

# LIOPRINT®

## A Breakthrough in Cholesterol Management

AELLSP-4/05

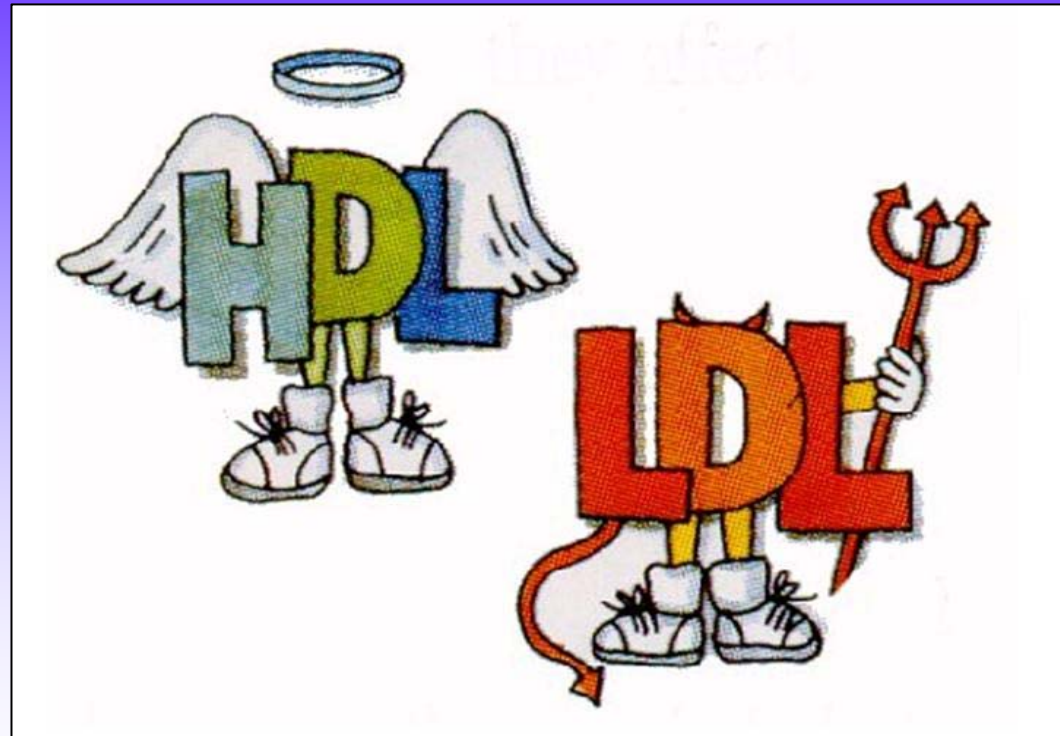
- Heart Disease is the leading cause of death in the United States
- Each year 1.5 million Americans suffer from a heart attack
- 500,000 deaths each year
- \$140 billion will be spent annually on operations and medical bills

Scientific studies have clearly demonstrated that lipids and lipoproteins are strongly associated with CAD along with other genetic and environmental factors

## NCEP ATP III guidelines for desirable lipid levels:

- Cholesterol < 200 mg/dl
- Triglycerides < 150 mg/dl
- LDL Cholesterol < 130 mg/dl
- HDL Cholesterol > 40 mg/dl

- LDL cholesterol  
(*Bad*)
- HDL cholesterol  
(*Good*)



[www.aeb.org/food/eggs-health.html](http://www.aeb.org/food/eggs-health.html)

## Traditional Risk Factors Don't Tell the Whole Story

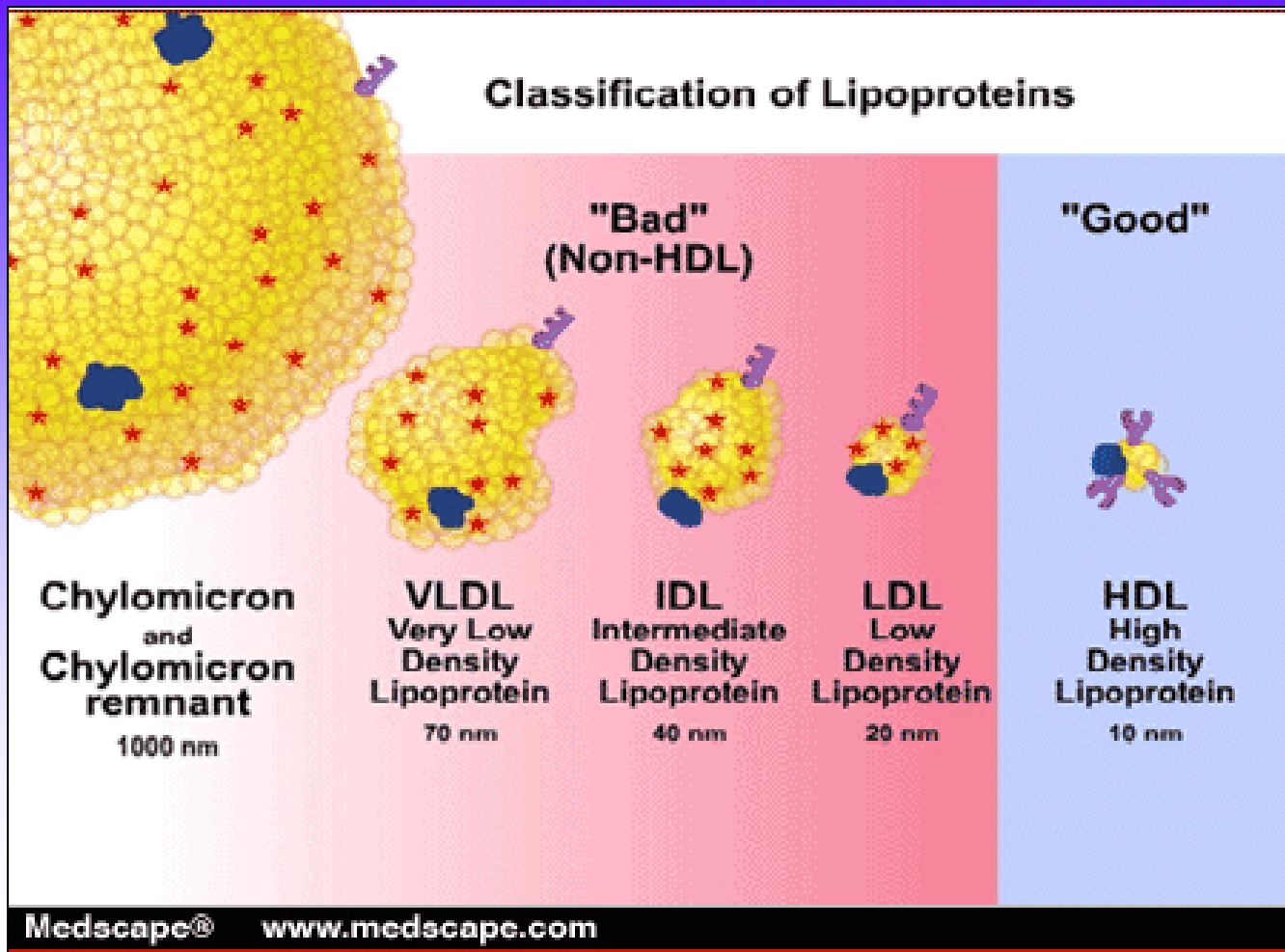
- The fact is that approximately 50% of CAD occurs among individuals with normal lipid levels
- Only 30% of all heart attacks can be explained on the basis of total cholesterol measurements alone
- Even with the most current ATP III guidelines most cases of CAD go unexplained

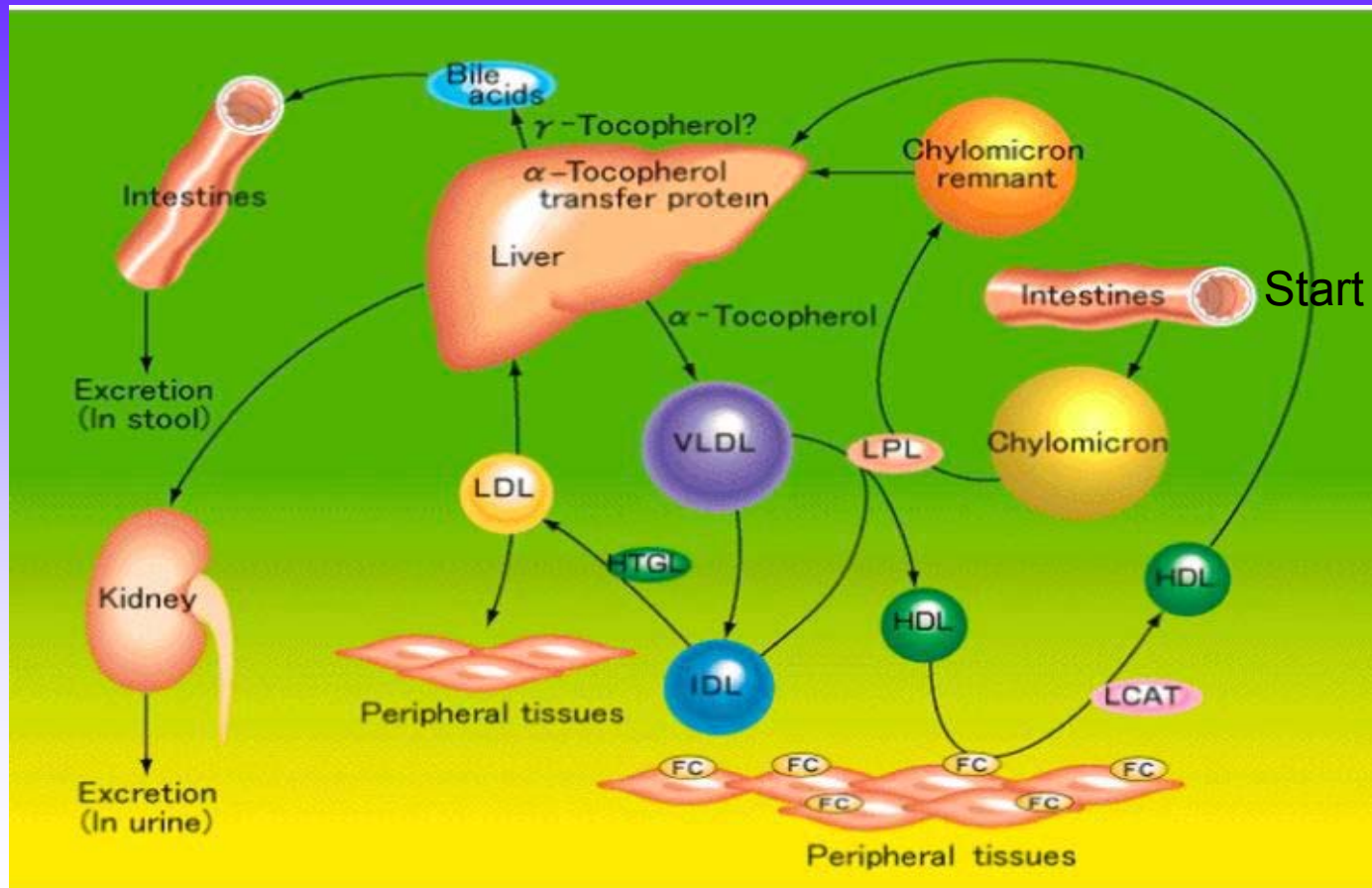
- Many individuals with normal LDL and HDL cholesterol levels are still at risk from cardiovascular disease
- These suggest that LDL and HDL measurement are not the best indicators for cardiovascular disease risk

## Lipoproteins have been classified into five major classes based on their density

- Chylomicrons
- VLDL (very low density lipoprotein)
- IDL (intermediate density lipoprotein)
- LDL “bad” (low density lipoprotein)
- HDL “good” (high density lipoprotein)



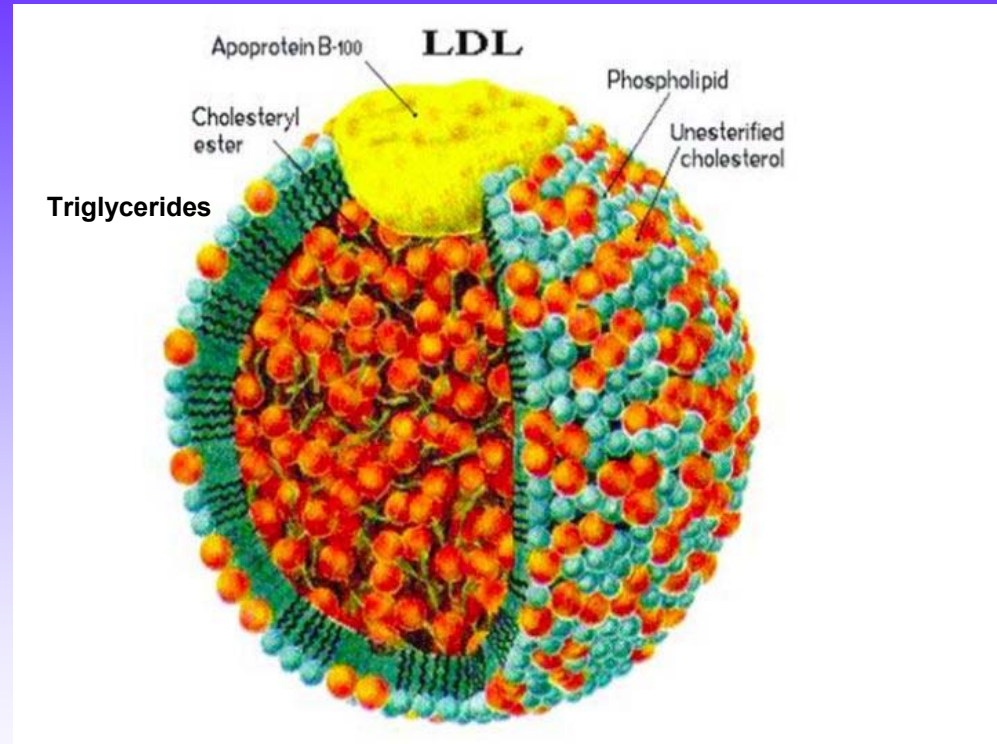




VARIABLE	CHYLOMICRON	VLDL	IDL	LDL	HDL	LP(a)
Density (g/mL)	<0.95	095 - 1.006	1.006 - 1.019	1.019 - 1.063	1.063 - 1.210	1.040 - 1130
Diameter (nm)	>70	25 - 70	22 - 24	19 - 23	4 - 10	25 - 30
Lipid: protein	99:1	90:10	85:15	80:20	50:50	75:25
Major lipids	trigs	trigs	trigs, chol. Esters	chol. Esters	phospholipids	chol. Esters, phospholipids
Major Proteins	A-I B-48 C-I, II, III	B-100 C-I, II, III E	B-100 E	B-100	A-I, II C-I, II, III E	(a) B-100

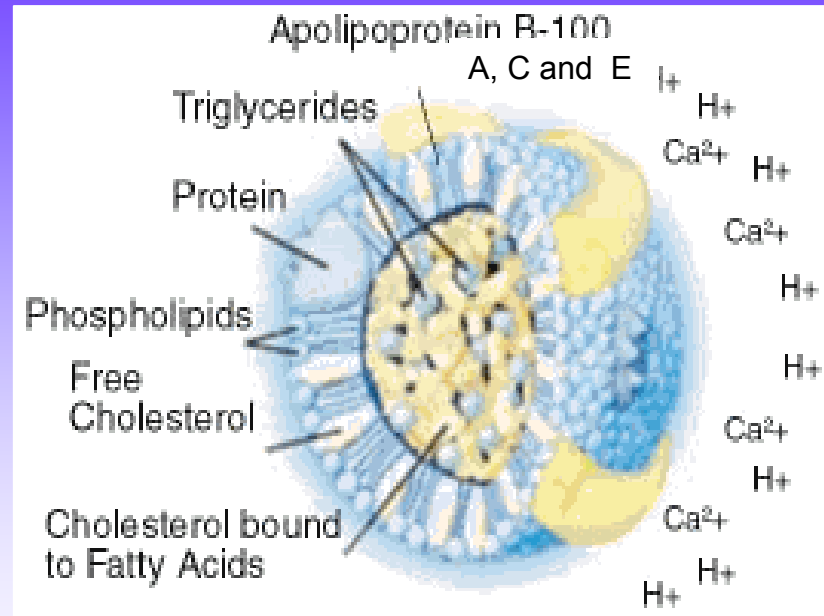
Source: Tietz Fundamentals of Clinical Chemistry (5th ed.); eds. C.A. Burtis, E.R. Ashwood; W.B. Saunders 2001

LDL particle is the primary carrier of cholesterol throughout the body

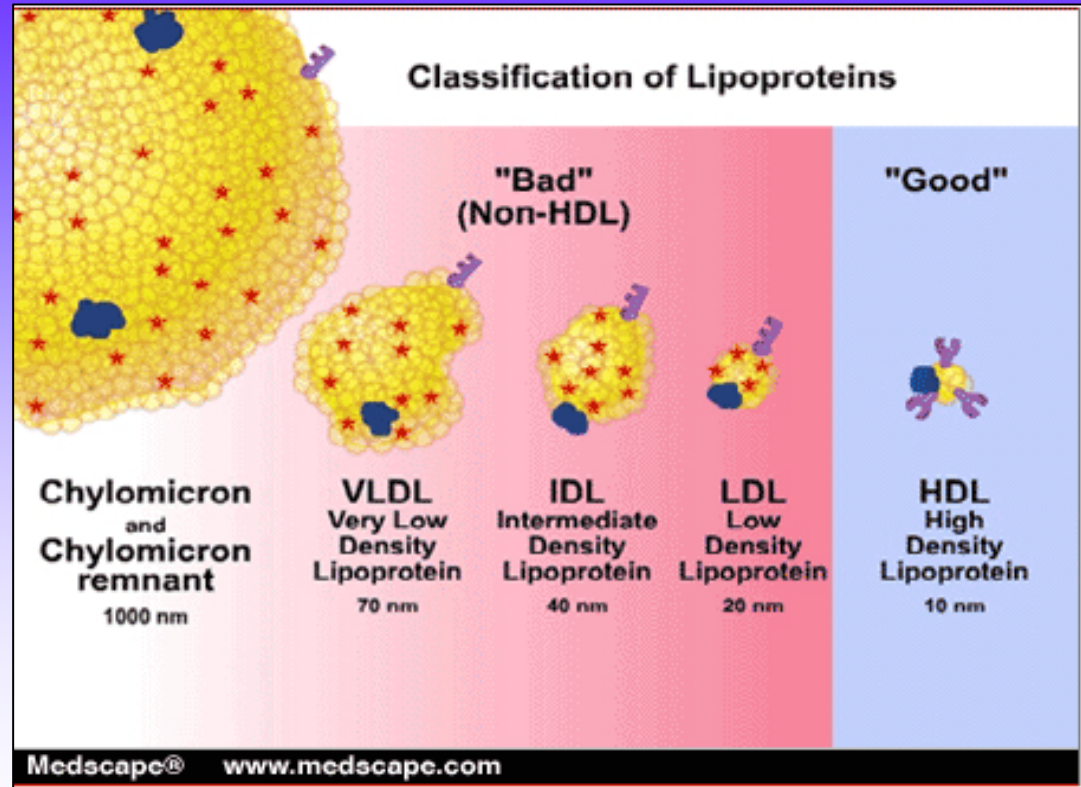


*Bioch.szote.u-szegedhu/astrojan/Prot/Ld.jpg*

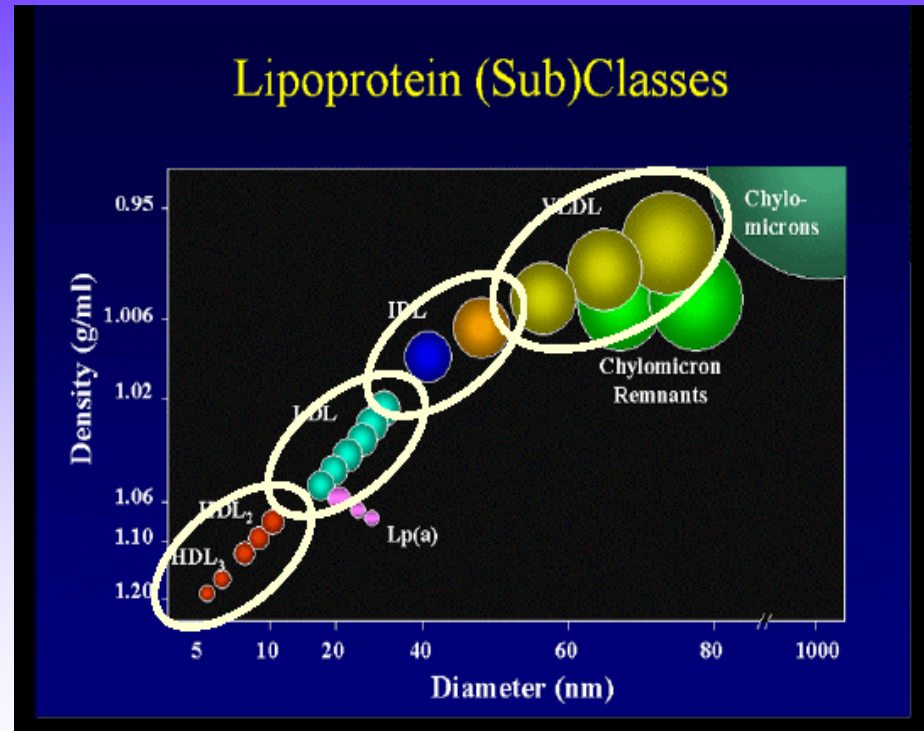
HDL particle is responsible for the removal of excess cholesterol from the body



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



- Chylomicrons: continuum of particles
- VLDL: continuum of particles
- IDL: three subclasses
- DL Seven subclasses
- HDL Up to ten subclasses



- Large LDL subclasses 1 and 2 are the “good or normal” LDL that are responsible for the transport of cholesterol
- Small LDL subclasses 3 through 7 are “bad or abnormal” and are easily oxidized and promote cardiovascular disease



## Continuous Gradient Ultracentrifugation (Gold Standard)

- Distribution of various LDL particles by their densities
- Used primarily in basic lipoprotein research

## Gradient Gel Electrophoresis (GGE - Legacy Method)

- LDL subfraction distribution and major LDL peak particle size
- Qualitative LDL subfraction analysis

## Nuclear Magnetic Resonance (NMR)

- Distribution LDL size determination by mathematical deconvolution of NMR signal
- Measures LDL particle number

## Vertical Analytical Profile (VAP) centrifugation

- LDL LDL distribution determined by deconvolution of the elution rate curve
- Qualitative phenotype classification of LDL sizes

**A test that measures all the  
lipoprotein classes and subclasses  
would be superior to the current  
lipid profile**

**THE LIOPRINT SYSTEM IS THE**  
**ONLY FDA-CLEARED**  
**DIAGNOSTIC TOOL FOR LDL SUBFRACTION**  
**TESTING**

## *Intended Use:*

The Quantimetrix Lipoprint System *LDL Subfractions* is a device intended to **measure lipoprotein cholesterol** (for lipoprotein fractions and subfractions from VLDL to HDL) in fasting serum or plasma with a Total Cholesterol concentration of  $\geq 100$  mg/dl.

## *Indications For Use:*

Lipoprotein subfraction cholesterol measurements are used as an aid in evaluating lipid metabolism disorders when used in conjunction with other lipid tests, patient risk assessment and clinical evaluation.

The Lipoprotein System utilizes non-denaturing, linear (non-gradient) polyacrylamide gel electrophoresis (PAGE) to separate the lipoprotein fractions and subfractions in serum or plasma on the basis of size.

The test uses a lipophilic dye that binds to the cholesterol in the lipoprotein particle prior to electrophoresis.

The electrophoresed gels are scanned to determine the relative area for each lipoprotein subfraction. The subfraction cholesterol is calculate by multiplying the relative area of each subfraction by the total cholesterol of the sample.

## System components:

- ↓ Chamber
- ↓ Prep Rack
- ↓ Prep Light
- ↓ Power Supply
- ↓ Digital Scanner
- ↓ iMac + software
- ↓ Printer





- Lipoprint Kit:**
- ↓ Gel Tubes
  - ↓ Loading Gel
  - ↓ Buffers



- Only fasting (12 hours) samples should be used
- Serum or EDTA plasma may be used
- Do not use heparin as anticoagulant
- Samples can be kept for up to 7 days at 2 - 8 C

## The four steps of the Lipoprint test:

- Electrophoresis
- Scan
- Analysis
- Report

## Loading Sample

- Place gel tubes in rack
- Apply 25  $\mu$ l serum or plasma to gel tube
- Add 200  $\mu$ l Loading Gel solution



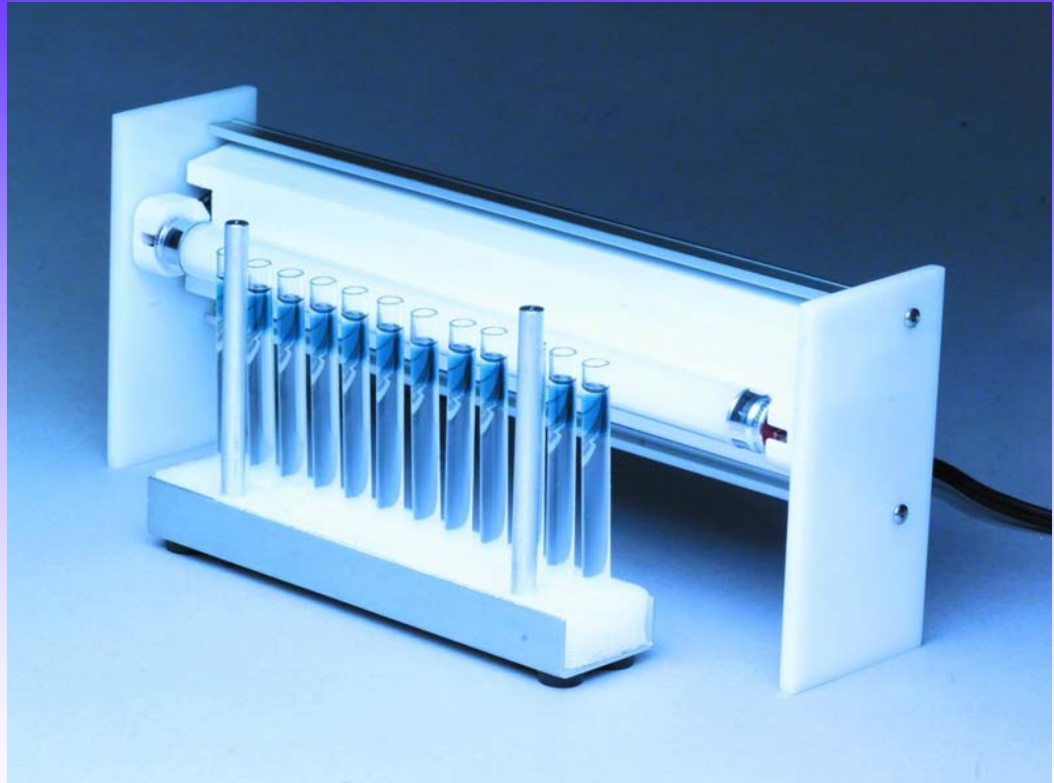
## Mixing Sample

- Place cover over gel tubes
- Mix sample with Loading Gel by inverting the gel tubes



## Photo-Polymerization

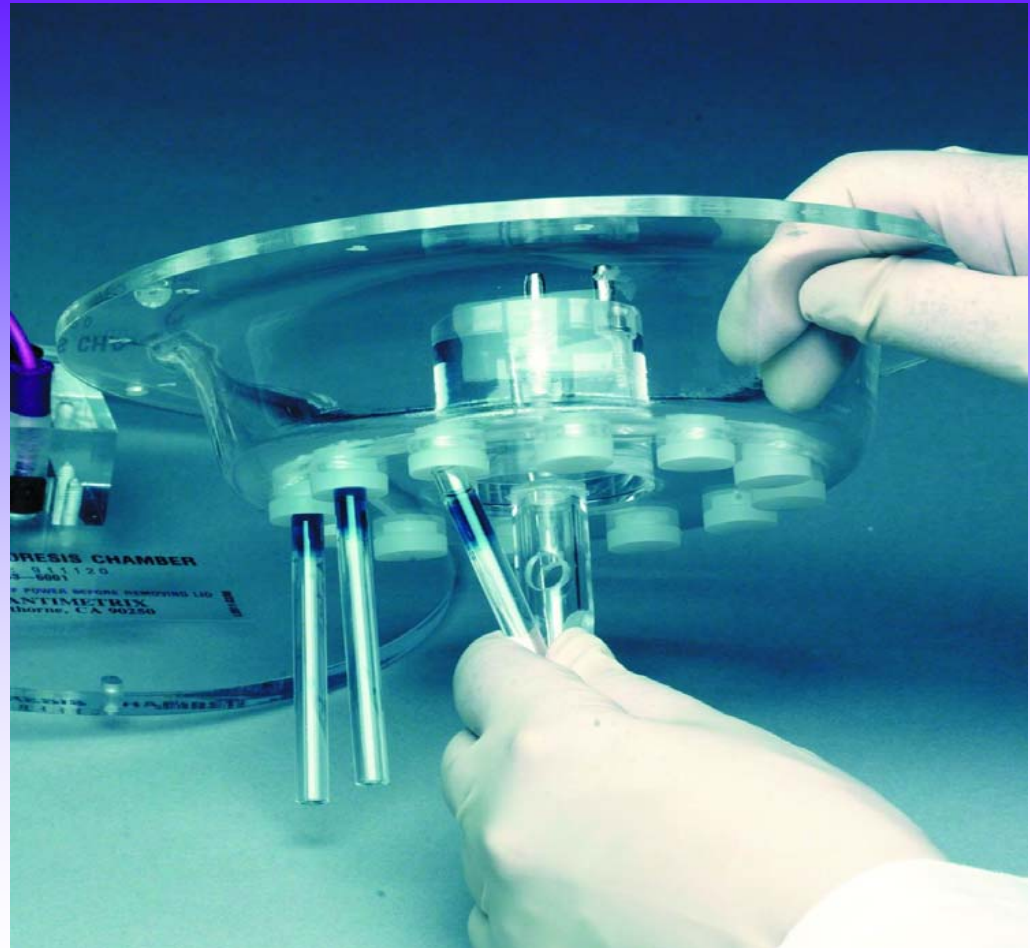
- Place gel tube in front of Preparation Light
- Photopolymerize gel tubes for 30 minutes



Stain binds to lipoprotein particles

## Loading Chamber

- Remove gel tubes from Preparation Rack
- Load gel tubes on the upper chamber



## Electrophoresis

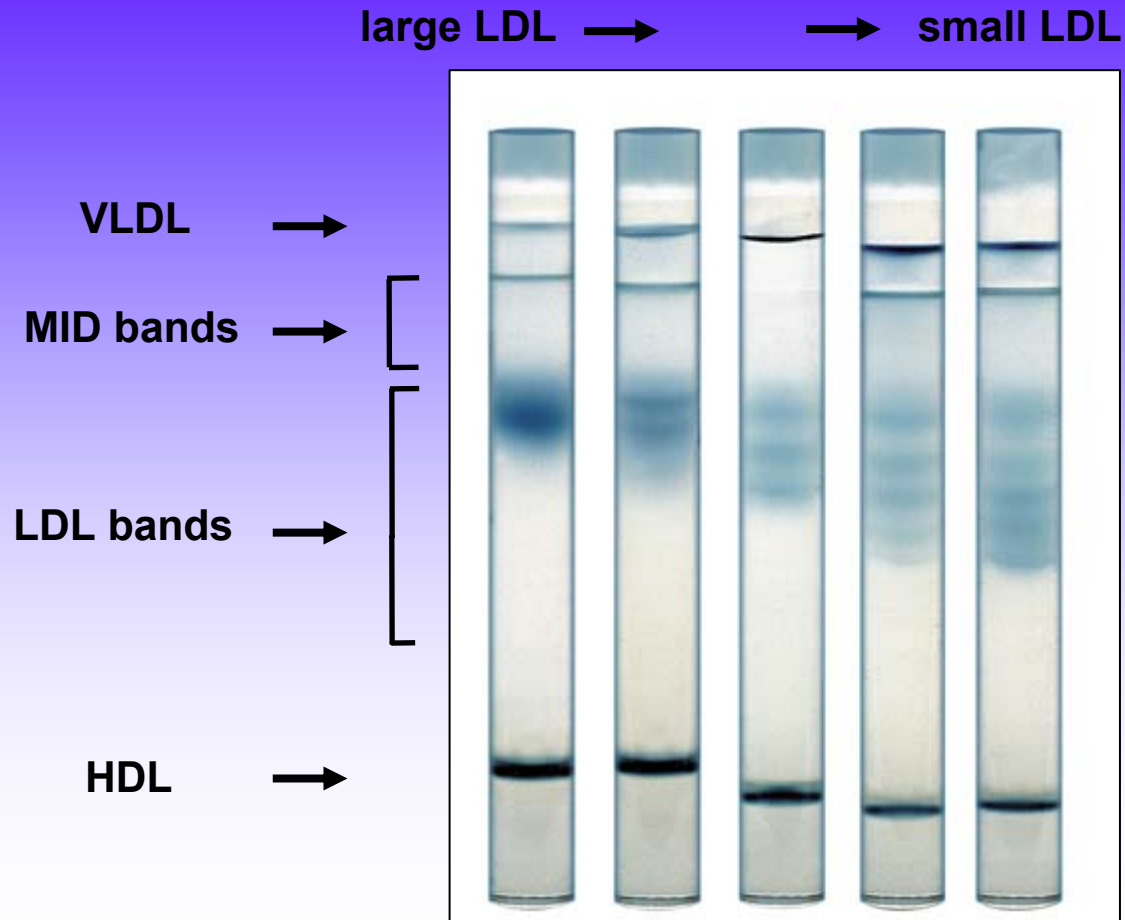
- Add Electrolyte buffer to Upper and lower Chambers
- Electrophores for 1 hour at 3 ma per gel tube





# LIPOPROTEIN SUBFRACTIONS ARE RESOLVED

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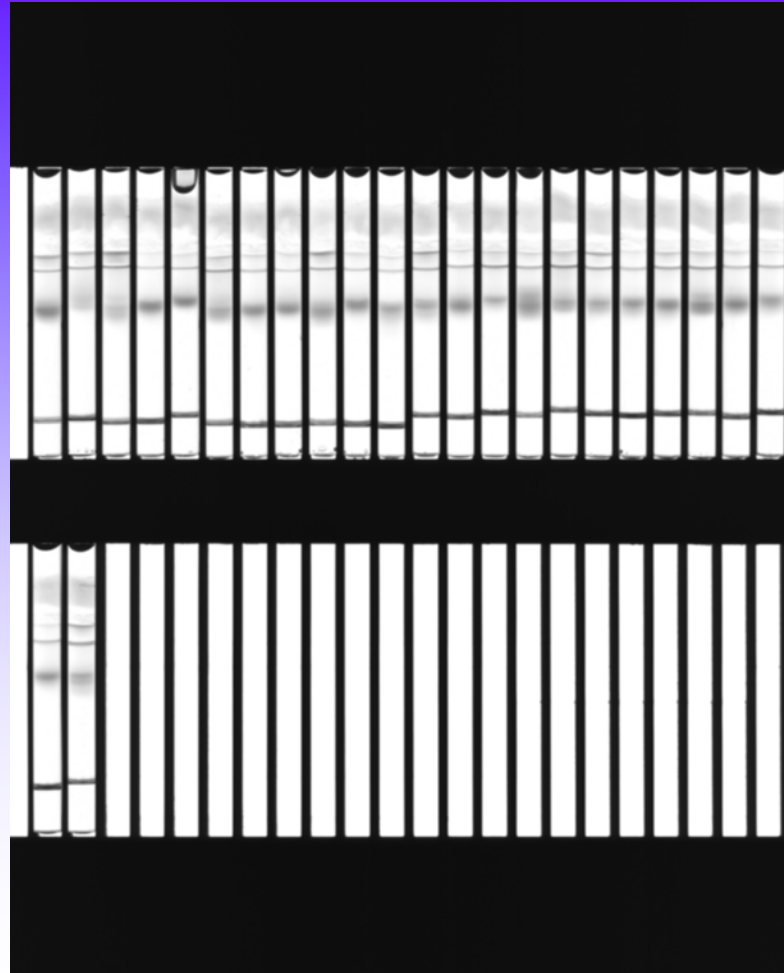


## Scan Gel Tubes

- Load electrophoresed gel tubes on Scanner
- Scan the gel tubes



A digitized image of the electrophoresed gel tubes is generated



## Automatic analysis of patient's results

- Enter patient's name and total cholesterol
- Run analysis of the scanned tubes
- Generate patient's profile
- Print patient's profile



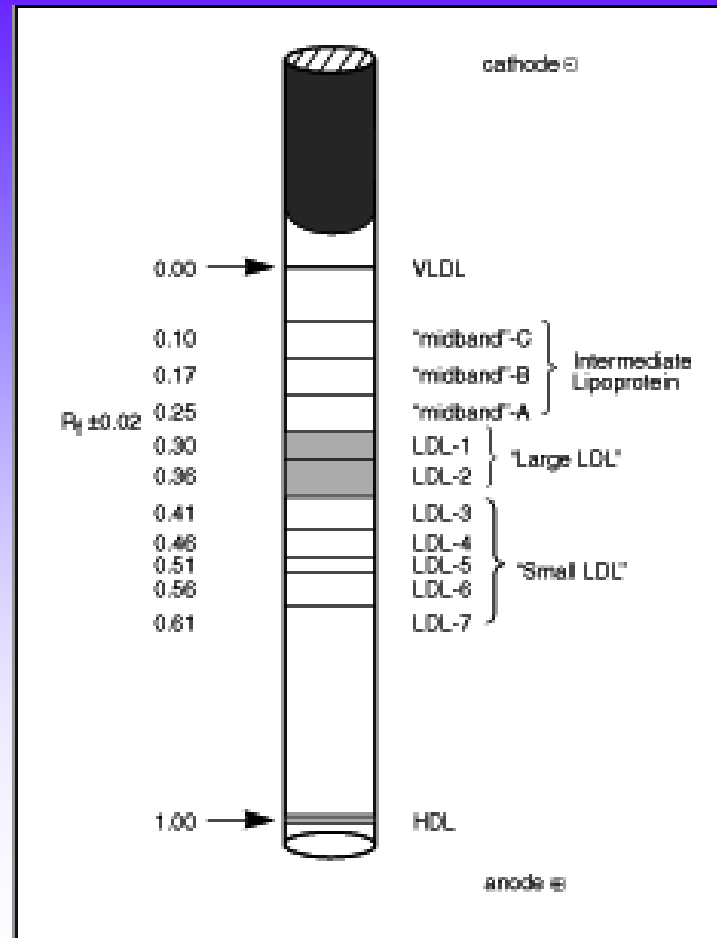
# Lipoproteins Bands Resolved

LIOPRINT®

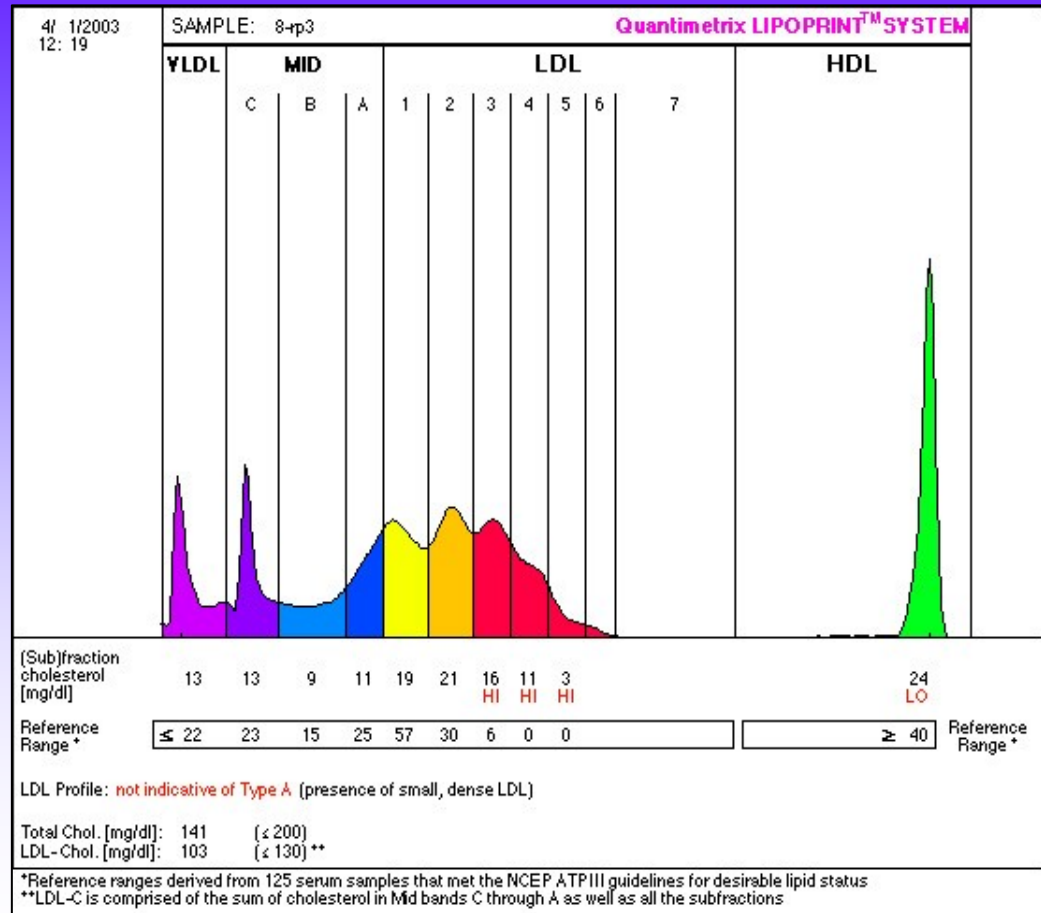
Up to 12 lipoproteins bands are resolved according to size (1 VLDL, 3 Mid Bands, 7 LDLs and 1 HDL)

VLDL migrates at the top of the separating gel  $R_f = 0$  and HDL migrates at the front  $R_f = 1$

Mid Bands and LDL subfractions migrate at specific rates between VLDL and HDL



An easy-to-interpret color graph of the patient's lipoprotein profile is generated and printed



## Run Analysis Of The Scanned Tubes

- The Lipoware software analyzes the samples
- Calculates the area percent for each subfraction
- Calculates the amount of cholesterol in each subfraction
- Creates a spreadsheet with all the results

	A	B	C	D	E	F	G	H	I	J
1		[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
2	Sample	VLDL	IDL-C	IDL-B	IDL-A	LDL-1	LDL-2	LDL-3	LDL-4	LDL-5
3										
4	tube 1	10.2	9.5	6.3	8.6	23.5	13.9	0	0	
5										
6	tube 2	10	10	6.7	9	23.7	12.9	0	0	
7										
8	tube 3	15.5	10.2	10	7.2	13.3	17	3.8	0	
9										
10	tube 4	17.4	8.2	14.3	12.9	19.8	5	0	0	
11										
12	tube 5	6.3	9.5	9.4	14.1	25.6	11.6	0	0	
13										
14	tube 6	6.2	9.1	9.1	13.5	25.2	12.1	0	0	
15										
16	tube 7	10.1	14.2	8.8	7.4	14.3	15.5	9.2	1.4	
17										
18	tube 8	8.9	12.7	8.2	7.6	14.2	15.3	10.6	3.4	
19										
20	tube 9	30.7	5.8	10.5	3.7	6.6	7.7	5.2	5.3	
21										
22	tube 10	4.9	15.5	9.8	6.1	10.8	9.1	6.8	7.4	
23										
24	tube 11	9.6	13.1	7	5.7	11	8.8	7.2	7.7	
25										
26	tube 12	10.2	12.6	6.8	5.4	11.4	9.5	7.6	8	
27										
28										

# UNDERSTANDING THE PROFILE



The Lipoprint System produces a “color-coded” easy-to-interpret patient profile, featuring lipoprotein subfraction distribution, cholesterol levels in each fraction, and a comparison to the NORMAL reference range.

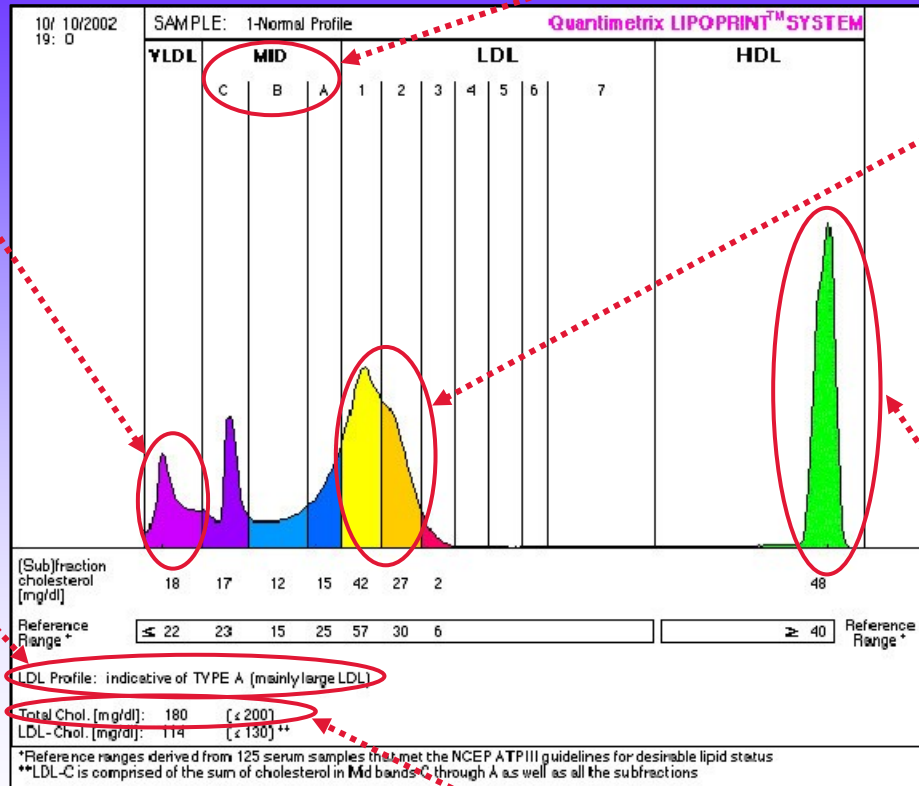
# NORMAL TYPE A PROFILE

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VLDL: Primárne lipoproteín bohatý na triglyceridy.

Mid Band C, B, A: Intermediate density lipoprotein (IDL)

Fenotyp: Type A (žiadúci)



LDL subfrakcie 1 a 2: Nájditelne takmer vo všetkých ľudských sérach, veľké čiastočky, vzostupný LDL (Žltá)

HDL ten Dobrý Cholesterol (Zelená)

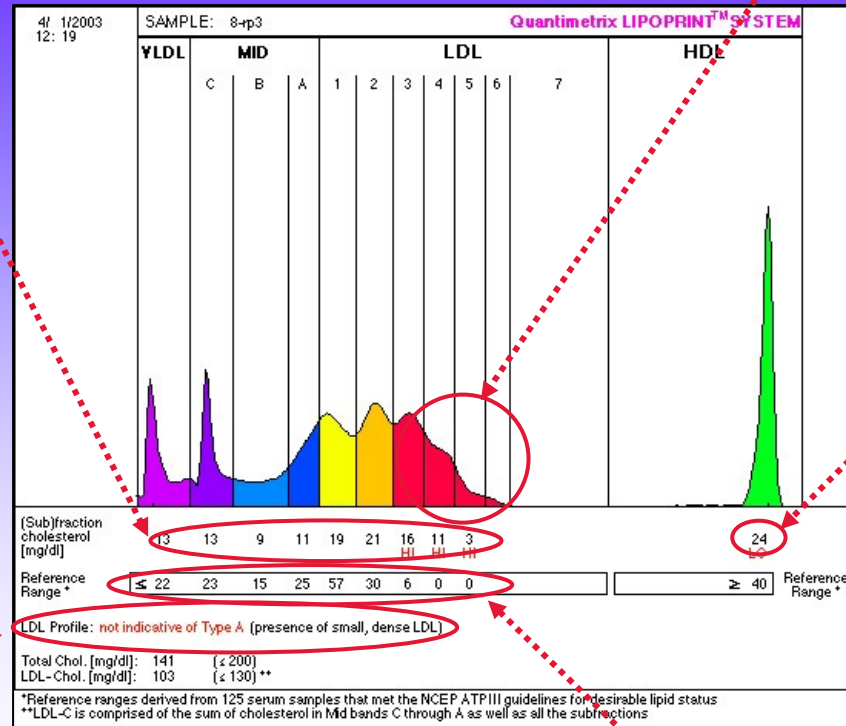
Celkový cholesterol: Meraný nezávisle

# ABNORMAL TYPE B PROFILE

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**Cholesterol mg/dl:** Quantifies the concentration of lipoprotein fractions and their subfractions

**Subfractions 3 – 7:** Small dense LDL, highly atherogenic (Red)



**HDL:** Direct measurement of total HDL (Green)

**Phenotype:** Not indicative of Type A (Predominantly small, dense LDL)

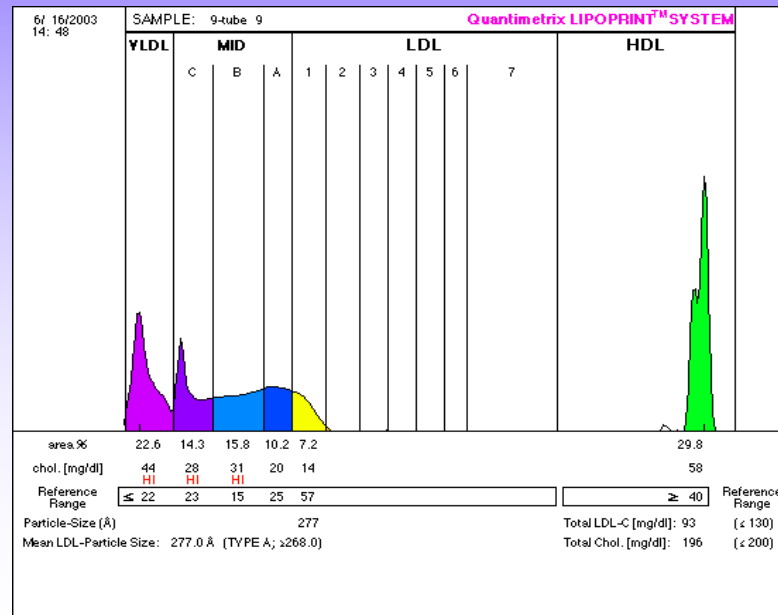
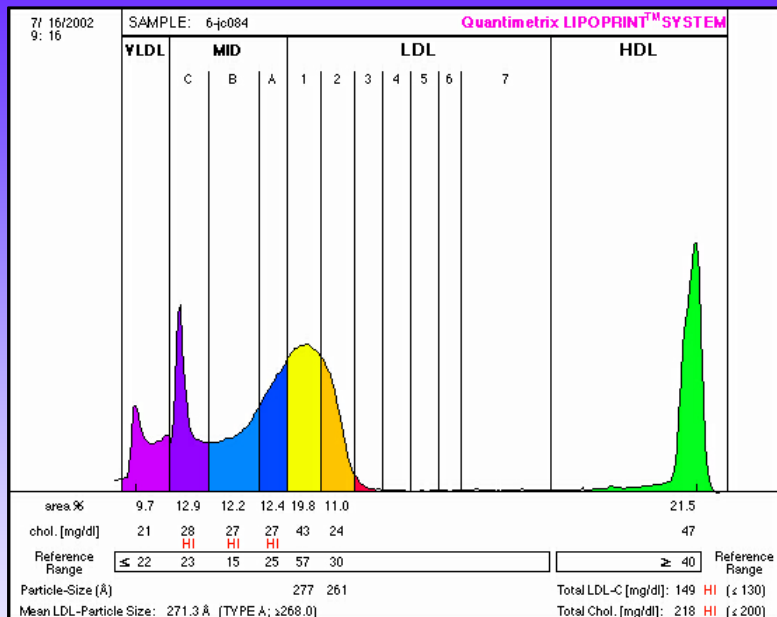
**Reference Ranges:** Flags all parameters that are above normal range – values based on ATP III

## Triglyceride Enriched Lipoproteins

- Chylomicron Remnants
- VLDL Remnants
- Intermediate Density Lipoproteins

# TYPE III DYSLIPIDEMIAS (ELEVATED IDL)

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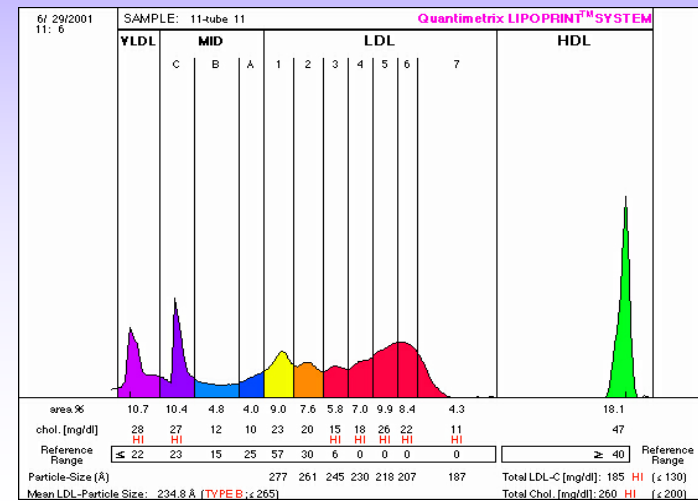
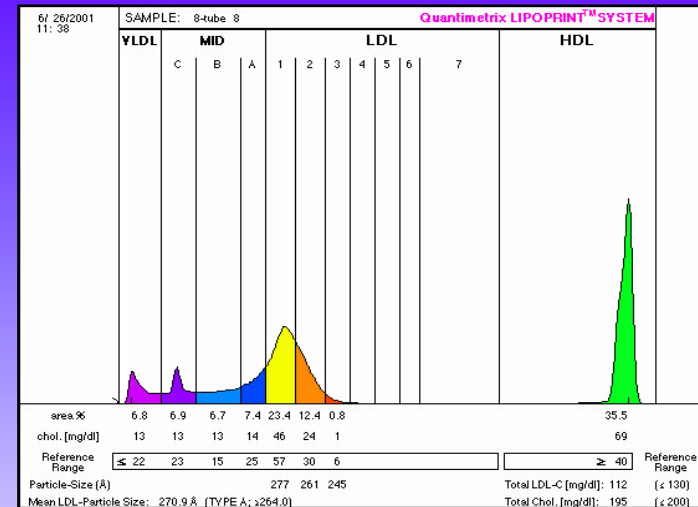
- Only FDA-cleared diagnostic test for separation and measurement of lipoprotein fractions and subfractionation
- Measures cholesterol (mg/dL) in all 12 lipoprotein fraction and subfraction
  - VLDL - associated with hypertriglyceridemia
  - Mid A, B, and C (IDL and VLDL remnants) Increased CVD risk
  - LDL 1 and 2 - associated with hypercholesterolemia
  - LDL 3 through 7 - associated with 3X increase of CVD
  - HDL - good cholesterol
  - LDL - bad Cholesterol

- Normal reference ranges based on the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III)
- Cholesterol values outside the normal reference range are flagged in red for ease of interpretation
- Color coded profile for ease of interpretation by doctors and patients.
- Lipoprotein Profile Classification – predominance of large LDL is classified as Type A and predominance of small dense LDL is classified as Not Indicative of Type A
- Test is accurate, easy, quick turn around time and can be performed at any laboratory
- Test is reimbursable by most insurances (CPT Code 83701)

# WHY MEASURE LDL SUBFRACTIONS?

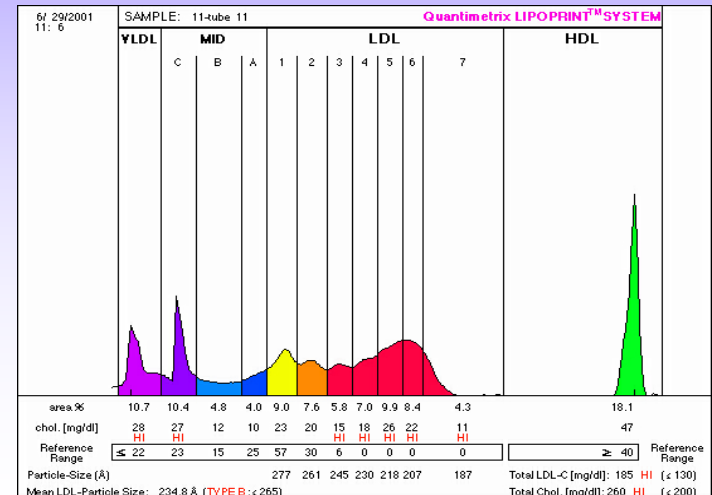
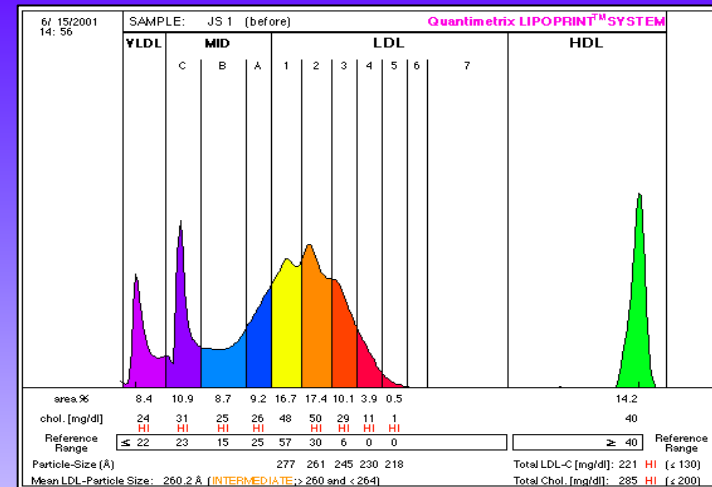


- LDL subfraction testing conveys a level of CVD risk not possible with conventional lipid profiles
- This risk could be present even with normal lipid levels



## Different profiles require different treatment

- Statins reduce the levels of total Cholesterol and the number of lipoprotein particle
- Niacin and fibrates change the particle distribution from small atherogenic to large less atherogenic
- Combined therapy
- The same is true for dietary therapy

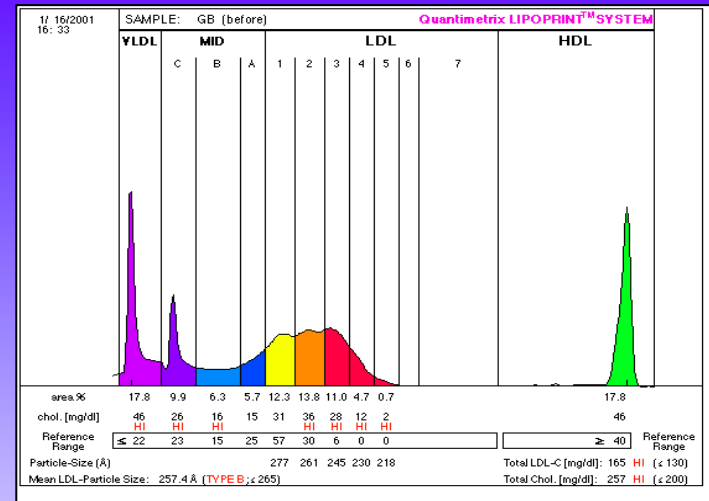


Drug Class	Generic Name	Brand Name	Dose Range (mg/d)
Nicotinic acid (niacin)	Nicotinic acid	Niaspan	500-2,000
HMG-CoA reductase inhibitors (statins)	Atorvastatin	Lipitor	10-80
	Simvastatin	Zocor	5-80
	Lovastatin	Mevacor	10-80
	Pravastatin	Pravachol	10-80
	Fluvastatin	Lescol	20-80
Bile acid sequestrants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

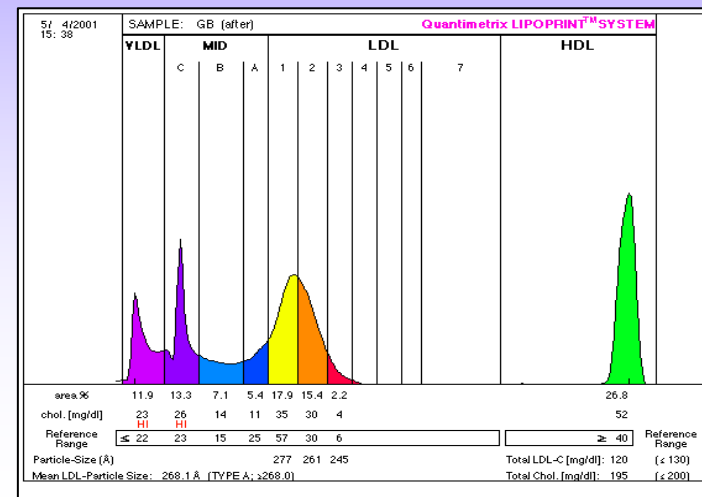
Adapted from: Nurse Practitioner's Drug Handbook, 4th Ed. Philadelphia: Lippincott, Williams, & Wilkins, 2002.

Help doctors and patients  
monitor the efficacy of  
treatment over time

Before treatment



After treatment



# CASE STUDIES

GB, a 65-year-old Caucasian male had his Lipoprint profile done in January 2001 (Figure 1). The profile contained small, dense LDL particles (red portion of the profile) indicating a lipid disorder.

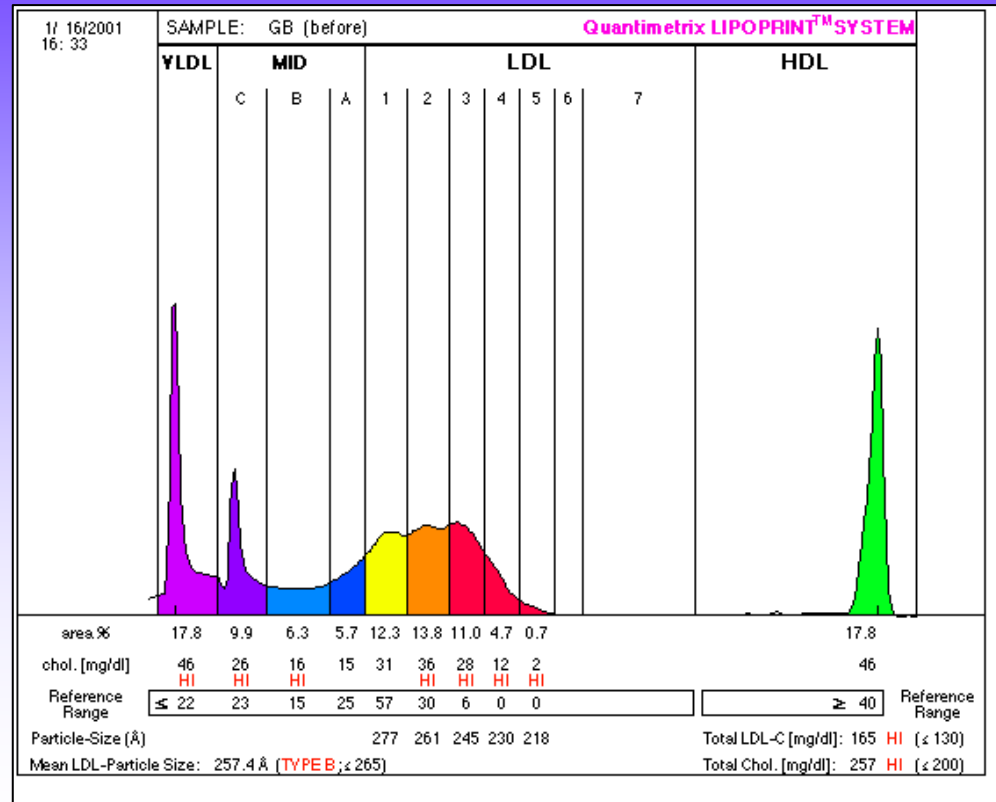


Figure 1. GB's Baseline Lipoprint Profile

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

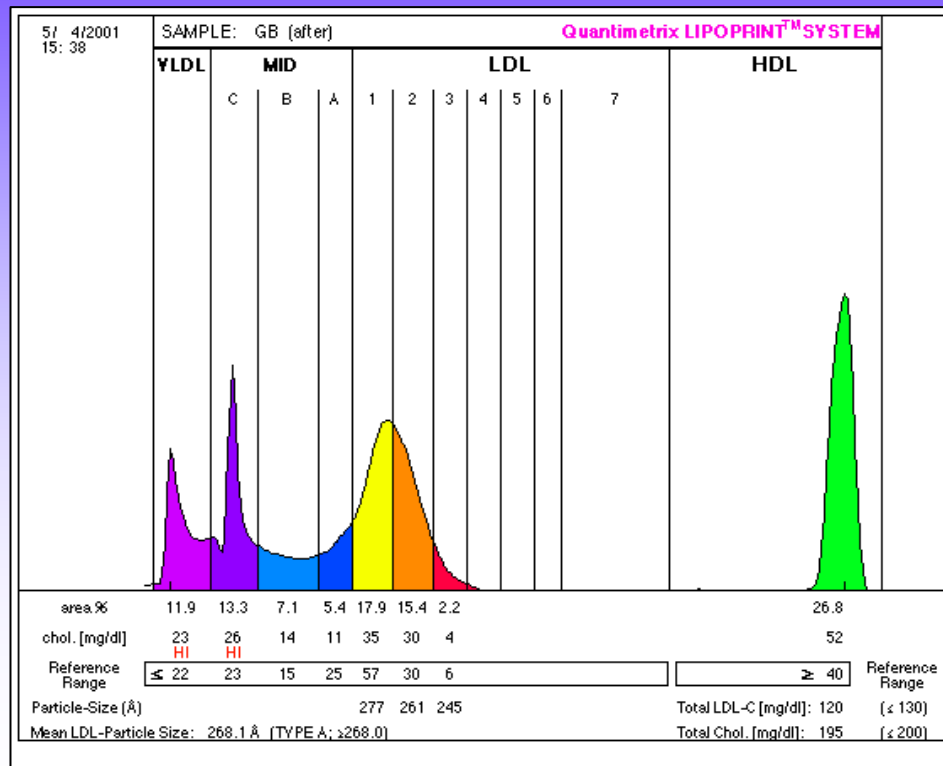


Figure 2. GB's after 3 Months of Intervention

JS, a 42 year old Indian male had his Lipoprint profile done in June 2001 (Figure 3). The profile contained high levels of cholesterol in many fractions and a distribution of lipoprotein particles shifted toward the smaller and denser particles (red portion of the profile) indicating a lipid disorder.

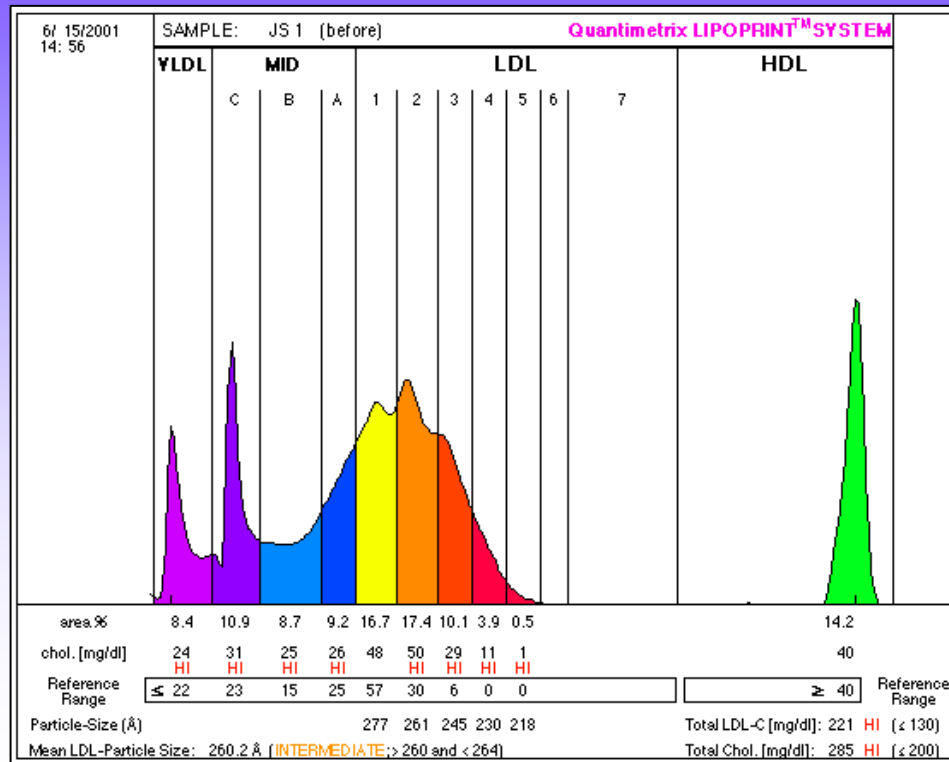


Figure 3. JS's Baseline Lipoprint Profile



After 2 months of a mainly vegetarian diet with emphasis on soy protein and increased exercise the patient presented with an essentially normal profile – only LDL-2 was still somewhat elevated (Figure 4).

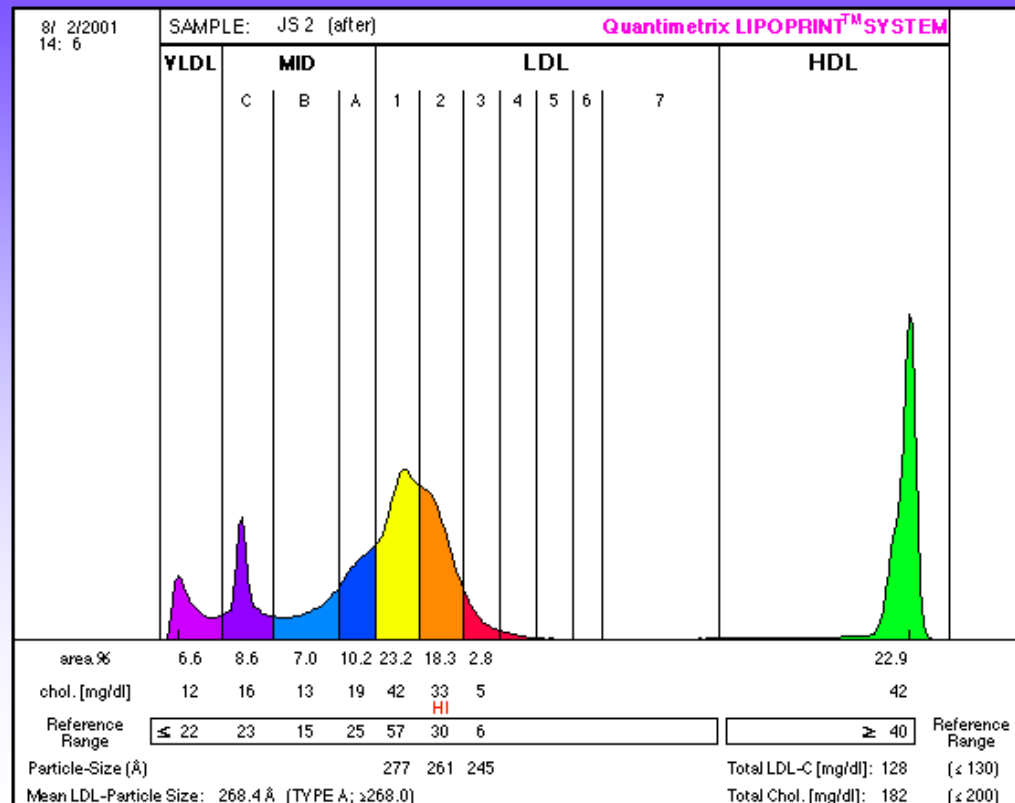
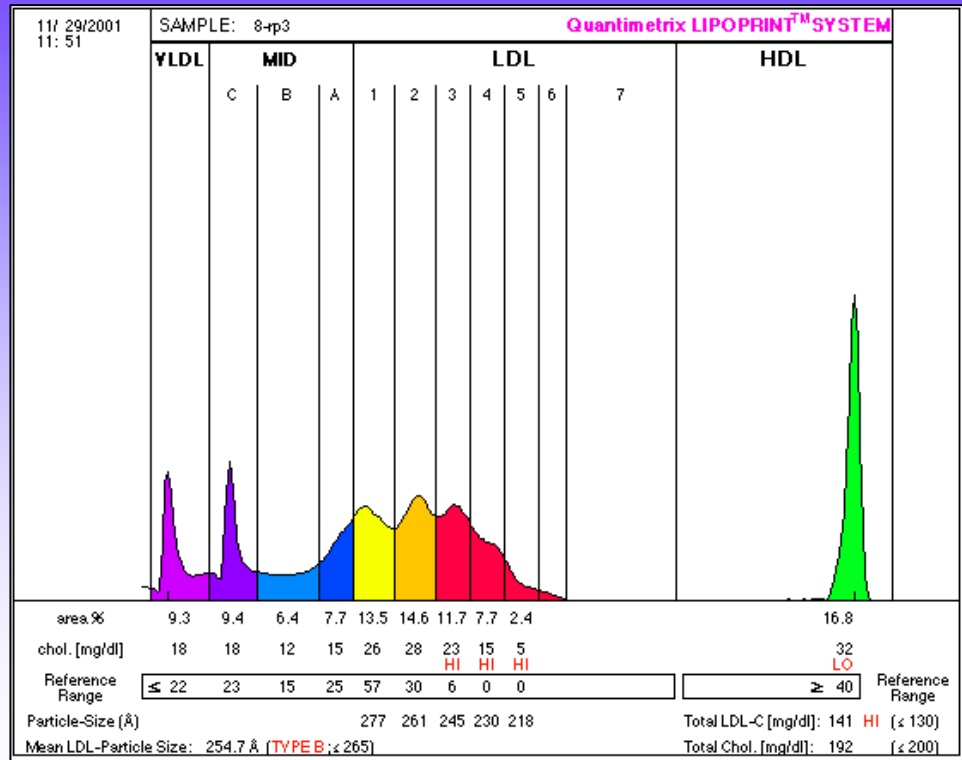


Figure 4. JS's after 2 Months of Diet and Exercise

The following Lipoprint profile was obtained 1.5 months before the individual, a fifty-year old, apparently healthy (non-smoker) Hispanic male, suffered a fatal myocardial infarction.



**Figure 5. RP's Baseline Profile, 6 Weeks before His Fatal Infarct**

# COMPETING TESTS

QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.

QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.

[www.lipoprintile.com](http://www.lipoprintile.com)

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## NMR LipoProfile®

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Raleigh, NC 27610  
www.lipoprofile.com

Page 2 of 2

Page 1 of 2

Patient Name		Sex	Age	Physician Name & Address	
		M	63		
Patient ID	Birth Date	Specimen ID		Phone: ( )	
	7-29-36	LM018820		FAX: ( )	
Date Collected	Date Received	Date Reported		Comments	
8-24-99	8-25-99	8-26-99			

### LIPOPROTEIN PANEL

	nmol/L	Coronary Heart Disease (CHD) Risk Categories			
		Optimal*	Desirable	Borderline-High	High Risk
LDL Particle Concentration	1654	less than 1100	1100 - 1399	1400 - 1799	greater than 1800
LDL Particle Size	nm	Pattern A (large LDL)		Pattern B (small LDL)	
		22.0 - 20.6	20.5 - 19.0		
		Lower-Risk	Higher-Risk		
Large HDL (cholesterol)	mg/dL	Negative Risk Factor		Positive Risk Factor	
		greater than 40	40 - 21	less than 21	
Large VLDL (triglyceride)	mg/dL	Lower-Risk		Higher-Risk	
		less than 7	7 - 27	greater than 27	

- Risk categories for LDL particle concentration correspond to NCEP categories for LDL cholesterol (on a percentile equivalence basis).
- LDL particle sizes are referenced to those measured by electron microscopy and are 5 nm smaller than gradient gel electrophoresis estimates. Pattern A and B definitions correspond to those of Austin et al. (JAMA 1988;260:1917).
- Large HDL is the protective component of HDL; levels < 21mg/dL (25th percentile) indicate higher risk and > 40 mg/dL (75th percentile) lower risk.
- Large VLDL levels are correlated with plasma triglyceride levels and delayed chylomicron clearance; values > 27 mg/dL (75th percentile) indicate higher risk and < 7 mg/dL (25th percentile) lower risk.

\*Goal for secondary prevention (patients with established CHD or diabetes)

### RISK ASSESSMENT PANEL

Elevated LDL Particle Concentration  >1400 nmol/L

Atherogenic Dyslipidemia (Lipid Triad)  ≥ 2 traits

Elevated LDL particle concentration and atherogenic dyslipidemia affect CHD risk interactively. Risk is highest when both are present.

#### Diagnostic Traits of Atherogenic Dyslipidemia

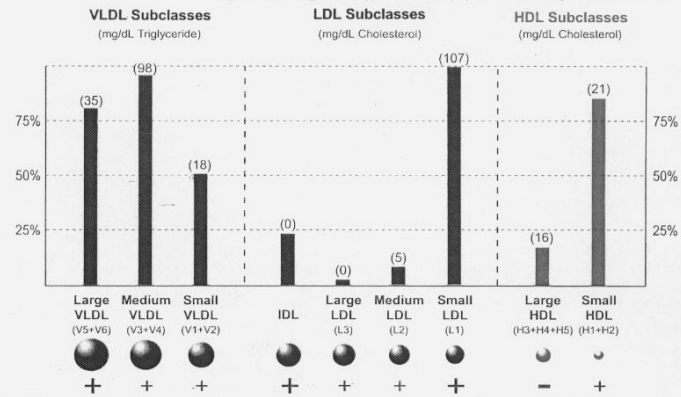
Small LDL Pattern B (≤ 20.5 nm)	Reduced Large HDL (< 21 mg/dL)	Elevated Large VLDL (> 27 mg/dL)
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

More on back →

Patient Name	Specimen ID	Date Reported
	LM018820	08-26-99

### SUBCLASS LEVELS

Lipoprotein subclass levels (mg/dL) are given in parentheses above each bar. The height of the bar gives the percent of the population\* with equal or lower levels.



The plus and minus signs shown above summarize current medical understanding of the relations between lipoprotein subclass levels and heart disease risk. Plus signs signify a positive association with disease (higher levels = higher risk). Larger plus signs signify especially high-risk subclasses. The minus sign signifies a negative association with disease (higher levels = lower risk).

\*Population percentile values are from NMR data obtained from analysis of 3,437 subjects in the Framingham Offspring Study.

### NMR LIPID PANEL

	mg/dL	Current NCEP Risk Categories		
		Desirable	Borderline-High	High
Total Cholesterol	185	less than 200	200 - 239	240 or greater
LDL Cholesterol	112	Optimal*	Desirable	Borderline-High
		less than 100	100 - 129	130 - 159
HDL Cholesterol	37	Negative Risk Factor		Positive Risk Factor
		60 or greater	59 - 35	less than 35
Triglycerides	181	Desirable	Borderline-High	High
		less than 200	200 - 400	400 - 1,000

NMR lipid panel values are determined by summation of directly-measured concentrations of VLDL, LDL, and HDL subclasses after conversion to cholesterol or triglyceride units. NMR values agree well with those determined by standard chemical tests, but may differ if the patient's lipoprotein particles have less than the normal amount of cholesterol. In such cases, NMR values will likely give a better indication of CHD risk.

\*Goal for secondary prevention (patients with established CHD or diabetes)

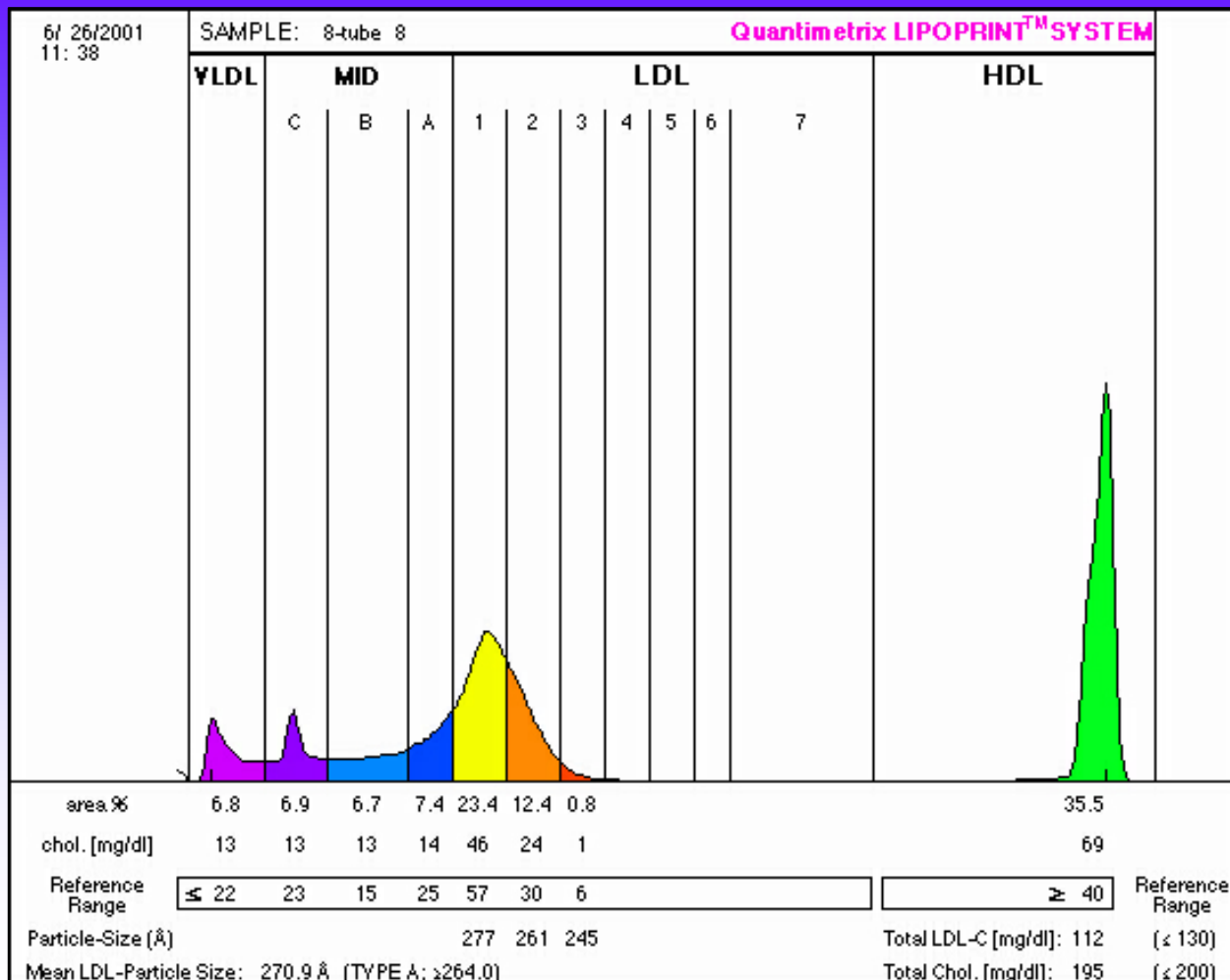
image taken from  
[www.lipoprofile.com](http://www.lipoprofile.com)

QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.

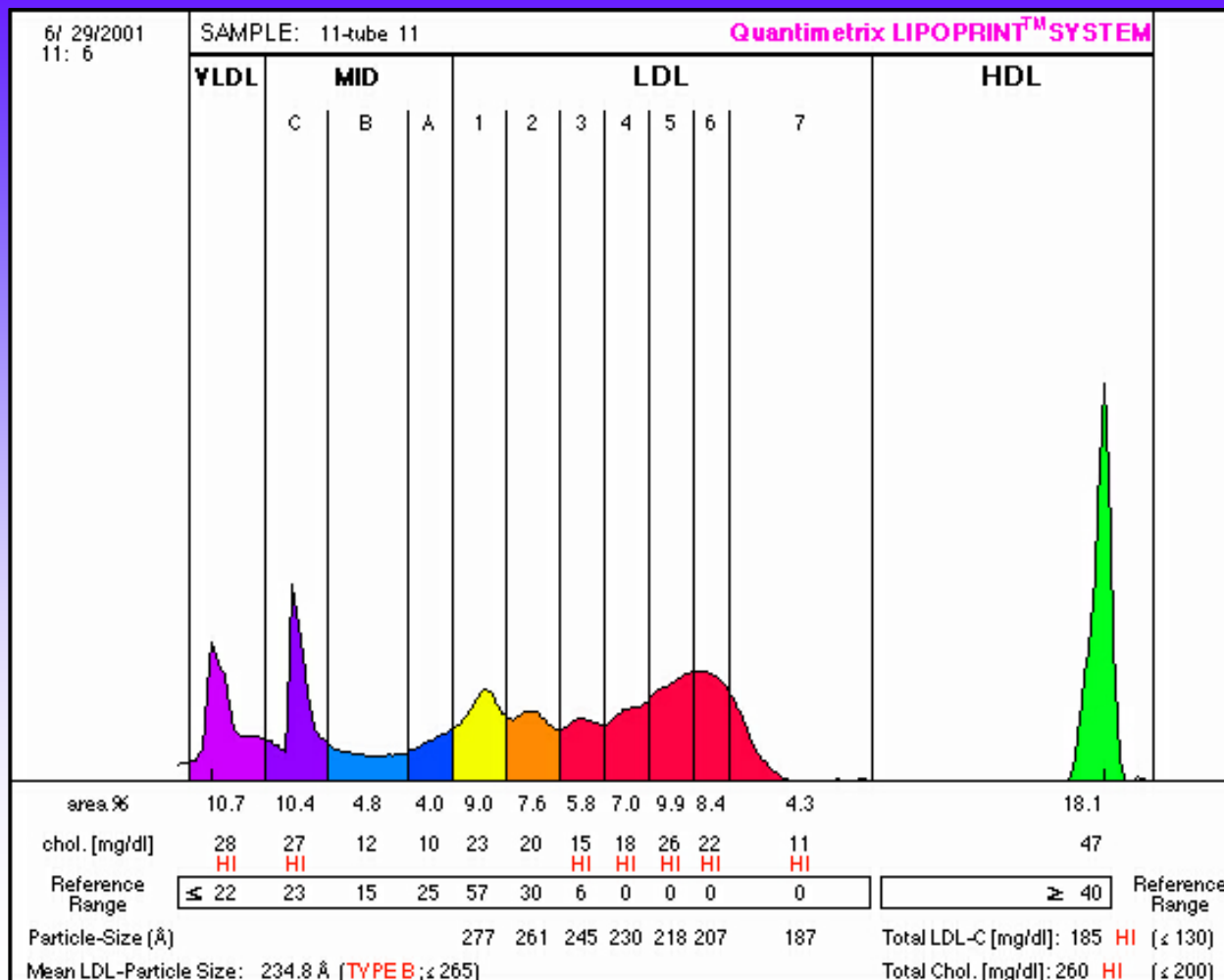
**More Information  
Easier Interpretation**

# PHENOTYPED FROM TYPE A...

LIPOPRINT®







COMING SOON!!!

# LIOPRINT HDL

Up to ten subfractions may be identified

Large HDL (subfractions 1 to 3)

Intermediate HDL (subfractions 4 to 7)

Small HDL (subfractions 8 to 10)

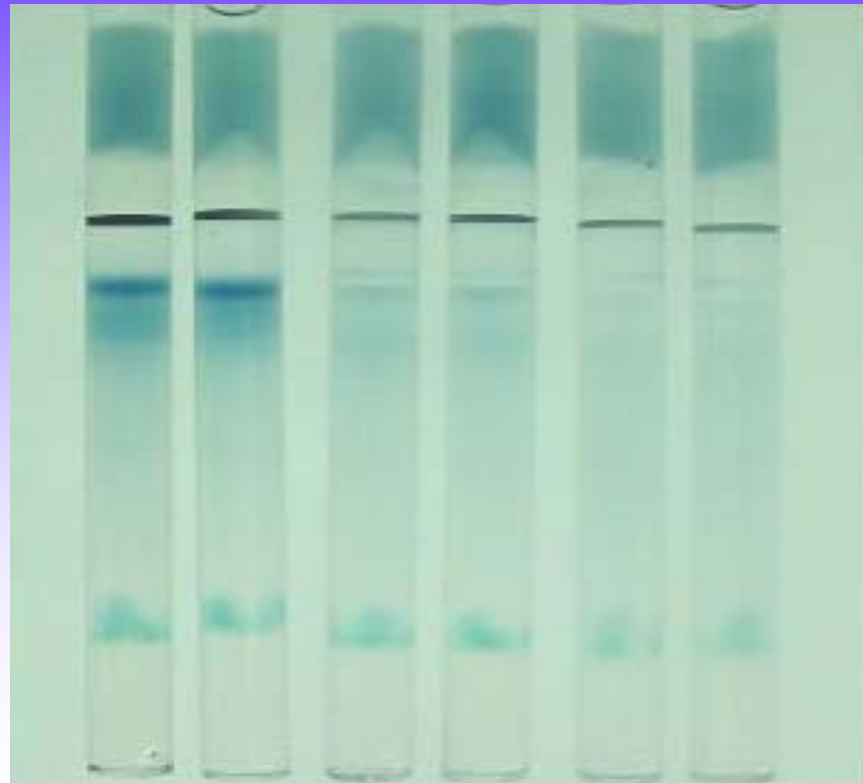
VLDL/LDL ----->

Large HDL ----->

Intermediate HDL -->

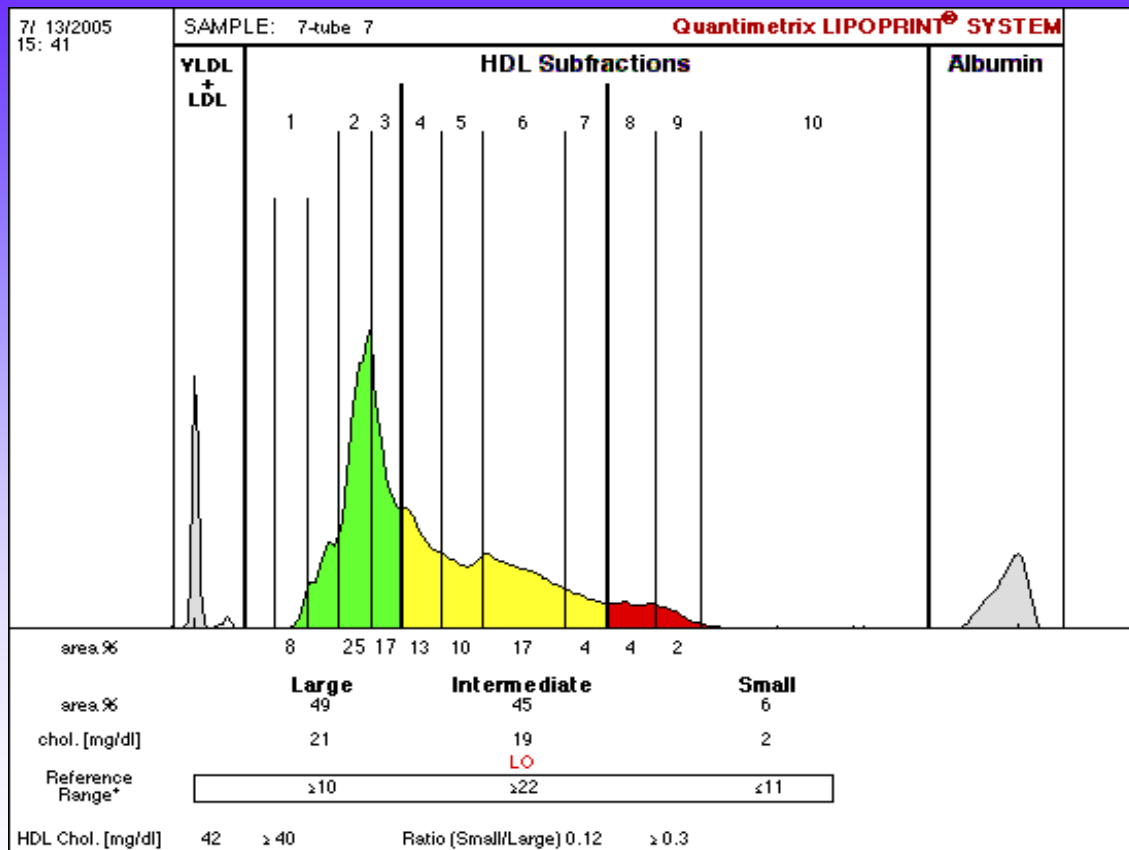
Small HDL ----->

Albumin ----->



# Primarily Large HDL particles

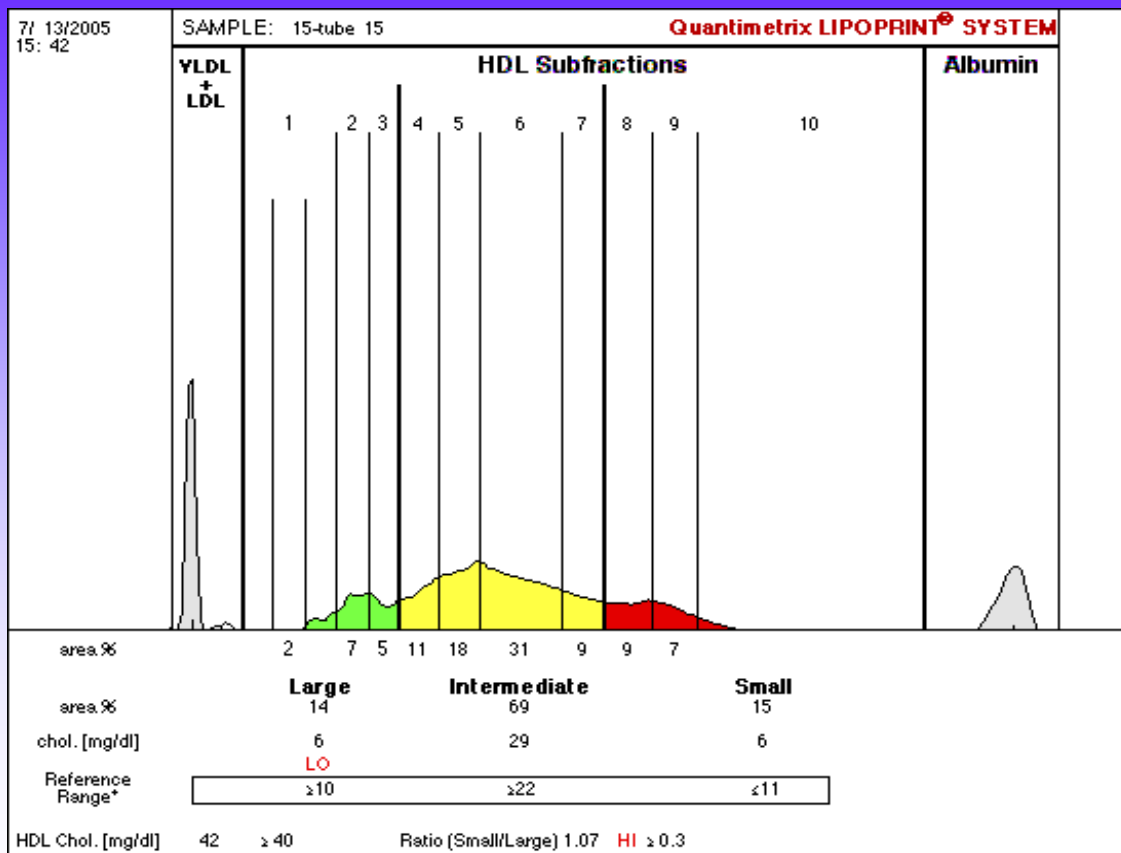
LIOPRINT®



\*Reference ranges derived from 123 serum samples that met the NCEP ATPIII guidelines for desirable lipid status

# Primarily Intermediate HDL particles

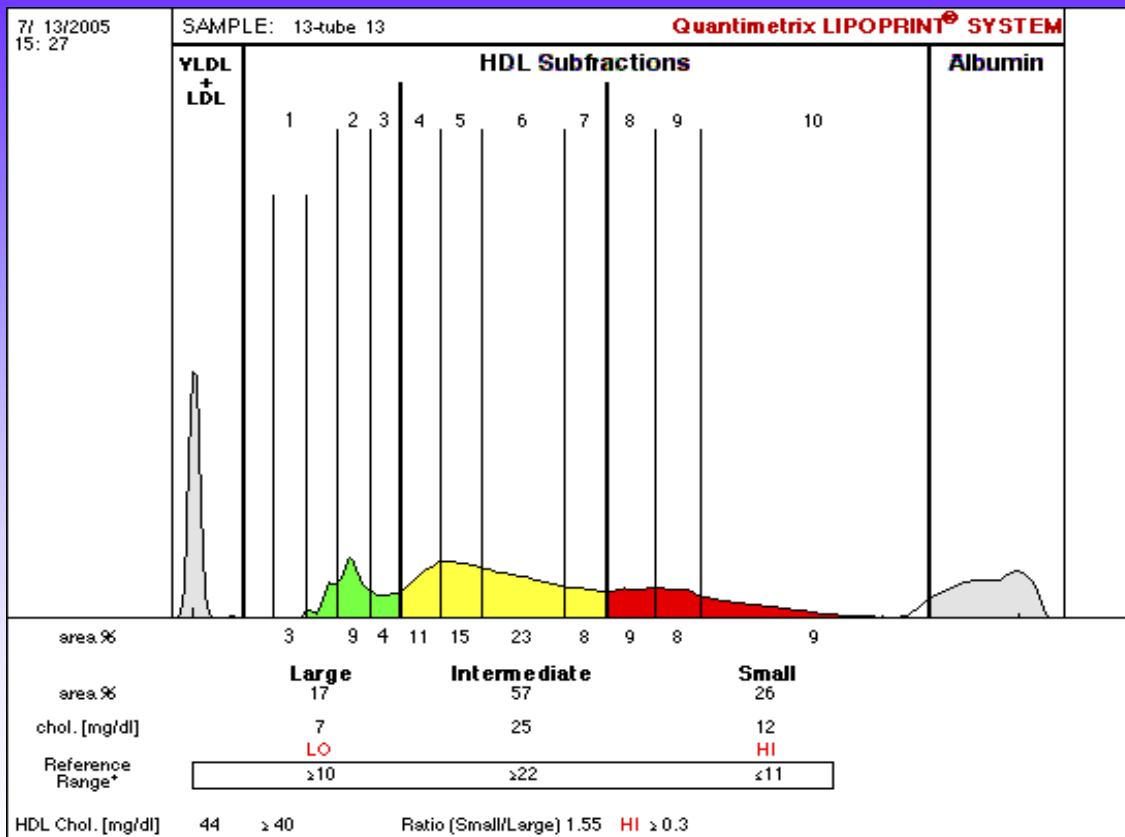
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\*Reference ranges derived from 123 serum samples that met the NCEP ATP III guidelines for desirable lipid status

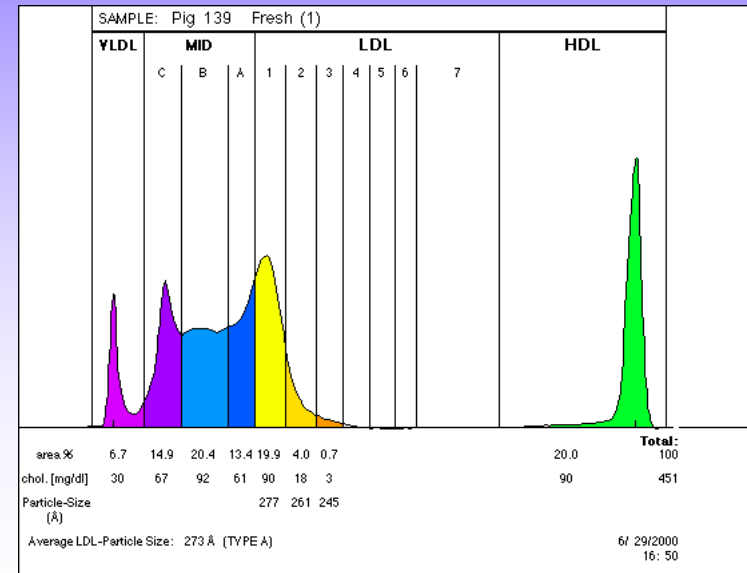
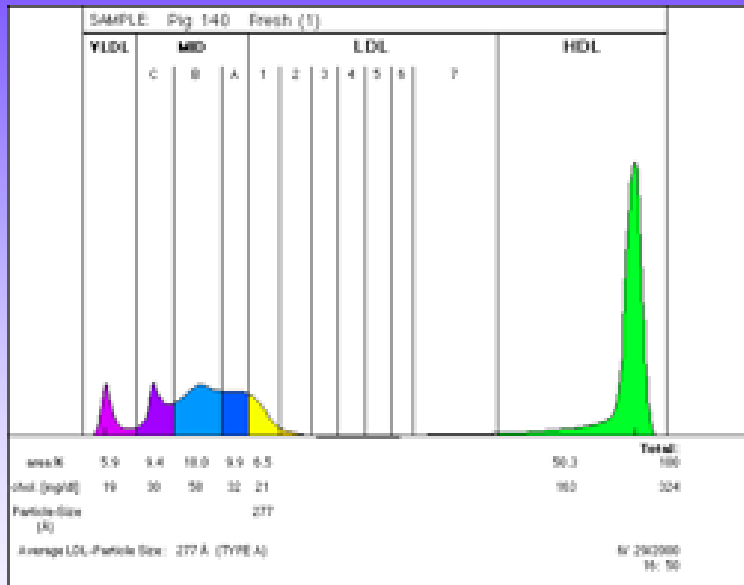
# Primarily Small HDL particle

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\*Reference ranges derived from 123 serum samples that met the NCEP ATPIII guidelines for desirable lipid status

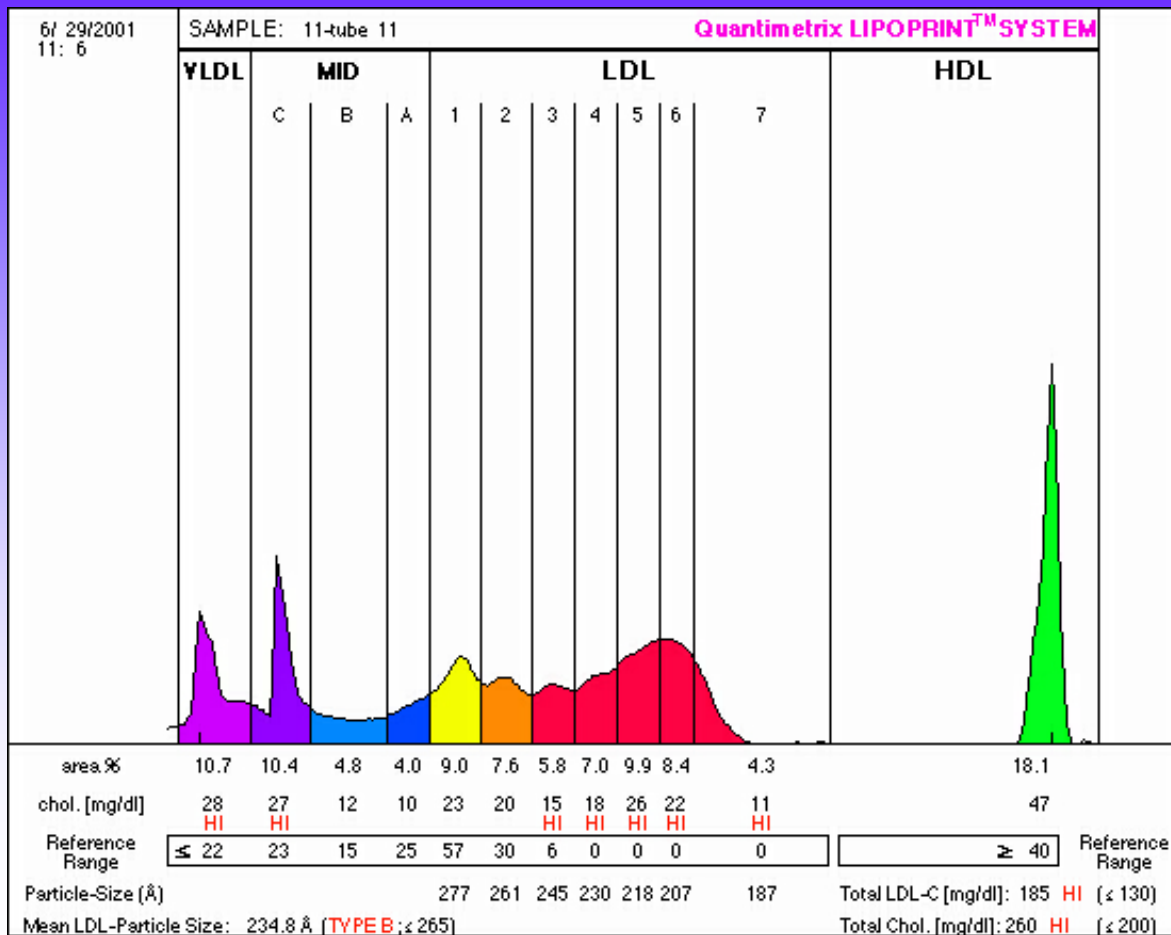
## Animal Research (Normal □ diet vs. high fat diet)





# Shouldn't you use Lipoprint?

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*The End*