concluded that a significant proportion of the risk for heart disease associated with small, dense LDL particles may be independent of variations in plasma lipid concentrations. Small LDL particles and elevated apo B levels were found to be the most predictive indications for ischemic heart disease.

Kholodova Y.D., Harris W.S., (1995). Identification and characteristic of LDL-subfractions in human plasma. Ukrain. Biochem.J. 67, 113.

Abstract: The cholesterol content of various LDL fractions, isolated by ultracentrifugation, was compared to the area under the curve for LDL fractions obtained with the Liposcreen System. 34 subjects, along with pooled serum samples, were studied.

Mack W.J., Krauss R.M., (1996). Lipoprotein subclasses in the monitored atherosclerosis regression study (MAR S). Arterioscler. Thromb. Vasc. Biol. 16, 697

Abstract: The effects of lovastatin treatment on the different LDL and HDL subclasses were evaluated. Triglyceride rich lipoproteins and HDL3 were identified as independent risk factors for the progression of CAD.

Rajman I., Maxwell S., et al., (1994). Particle size: the key to the atherogenic lipoprotein? Q.J.Med. 87, 709.

Yoshino G., Saburo N., et al (2012). Rosuvastatin Reduces Plasma Small Dense LDL-Cholesterol Predominantly in Non-Diabetic Hypercholesterolemic Patients. Pharmacology & Pharmacy 3:72-78

Brown L., Rosner B., Willett W., Sacks F. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr . vol. 69 no. 1:30-42

Chen P.R., Chien K.L., Su T.C. et al (2005). Dietary sesame reduces serum cholesterol and enhances antioxidant capacity in hypercholesterolemia. Nutrition Research, Vol. 25, 6:559–567

Gladys Castaño G., Rosa Más R., Nodarse M., et al. (1995). One-year study of the efficacy and safety of policosanol (5 mg twice daily) in the treatment of type II hypercholesterolemia. Current Therapeutic Research, Vol. 56, 3:296–304.

Heber D., Yip I., Ashley J.M., Flashoff D.A., Elashoff R.M., Go Y.L. (1999). Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. Am J Clin Nutr. vol. 69 no. 2:231-236.

Yancy W.S., Olsen M.K., Guyton J.R., Bakst R.P., Westman E.C. (2004). A Low-Carbohydrate, Ketogenic Diet versus a Low-Fat Diet To Treat Obesity and Hyperlipidemia: A Randomized, Controlled Trial. Ann Intern Med. 140(10):769-777.