Valid edition of the instructions for use: 2017-08. If you do not have this edition in the language you require, you can obtain it free of charge from **https://quantimetrix.com/resources/technical-documents/** or call toll-free: **+1-800-624-8380.**

Lipoprint®
LDL Subfractions Kit

Quantimetrix

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English

INTENDED USE AND INDICATIONS FOR USE

The Quantimetrix Lipoprint System LDL Subfractions Kit “Lipoprint LDL Kit” 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 >100 mg/dL. Lipoprotein 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.

SUMMARY AND EXPLANATION OF THE TEST

Plasma lipoproteins are spherical particles responsible for the transport of cholesterol, triglycerides and phospholipids. There are five major lipoprotein classes: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Low HDL cholesterol is a strong independent predictor of coronary heart disease (CHD) [1]. Increased LDL cholesterol (LDL-C) has been identified as a major risk factor for CHD [2]. It is known that the lipoprotein classes are heterogeneous, consisting of multiple subfractions that vary with respect to particle size, density and chemical composition. Lipoprotein heterogeneity has been demonstrated by various analytical methods such as density gradient ultracentrifugation [3], nuclear magnetic resonance (NMR) [4], non-denaturing gradient gel electrophoresis (GGE) [5] and the Lipoprint System, a linear, polyacrylamide gel electrophoresis system [6].

LDL can be resolved into a maximum of seven LDL subfractions with the Lipoprint System. The LDL subfractions have been named LDL-1, consisting of the largest particles, through LDL-7, consisting of the smallest particles.

Genetic as well as environmental factors are responsible for the differences in the degree of LDL heterogeneity among subjects. Age, gender and lipid status are known to affect the LDL subfraction profile [7]. Individuals exhibiting lipoprotein profiles, consisting primarily of the larger, buoyant LDL-1 and LDL-2 subfractions, have been designated as **Pattern A** while profiles with predominantly smaller and denser subfractions (LDL-3 through LDL-7) have been designated as **Pattern B** [5]. (Fig. 1)

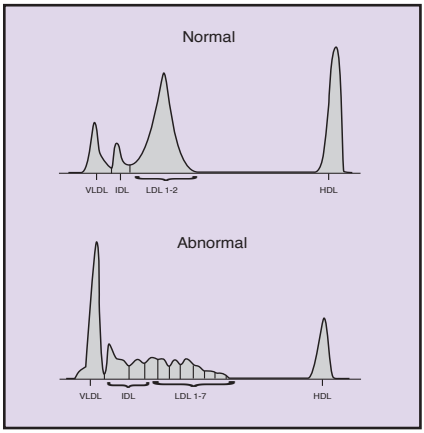

Figure 1. Normal (Pattern A) and abnormal (Pattern B) lipoprotein profiles

In a study of 109 patients with myocardial infarction (MI) Austin et al. [5] showed that LDL subfraction Pattern B was associated with a threefold increased risk of MI, independent of sex, age and relative weight. Krauss [8] reported similar findings. Rajman et al. [6] also reported on the risk factor associated with low density lipoprotein subfractions in normotriglyceridaemic men.

TEST PRINCIPLE

The Lipoprint LDL Kit consists of:

- Precast linear polyacrylamide gel (stacking gel and separating gel) in a glass tube (Fig. 2)
- Liquid loading gel with a lipophilic dye
- Buffer salts

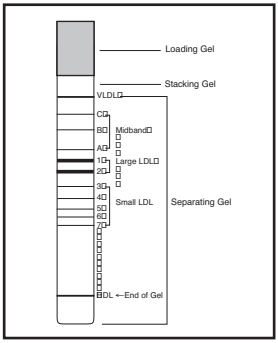

Figure 2. Lipoprint gel tube schematic

The dye binds proportionally to the relative amount of cholesterol in each lipoprotein [9]. The prestained lipoproteins subsequently undergo electrophoresis. During the first phase of the electrophoresis, the lipoprotein particles are concentrated by the loading and stacking gels into a sharp narrow band. As the lipoprotein particles migrate through the separating gelmatrix, they are resolved into lipoprotein bands according to their particle sizes from largest to smallest due to the sieving action of the gel: HDL migrates the farthest, followed by smalldense LDL, larger-buoyant LDL, Midbands (comprising primarily IDL) and VLDL. Chylomicrons, if present, will appear above the stacking gel or remain in the loading gel.

A typical Lipoprint profile consists of 1 VLDL band, 3 Midbands, up to 7 LDL bands and 1 HDL band. After the electrophoresis is completed, the various stained lipoprotein fractions (bands) present in the sample are identified by their mobility (Rf) using VLDL as the starting reference point (VLDL = 0) and HDL as the leading reference point (HDL = 1). (Fig. 3)

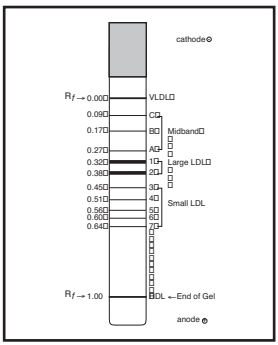

Figure 3. Mobilities of the lipoprotein bands

The relative area for each lipoprotein band is determined and multiplied by the total cholesterol concentration of the sample to yield the amount of cholesterol for each band in mg/dL. The total cholesterol concentration of the sample needs to be measured independently, e.g., a clinical analyzer or a point of care instrument.

PRODUCT DESCRIPTION

The Lipoprint LDL Kit consists of precast, high resolution polyacrylamide gel tubes, a loading gel solution containing a lipophilic dye and the buffer salts.

Reagents and Materials (Provided, See Quantimetrix Catalog No. 48-7002)

A 100 test kit consists of:

1.	Lipoprint LDL Gel Tubes Polyacrylamide, Buffer, Preservative	100 tubes
2.	Lipoprint LDL Loading Gel Acrylamide N, N-methylenebisacrylamide Lipophilic dye Catalyst Stabilizer Buffer	24 mL
3.	Lipoprint LDL Buffer Salts Tris (hydroxymethyl) aminomethane Boric Acid	6 vials
4.	Lipoprint LDL Product Insert	1 each

Lipoprint System (Not Provided, Product No. 48-9150/9152)

1. Computer (includes Lipoware Analysis Program)
2. Color Printer
3. Digital Scanner
4. Electrophoresis Chamber
5. Power Supply (120V/220V)
6. Preparation Rack
7. Preparation Light
8. Rimming Tool

Liposure - Lipoprotein Control (Not Provided, see Quantimetrix Catalog No. 48-7060-Level 1)

Materials Required (Not Provided)

1. Distilled or Deionized Water
2. 25 µL Automatic Pipettor
3. 200 µL Automatic Pipettor
4. Magnetic Stirrer
5. Parafilm™
6. Graduated Cylinders

Reconstitution of Reagents

The electrolyte buffer solution is reconstituted by dissolving one vial of Lipoprint LDL Kit Buffer salts in 1200 mL of distilled or deionized water.

Storage and Stability

Gel tubes, loading gel and buffer salts should be stored at 2-8°C. **Do not freeze.** With proper storage the reagents, opened or unopened, are stable until the date of expiration.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only

- Use the Lipoprint LDL Kit only in accordance with the Lipoprint Insert instructions.
- The loading gel solution contains acrylamide which is toxic when in contact with skin or swallowed. Avoid inhalation and prolonged exposure to the loading gel solution.
- The loading gel is light sensitive and is packaged in an amber glass bottle.
- Avoid pipetting by mouth and any physical contact with reagents or specimens.
- All samples, reagents and controls should be treated as potentially infectious if ingested or absorbed through prolonged skin contact. Precautions, as they apply to your facility, should be used for handling and disposal of materials at all times.

SPECIMENS AND SPECIMEN COLLECTION

- Only fasting (12 hours) samples should be used.
- Serum or EDTA plasma may be used (see p. 15).
- Do not use heparin as anticoagulant.
- Samples can be kept for up to 7 days at 2-8°C.
- Freezing of the sample is not recommended. However, if a sample needs to be frozen it should be frozen cryogenically (-70°C or below).

ASSAY PROCEDURE

1. Prepare the electrolyte buffer solution as described by dissolving one vial of the buffer salts in 1200 mL of deionized/distilled water.
2. Remove the Gel Tubes from the jar, wipe off and place them in the Preparation Rack with the unfilled end up (Fig. 4). Avoid touching the ends of the Gel Tube or exerting any pressure on the gels since this will cause air bubbles to be introduced into the gel. Do not use the Gel Tube if air bubbles appear inside or gel protrudes.
3. Remove the storage buffer completely from the top of the gels by shaking the rack while inverted. If necessary, blot the end of the tube while the tubes are inverted in order to remove excess buffer from inside the tube.
4. Apply 25 µL of sample to each tube. (Fig. 4)

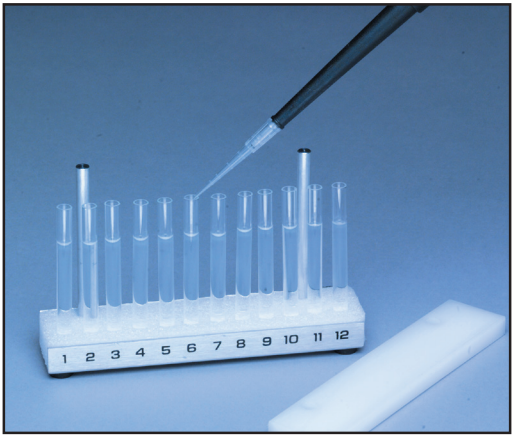

Figure 4. Sample application

5. Add 200 µL of Lipoprint Loading Gel to each tube.

6. Place a strip of Parafilm between the Gel Tubes and Preparation Rack Cover to avoid contamination. Mix the Loading Gel with the specimen by inverting the Preparation Rack several times. (Fig. 5)

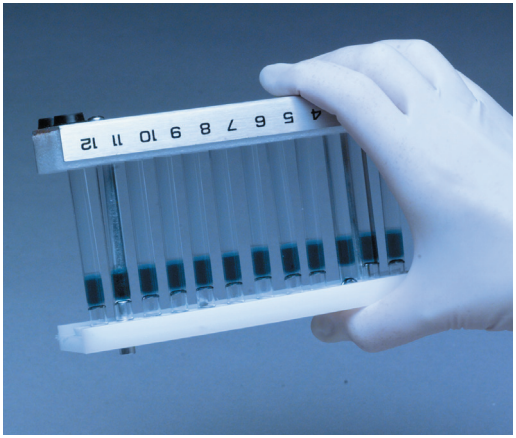

Figure 5. Mixing loading gel and specimens

7. Set the Preparation Light upright with the bulb at the top. Place the loaded Preparation Rack so the Loading Gel is touching the bulb. (Fig. 6) Photopolymerize the Loading Gels for 30 minutes (but no longer than 40 minutes).

Figure 6. Photopolymerization

8. After the photopolymerization is complete, remove each Gel Tube from the Preparation Rack and carefully insert it into the silicone adapter of the upper chamber. While holding the Gel Tube by the side, push it up until the loading gel end of the tube is flush with upper side of adapter. Wetting the top of the Gel Tube makes insertion easier. Avoid touching either end of the tube during this step. If running less than a full chamber, plug empty adapters with small

glass tubes provided for this purpose. Push tubes from top until flush with lower side of adapter. (Fig. 7)

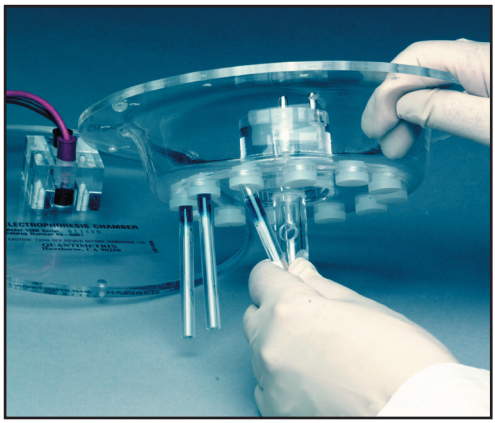

Figure 7. Loading tubes

9. Place 1000 mL of electrolyte buffer solution in the lower chamber and 200 mL in the upper chamber. The lower buffer may be reused up to five times. **Use only fresh buffer in the upper chamber.** The buffer must be at room temperature (RT: 18-27°C).
10. After both chambers are assembled and filled with buffer, thoroughly examine each tube for air bubbles. Dislodge any bubbles with a pipettor tip. Bubbles could obstruct the passage of electrical current.
11. Put the electrophoresis chamber lid in place and connect it to the power source. (Fig. 8) Adjust the power source to deliver the current of **3 mA per each Gel Tube** (e.g., 36 mA for 12 tubes, 18 mA for 6 tubes etc). The voltage should be set at maximum delivery (500V).

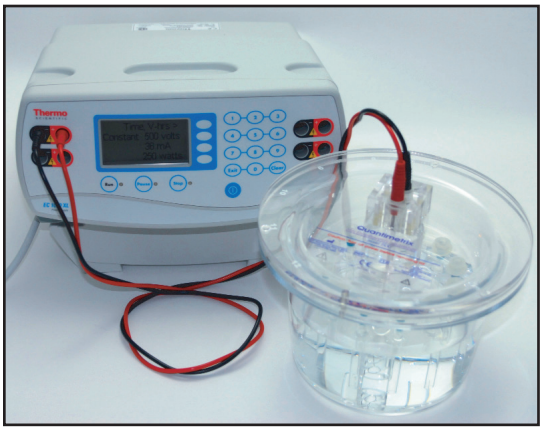

Figure 8. Assembled chamber

12. Electrophoresis time is approximately 60 minutes. Stop the electrophoresis when the HDL fraction is about 1 cm from the bottom of the fastest migrating Gel Tube.
13. When the electrophoresis is complete, turn the power OFF, remove the chamber lid and discard the electrolyte buffer in the upper chamber. The lower buffer may be retained and reused up to 5 times. Discard after 7 days.

14. Before removing the Gel Tubes from the electrophoresis chamber, wipe off excess buffer, then place in Preparation Rack for transport to the scanner for analysis. Allow the gel tubes to rest for at least 30 minutes but no longer than 2 hours before scanning.

Procedural Note: *Distortion of the separating gel surface and the VLDL band may occur during electrophoresis. The distortion is corrected after the gels are removed from the electrophoresis chamber by carefully introducing the rimming tool from the top of the loading gel, alongside the glass. In a circular motion rim the surface of the separating gel. The gel will return to its normal shape. Avoid dislodging the loading gel when withdrawing the tool (should the gel become dislodged fill the void with deionized/distilled water).*

QUALITY CONTROL

Reliability of test results should be routinely monitored with control material that reasonably emulates performance of patient specimens. Use of quality control material is recommended with each run of patient samples. Control materials are intended only as monitors of accuracy and precision. The recovery of control values within the appropriate ranges should be the criteria in validating assay performance.

Quality control should be performed in conformance with local, state and federal regulations or accreditation requirements. An appropriate quality control material, Liposure, is available from Quantimetrix Corporation.

QUALITATIVE RESULTS

Note: *The various bands of a Lipoprint profile may be identified using the Lipoprint Template for a qualitative assessment (homogeneous LDL distribution vs. heterogeneous distribution).*

The lipoprotein profile reflects the lipid status of the patient sample. (Fig. 9) A normal lipoprotein profile (Pattern A) will typically exhibit bands for VLDL; Midbands-C, B and A (these comprise IDL); LDL-1 and 2 and HDL. The presence of additional LDL subfractions (LDL-3 through 7) is indicative for heterogeneous LDL (i.e., Pattern B).

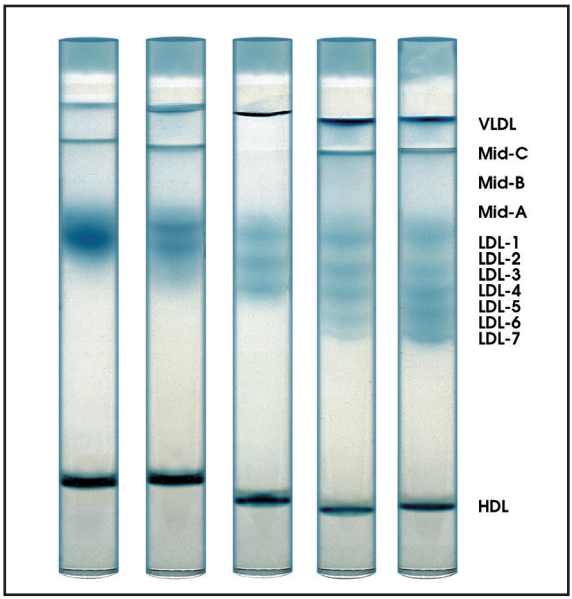

Figure 9. Lipoprotein subfraction distributions for five individuals - from a homogeneous LDL pattern on the left to a progressively more heterogeneous pattern on the right.

Using the Lipoprint Template
The Lipoprint LDL System Template is used to identify the lipoprotein subfractions present on the gel. (Fig. 10)

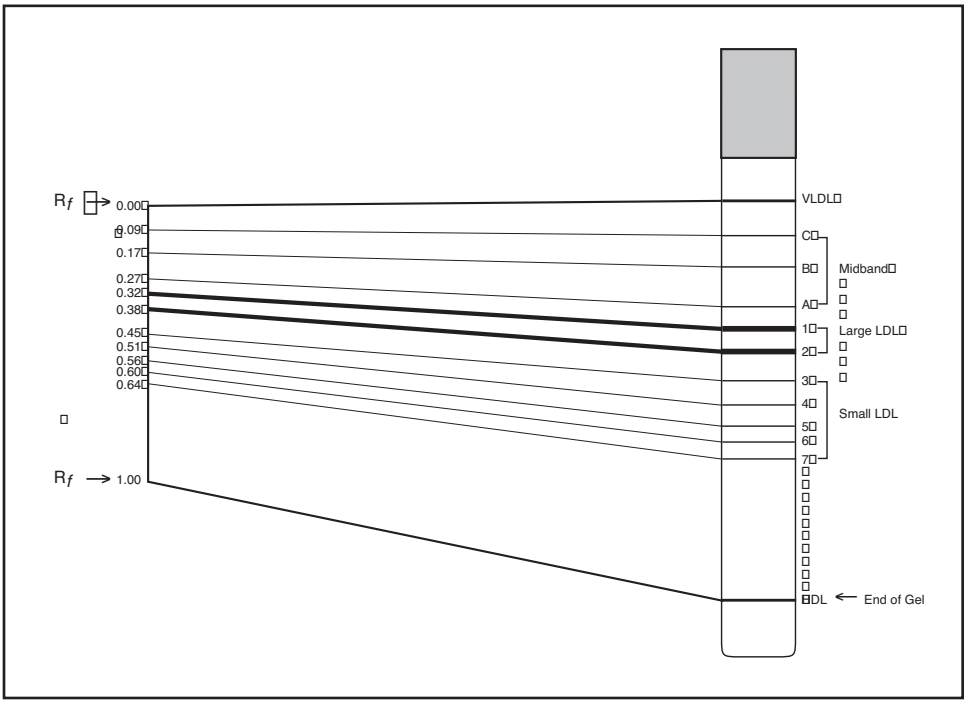

Figure 10. Lipoprint System LDL Subfractions Template

1. Mark the center of each lipoprotein band present with a marking pen on the glass tube.
2. Align the VLDL fraction at the top of the separating gel with the line on the template marked “VLDL.”
3. Slide the gel along the template until the HDL band is superimposed over the line marked “HDL.”
4. Determine the lipoprotein subfractions present in the sample by matching the bands on the gel to the corresponding lines on the template.

QUANTITATIVE RESULTS
Note: *A total cholesterol value for each sample to be analyzed must be obtained using a clinically approved cholesterol method prior to generating quantitative results.* ***The total cholesterol concentration of the sample must be >100 mg/dL.***

The Gel Tubes are scanned and the relative area of each lipoprotein subfraction is established by dropping vertical lines at predetermined cut-off ranges for each band. The amount of cholesterol in each lipoprotein band is calculated by multiplying the relative area of each band by the total cholesterol of the sample. LDL cholesterol is calculated as the sum of the cholesterol concentrations of all the LDL subfractions plus the Midbands A, B and C.

Traditionally lipoprotein subfraction profiles have been classified into Type A (normal) and Type B (abnormal) based on the average particle size of the LDL particles [5]. A normal Lipoprint profile with predominately large LDL (LDL-1 and LDL-2) is consistent with Type A. (Fig. 11)

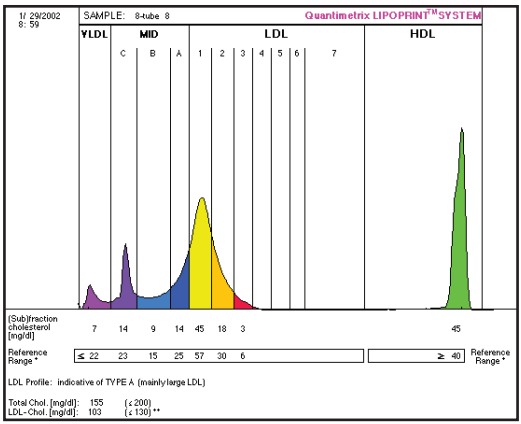

Figure 11. Typical normal Lipoprint profile

Also, an abnormal Lipoprint profile with predominantly small LDL (LDL-3 to LDL-7) is consistent with Type B, as described in the literature. (Fig. 12)

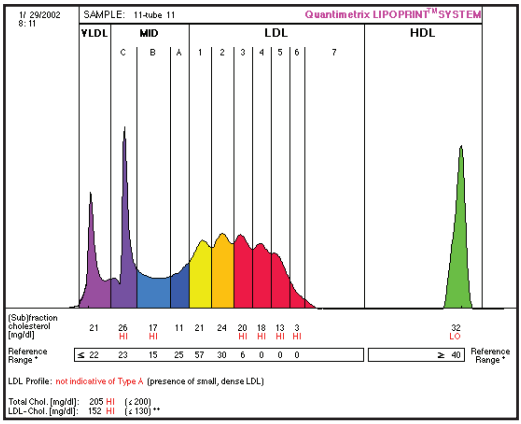

Figure 12. Typical abnormal Lipoprint profile

LIMITATIONS

- The Lipoprint LDL test should be used in conjunction with other data (e.g., additional clinical testing, physician observations, family history, etc.)
- Heparin interferes with the separation of LDL subfractions.
- Total cholesterol in the sample must be above 100 mg/dL to avoid overestimation of VLDL cholesterol.
- Chylomicrons in sample will invalidate the subfraction cholesterol measurement. Appearance of the specimen (i.e., cloudiness, turbidity or a creamy layer on top of the sample) after overnight refrigeration should be used as the confirming test for presence of chylomicrons.
- Lipoprint results have not been evaluated for testing lipoproteins during pregnancy.
- NCEP has not published guidelines for optimal/ desirable values for LDL subfractions.

EXPECTED VALUES

HDL-C and LDL-C

The National Cholesterol Education Program (NCEP) had established risk-related cut-off points for LDL cholesterol and HDL cholesterol. These were reaffirmed in May 2001 by the third report on the NCEP Adult Treatment Panel (ATP III) [10]:

Fraction	Range	Status
HDL-C	≥ 40 mg/dL	Decreased Risk at Higher Levels
	< 40 mg/dL	Increased Risk
LDL-C	≤ 100 mg/dL	Optimal for Patients with Coronary Heart Disease (CHD)
	< 130 mg/dL	Desirable
	130 - 159 mg/dL	Borderline High Risk
	≥ 160 mg/dL	High Risk

LDL-C includes all the lipoprotein particles with d > 1.006 to 1.063 kg/L, such as VLDL remnants, IDL, Lp(a) and LDL [2]. These particles correspond to the sum of Midbands and LDL subfractions resolved by the Lipoprint System.

Subfraction Cholesterol

Expected normal values for the individual subfractions on the Lipoprint System were established as follows: Self declared healthy individuals, N = 273 (aged 18 to 85 years, 166 females and 107 males, 47% Caucasian, 18% Hispanic, 16% Asian, 5% Black and 14% undeclared) were recruited. These volunteers were required to fast for 12 hours. Whole blood was collected into a serum tube via venipuncture. Serum samples were then used to test for indicated lipid parameters and to generate the lipoprotein profiles with the Lipoprint LDL system.

Exclusion criteria included diabetics, individuals taking lipid lowering medication and those with recent heart attacks. Additionally, pregnant women were excluded from the study due to their changed lipid status [12].

Only samples that met the NCEP guidelines (ATP III) for desirable lipid status [10], namely TC < 200 mg/dL, LDL < 130 mg/dL, HDL > 40 mg/dL and Triglycerides < 150 mg/dL were used to determine the expected values. The normal samples, N =114 (aged 18 to 84 years, 32% male and 68% female) were used to establish expected normal values, defined as the 95% confidence interval (mean ± 2 SD) for each lipid parameter as obtained on the Lipoprint LDL System. (Table 1)

Table 1. Normal population

		Midband Cholesterol			LDL Subfraction Cholesterol					
	VLDL (mg/dL)	Mid-C (mg/dL)	Mid-B (mg/dL)	Mid-A (mg/dL)	LDL-1 (mg/dL)	LDL-2 (mg/dL)	LDL-3 (mg/dL)	Total LDL (mg/dL)	HDL (mg/dL)	TC (mg/dL)
Range	6 - 26	9 - 24	5 - 17	6 - 26	24 - 59	4 - 32	0 - 4	59 - 128	40 - 103	123 - 199
Mean	12.9	16.5	10.1	16.6	41.1	14.3	1.9	95.7	56.8	168.1
SD	4.12	2.82	2.40	4.26	7.85	6.82	0.81	16.56	11.29	18.45
95% Range	4.7 - 22.1	10.9 - 22.1	5.3 - 14.9	8.1 - 25.1	25.4 - 56.8	0.7 - 28.6	0 - 3.6	62.5 - 128.8	40.0 - 79.4	131.2 - 200.0
N*	114	114	114	114	114	114	44	114	114	114

* Number of samples showing the respective fractions.

It is recommended that each laboratory establish its own normal range, which may be unique to the population it serves, depending on geographical, patient or environmental factors.

Data for the population outside the NCEP group, N = 141 (aged 18 to 77 years, 46% male and 54% female) are shown in Table 2.

Table 2. Population outside NCEP guidelines

		Midband Cholesterol			LDL Subfraction Cholesterol							
	VLDL (mg/dL)	Mid-C (mg/dL)	Mid-B (mg/dL)	Mid-A (mg/dL)	LDL-1 (mg/dL)	LDL-2 (mg/dL)	LDL-3 (mg/dL)	LDL-4 (mg/dL)	LDL-5 (mg/dL)	Total LDL (mg/dL)	HDL (mg/dL)	TC (mg/dL)
Range	5 - 69	9 - 40	5 - 37	5 - 34	9 - 77	7 - 55	0 - 35	0 - 28	0 - 11	58 - 215	26 - 137	104 - 319
Mean	24.4	23.2	14.6	18.6	46.5	31.5	9.2	4.7	5.5	134.8	54.2	219.4
SD	13.50	5.65	5.21	6.14	15.13	10.98	7.50	6.05	3.6	27.17	18.05	35.04
95% Range	0 - 51.4	12.0 - 34.7	4.2 - 25.0	6.4 - 30.9	16.2 - 76.8	9.5 - 53.5	0 - 24.2	0 - 16.8	0 - 16.8	80.5 - 189.1	18.2 - 90.2	148.6 - 290.2
N*	141	141	141	141	141	141	115	44	8	141	141	141

* Number of samples showing the respective fractions.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Four samples were tested for intra-assay and inter-assay variability. Samples with low, medium and high HDL-C and LDL-C were selected:

Sample 1: low LDL-C, high HDL-C and homogeneous LDL pattern (LDL-1 and 2 only)
Sample 2: medium LDL-C, medium HDL-C and slightly disperse LDL pattern (LDL-1, 2, 3)
Sample 3: high LDL, low HDL and disperse LDL pattern (LDL-1, 2, 3 and 4)
Sample 4: high LDL-C, intermediate HDL-C and disperse LDL pattern (LDL-1 through 7)

Intra-assay Precision

Samples were tested in replicates of 12 (the maximum capacity of the electrophoresis chamber). Precision results for HDL-C, LDL-C (sum of Midbands C, B, A and LDL subfractions) and VLDL-C are shown in Table 3.

Table 3. Intra-assay precision data for **HDL**, **LDL** and **VLDL**

Sample	N	HDL-C Mean (mg/dL)	SD	CV (%)	LDL-C Mean (mg/dL)	SD	CV (%)	VLDL-C Mean (mg/dL)	SD	CV (%)
1	12	55	1.47	2.68	86	0.90	1.05	17	0.99	5.86
2	12	42	0.78	1.87	120	1.43	1.20	15	0.99	6.43
3	12	31	0.90	2.87	133	2.02	1.52	35	1.97	5.58
4	12	48	1.37	2.84	180	2.02	1.12	23	1.66	7.28

Precision data for Midbands C, B, A and LDL subfractions 1-7 are seen in Tables 4 and 5.

Table 4. Intra-assay precision data for **MIDBAND** subfractions

Sample	N	Mid-C Mean (mg/dL)	SD	CV (%)	Mid-B Mean (mg/dL)	SD	CV (%)	Mid-A Mean (mg/dL)	SD	CV (%)
1	12	16	0.76	4.75	9	0.60	6.67	14	1.56	11.14
2	12	17	1.44	8.47	13	0.51	3.92	14	0.72	5.14
3	12	22	1.21	5.50	16	0.47	2.94	13	0.50	3.85
4	12	30	1.44	4.80	15	1.36	9.07	10	0.77	7.70

Table 5. Intra-assay precision data for **LDL** subfractions

Sample	N	LDL-1 Mean (mg/dL)	SD	CV (%)	LDL-2 Mean (mg/dL)	SD	CV (%)	LDL-3 Mean (mg/dL)	SD	CV (%)	LDL-4 Mean (mg/dL)	SD	CV (%)
1	12	36	0.60	1.67	10	1.68	16.80	N/A	-	-	N/A	-	-
2	12	28	0.91	3.25	32	0.70	2.19	14	1.65	11.79	N/A	-	-
3	12	21	0.51	2.43	19	0.88	4.63	18	0.38	2.11	19	0.86	4.53
4	12	24	0.86	3.58	22	0.80	3.64	17	0.28	1.65	20	0.49	2.45

Sample	N	LDL-5 Mean (mg/dL)	SD	CV (%)	LDL-6 Mean (mg/dL)	SD	CV (%)	LDL-7 Mean (mg/dL)	SD	CV (%)
1	12	N/A	-	-	N/A	-	-	N/A	-	-
2	12	N/A	-	-	N/A	-	-	N/A	-	-
3	12	N/A	-	-	N/A	-	-	N/A	-	-
4	12	24	0.41	1.72	16	0.72	4.62	4	0.68	17.89

Inter-assay Precision

Four samples were tested in duplicate, twice a day for 5 days, in 4 electrophoresis chambers using a single lot of Gel Tubes. The corresponding precision results for inter-assay are shown in Tables 6 - 8.

Table 6. Inter-assay precision data for **HDL**, **LDL** and **VLDL**

Sample	N	HDL-C Mean (mg/dL)	SD	CV (%)	LDL-C Mean (mg/dL)	SD	CV (%)	VLDL-C Mean (mg/dL)	SD	CV (%)
1	80	60	1.49	2.49	94	1.41	1.50	10	0.90	9.40
2	80	46	1.45	3.15	137	1.73	1.26	11	0.91	8.27
3	80	32	1.52	4.75	160	2.03	1.27	33	2.35	7.12
4	80	50	2.32	4.69	178	2.79	1.57	23	1.97	8.57

Table 7. Inter-assay precision data for **MIDBAND** subfractions

Sample	N	Mid-C Mean (mg/dL)	SD	CV (%)	Mid-B Mean (mg/dL)	SD	CV (%)	Mid-A Mean (mg/dL)	SD	CV (%)
1	80	14	1.23	8.79	10	0.87	8.27	19	2.00	10.90
2	80	18	1.99	11.06	13	0.86	6.62	16	1.33	8.31
3	80	21	2.84	13.63	22	1.04	4.73	17	1.27	7.47
4	80	28	1.87	7.79	13	0.91	7.00	10	0.81	8.10

Table 8. Inter-assay precision data for **LDL** subfractions

Sample	N	LDL-1 Mean (mg/dL)	SD	CV (%)	LDL-2 Mean (mg/dL)	SD	CV (%)	LDL-3 Mean (mg/dL)	SD	CV (%)	LDL-4 Mean (mg/dL)	SD	CV (%)
1	80	41	1.61	3.92	11	1.47	13.50	N/A	-	-	N/A	-	-
2	80	37	1.43	3.86	39	1.50	3.85	14	2.69	19.21	N/A	-	-
3	80	29	1.10	3.79	24	1.73	7.21	20	1.33	6.65	20	1.21	6.05
4	80	24	0.88	3.67	22	1.48	6.73	17	0.95	5.59	20	0.69	3.45

Sample	N	LDL-5 Mean (mg/dL)	SD	CV (%)	LDL-6 Mean (mg/dL)	SD	CV (%)	LDL-7 Mean (mg/dL)	SD	CV (%)
1	80	N/A	-	-	N/A	-	-	N/A	-	-
2	80	N/A	-	-	N/A	-	-	N/A	-	-
3	80	N/A	-	-	N/A	-	-	N/A	-	-
4	80	24	0.62	2.58	17	2.05	12.06	4	1.39	33.90

Linearity
Linearity studies were conducted with two serum samples fortified with either HDL-C or LDL-C isolated by ultracentrifugation.

Sample 1
LDL-C: 695 mg/dL
HDL-C: 260 mg/dL
VLDL-C: 140 mg/dL

Sample 2
LDL-C: 163 mg/dL
HDL-C: 178 mg/dL
VLDL-C: 38 mg/dL

Serial dilutions were prepared using a physiological human serum albumin solution. The concentrations observed for total LDL-C, HDL-C and VLDL-C were compared to the expected values. (Tables 9 - 11) Percent recovery was calculated as follows:
% recovery = (observed value/expected value) x 100.

Table 9. Dilution linearity for **LDL**

Dilution (%)	Observed LDL (mg/dL)	Expected LDL (mg/dL)	Recovery (%)
0%	695	695	100.0
10%	627	626	100.2
30%	466	486	95.9
50%	351	348	100.9
70%	195	209	93.3
95%	59	69	85.6

Dilution (%)	Observed LDL (mg/dL)	Expected LDL (mg/dL)	Recovery (%)
0%	163	163	100.0
5%	155	157	98.7
10%	151	148	102.0
50%	84	89	94.4
90%	15	15	100.0
95%	13	13	100.0

Table 10. Dilution linearity for **HDL**

Dilution (%)	Observed HDL (mg/dL)	Expected HDL (mg/dL)	Recovery (%)
0%	260	260	100.0
10%	218	234	93.2
30%	174	182	95.9
50%	117	130	90.0
70%	73	78	93.6
90%	25	26	96.2

Dilution (%)	Observed HDL (mg/dL)	Expected HDL (mg/dL)	Recovery (%)
0%	178	178	100.0
5%	170	169	100.6
10%	162	160	101.3
50%	95	89	106.7
95%	11	9	122.2
97%	5	5.3	94.3

Table 11. Dilution linearity for **VLDL**

Dilution (%)	Observed VLDL (mg/dL)	Expected VLDL (mg/dL)	Recovery (%)
0%	140	140	100.0
10%	129	126	102.4
30%	99	98	101.0
50%	75	70	107.1
70%	48	42	115.5
90%	22	14	157.1

Dilution (%)	Observed VLDL (mg/dL)	Expected VLDL (mg/dL)	Recovery (%)
0%	38	38	100.0
5%	36	37	97.3
10%	34	35	97.1
50%	24	24	100.0
90%	17	13	130.8
95%	13	11.4	114.0

Dose-Response

Serum sample A (containing a wide range of subfractions) was blended with an equal volume of sample B (containing primarily LDL-2) to give AB. The samples were analyzed with the Lipoprint System, the cholesterol concentrations of the individual fractions were determined and the observed values were compared with the expected values. (Table 12)

Similarly, a serum sample A (containing a wide range of subfractions) was blended with an equal volume of serum sample C (containing primarily LDL-1) to give AC. The samples were analyzed with the Lipoprint System and the cholesterol concentrations of the individual fractions were determined and compared with the expected values. (Table 13)

Table 12.
Observed vs. expected values for blended Probe AB

	A	B	AB		
	mg/dL	mg/dL	Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
VLDL	35	20	28	29	103.6
Mid-C	37	20	29	28	97.6
Mid-B	35	15	25	21	84.0
Mid-A	15	15	15	15	100.0
LDL-1	21	29	25	26	104.0
LDL-2	17	49	33	37	112.1
LDL-3	14	28	21	25	119.0
LDL-4	14	5	10	12	120.0
LDL-5	12	-	6	5	83.8
LDL-6	9	-	4	2	50.0
LDL-7	15	-	7	2	28.8
HDL	29	35	32	35	109.4

Table 13.
Observed vs. expected values forblended sample AC

	A	C	AC		
	mg/dL	mg/dL	Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
VLDL	35	13	24	25	104.2
Mid-C	37	16	27	25	92.6
Mid-B	35	11	23	18	78.3
Mid-A	15	16	16	14	87.5
LDL-1	21	54	38	36	94.8
LDL-2	17	38	28	34	121.4
LDL-3	14	7	11	16	145.4
LDL-4	14	-	7	11	157.1
LDL-5	12	-	6	5	83.3
LDL-6	9	-	4	2	50.0
LDL-7	15	-	7	2	28.6
HDL	29	40	35	37	105.7

Sensitivity

Sensitivity of the Lipoprint System was defined as the minimum concentration of HDL-C and total LDL-C that can be detected reliably. It was determined as the intersection of the lower 95% confidence interval of the mean with the x-axis when plotting a dilution series of expected vs. actual values for the individual lipoprotein parameters: VLDL sensitivity is ≥ 2.02 mg/dL, HDL sensitivity is ≥ 3.65 mg/dL and Total LDL sensitivity is ≥ 8.30 mg/dL.

Interferences

Potentially interfering substances were spiked into serum samples at the concentrations listed in Table 14 and tested next with uncompromised samples (4 replicates). Hemoglobin at concentrations above 200 mg/dL and heparin at typical concentrations found in heparinized plasma collection tubes were found to interfere with the Lipoprint test.

Table 14. Interferences

Interferant	Conc. (mg/dL)	VLDL-C (mg/dL)	Recovery (%)	LDL-C (mg/dL)	Recovery (%)	HDL-C (mg/dL)	Recovery (%)
None	-	17±1.1	100	115±1.9	100	42±2.4	100
Bilirubin	20	15±0.9	89	115±2.2	100	45±1.6	107
Hemoglobin	500	28±0.3	165	106±2.0	92	40±2.0	95
Hemoglobin	200	18±0.2	106	116±0.7	101	40±0.6	95
Niacin	2.5	16±0.7	94	115±1.5	100	43±2.0	102
EDTA	200	16±1.0	94	114±0.4	99	44±1.2	105
Heparin	14 U/mL	20±0.4	118	113±1.4	98	41±1.9	98

Serum vs. Plasma Comparison
Serum and EDTA plasma samples from 37 patients were compared on the Lipoprint System. The cholesterol values (mg/dL) of all lipoprotein fractions and subfractions (N = 322), up to 12 per sample, generated by the Lipoprint system were plotted and correlated:

$$\textbf{Cholesterol}_{plasma} = 0.995\ (\textbf{Cholesterol}_{serum}) + 1.158 \qquad (r^2 = 0.971)$$

Sample Stability
Serum samples (N = 22) covering a wide range of cholesterol concentrations and containing 7 - 12 fractions and subfractions were kept in the refrigerator for 7 days. The Lipoprint profiles for all samples were generated on day 3 and day 7 and the resulting cholesterol values of all lipoprotein fractions and subfractions (N = 206) were compared:

$$\textbf{Cholesterol}_{day\ 3} = 0.996\ (\textbf{Cholesterol}_{day\ 0}) + 0.075 \qquad (r^2 = 0.976)$$

$$\textbf{Cholesterol}_{day\ 7} = 0.964\ (\textbf{Cholesterol}_{day\ 0}) + 0.758 \qquad (r^2 = 0.961)$$

It was concluded that serum and EDTA plasma are equally suitable as specimens and that the samples may be stored refrigerated (2 - 8°C) for up to 7 days.

Accuracy by Correlation
The Lipoprint Test System was compared to a direct HDL method (EQUAL Diagnostics HDL Direct Liquid Select) and a direct LDL method (EQUAL Diagnostics LDL Direct Liquid Select). A population of 268 serum samples with LDL-C ranging from 55 - 218 mg/dL (Lipoprint) and 54 - 215 mg/dL (direct LDL) as well as HDL-C ranging from 24 - 129 mg/dL (Lipoprint) and 26 - 137 mg/dL (direct HDL) was evaluated. (Tables 15 and 16)

Table 15.

	Lipoprint HDL	Direct HDL
N	268	268
Mean (mg/dL)	53.2	54.8
SD (mg/dL)	15.13	15.43
Regression	Lipoprint HDL = 0.9361 (HDL_{direct}) + 1.8607	
r^2	0.912	

Table 16.

	Lipoprint LDL	Direct LDL
N	268	268
Mean (mg/dL)	121.9	116.3
SD (mg/dL)	30.73	29.58
Regression	Lipoprint LDL = 0.998 (LDL_{direct}) + 5.7995	
r^2	0.923	

A similar comparison was performed between Ultracentrifugation (ß-Quantification) and the Lipoprint LDL System. A population of 40 serum samples with LDL-C ranging from 66 - 211 mg/dL (Lipoprint) and 68 - 218 mg/dL (ß-Quantification), HDL-C ranging from 29 - 91 mg/dL (Lipoprint) and 28 - 90 mg/dL (ß-Quantification) as well as VLDL-C ranging from 9.5 - 49 mg/dL (Lipoprint) and 6 - 57 mg/dL (ß-Quantification) was evaluated. (Tables 17 - 19.)

Table 17.

	Lipoprint LDL	ß-Quant LDL
N	40	40
Mean (mg/dL)	130.8	130.0
SD (mg/dL)	30.14	30.42
Regression	Lipoprint LDL = 0.933 ($LDL_{ß\text{-}Quant}$) + 9.430	
r^2	0.887	

Table 18.

	Lipoprint HDL	ß-Quant HDL
N	40	40
Mean (mg/dL)	53.5	53.5
SD (mg/dL)	15.29	15.71
Regression	Lipoprint HDL = 0.944 ($HDL_{ß\text{-}Quant}$) + 3.030	
r^2	0.941	

Table 19.

	Lipoprint VLDL	ß-Quant VLDL
N	40	40
Mean (mg/dL)	24.7	22.9
SD (mg/dL)	10.34	12.61
Regression	Lipoprint VLDL = 0.689 ($VLDL_{ß\text{-}Quant}$) + 7.990	
r^2	0.8216	

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