



# Lipoprint<sup>®</sup> System HDL subfractions kit

**REF** 48-9002 *For Research Use Only*

## INTENDED USE

The Quantimetrix Lipoprint System HDL Subfractions Kit is a polyacrylamide gel electrophoresis test intended to separate and measure HDL subfractions cholesterol in fasting serum or plasma. The HDL kit is intended for Research Use Only (RUO) - Not for use in diagnostic procedures.

## SUMMARY AND EXPLANATION OF THE TEST

Coronary artery disease (CAD) is one of the leading causes of illness and death [1]. Serum lipid and lipoprotein levels are the most highly correlated factors contributing to increased risk of coronary artery disease. The utility of these biochemical parameters in predicting coronary risk was demonstrated by the Framingham lipid study [2,3].

The National Cholesterol Education Program (NCEP) Adult Treatment Panel recommends that individuals at moderate or high risk undergo more extensive lipoprotein analysis, including the determination of low-density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDL), and very low density lipoprotein cholesterol (VLDLC).

HDL cholesterol has been shown to have an inverse relationship with coronary disease risk. As a general rule, HDL cholesterol levels below 35 mg/dL are considered as an independent risk factor. Dietary and drug therapy has been recommended for all individuals in the high-risk category.

Using analytical ultracentrifugation, two major subclasses of HDL have been identified named HDL<sub>2</sub> and HDL<sub>3</sub> [4]. These subfractions have also been separated by means of various precipitation techniques [5,6] and pore gradient polyacrylamide gel electrophoresis [7]. Ballatyne et al [8] showed that an inverse correlation exist between two HDL<sub>2</sub> levels and myocardial infraction. Patsch et al demonstrated a negative relationship between HDL<sub>2</sub> and postprandial lipemia [9]. The significance of the HDL<sub>3</sub> subfraction and coronary risk is not well understood.

More recent studies using 4-30% gradient polyacrylamide gel electrophoresis show that HDL<sub>2</sub> and HDL<sub>3</sub> are in themselves heterogeneous, consisting of at least two and three subfractions, respectively [10]. Using the Lipoprint HDL polyacrylamide gel system, up to ten subfractions of HDL have been resolved, which are grouped into three categories: Large, Intermediate and Small relative to particle size.

The following symbols are used in labeling for Quantimetrix Corporation.

**Cont.**

Contents of kit



Manufactured by

**REF**

Catalog No.



See Product Insert

**EC REP**

Authorized Representative



Store at



- |                                       |         |
|---------------------------------------|---------|
| Stabilizer                            |         |
| Buffer                                |         |
| 3. Lipoprint Electrolyte Buffer Salts | 6 vials |
| Tris (hydroxymethyl) aminomethane     |         |
| Boric Acid                            |         |
| 4. Lipoprint HDL Product Insert       | 1 each  |

**Lipoprint System** (Not Provided, see Quantimetrix Catalog No. 48-9150/9152)

1. Computer (includes Lipoware HDL RUO 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

**Materials Required** (Not Provided)

1. Distilled or Deionized Water
2. 25  $\mu$ L Automatic Pipettor
3. 300  $\mu$ L 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 Electrolyte Buffer salts in 1200 mL of distilled or deionized water.

**Storage and Stability**

Gel tubes, loading gel and electrolyte 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 Research Use Only**

- Use the Lipoprint HDL Kit only in accordance with the Lipoprint HDL 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.
- 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  $\mu\text{L}$  of sample to each tube. (Fig. 4)

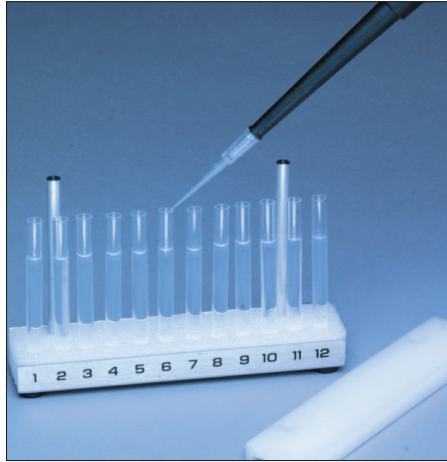


Figure 4. Sample application

5. Add 300  $\mu\text{L}$  of Lipoprint HDL 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)

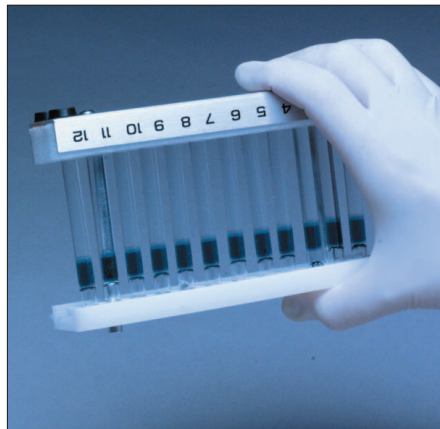


Figure 5. Mixing loading gel and specimens

7. After mixing, place the loaded Preparation Rack against the preparation light. (Fig. 6) Allow the loading gel to photopolymerize 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)

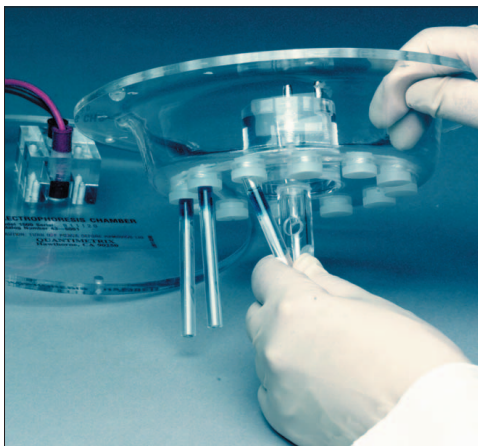


Figure 7. Loading tubes

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 (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).

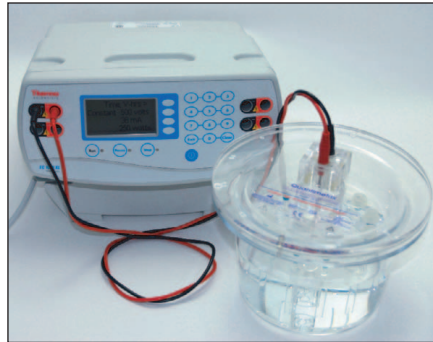


Figure 8. Assembled chamber

12. Electrophoresis time is approximately 50 minutes. Stop the electrophoresis when the albumin band has migrated to a distance of approximately 35 mm from the top of the separating gel.
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.

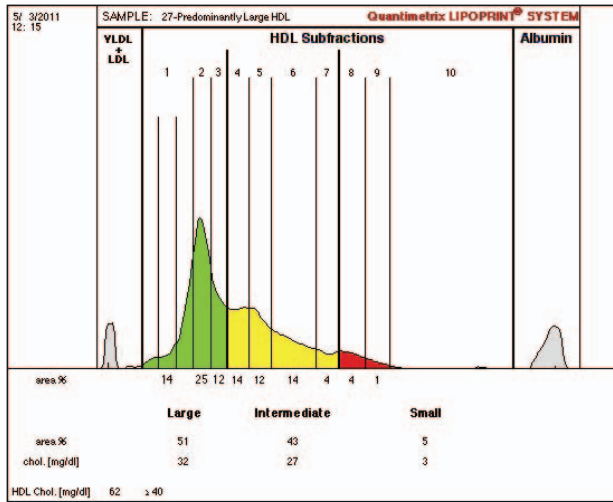
#### QUANTITATION

The electrophoresed HDL gels are scanned and analyzed using the Lipoprint LDL/HDL subfractionation system. General instructions for scanning and sample analysis can be found in Chapter 3: Sample Analysis of the Lipoprint System User's Manual.

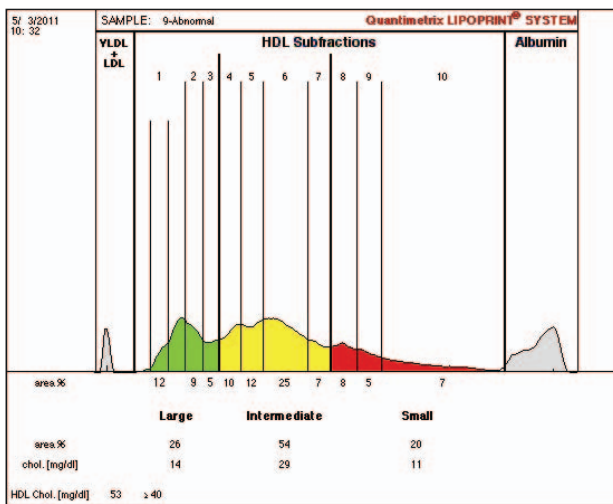
#### QUANTITATIVE RESULTS

Using the Lipoprint HDL System, up to ten subfractions of HDL can be separated, which are grouped into Large, Intermediate and Small groups. The number of bands and the staining intensity of each band may vary from patient to patient. Other subfractions with different migration rates may be identified in some patients.

The subfractions obtained by the Lipoprint HDL system have not been correlated to the HDL subspecies reported in the literature [12]. However, Quantimetrix' studies regarding the effect of gender, increased age, triglyceride, and total cholesterol on the distribution of HDL subfractions appear to be in agreement with the results reported by other investigators [5,8,13,14].



HDL Profile Showing Predominantly Larger Subfractions



HDL Profile Showing Predominantly Intermediate and Small HDL Subfractions

## REFERENCES

1. Stamler J, Wentworth D, Neaton JD: JAMA 1986; 256: 2823-28.
2. Gordon T, Kannel WB, Castelli WP, Dawber TR: Arch inter Med 1981; 141: 1128-31
3. Strong JP, Solberg LA, Restrepo C: Lab Invest 1968; 18: 527-37.
4. De Lalla O, Gofman J: Ultracentrifugal Analysis of Serum Lipoproteins. Methods Biochem Anal 1954; 1: 459-78
5. Alger V, Wirbel E, Roche D, Apfelbaum M, Burstein, Girard-Globa A: Distribution of HDL<sub>2</sub> and HDL<sub>3</sub> in a Random Population of Healthy French Males and Females – Evaluation by Two-Step Precipitation Procedure. Clinica Chimica Acta 1990; 189: 111-122.
6. Kostner GM, Molinari E, Pichler P: Evaluation of a New HDL<sub>2</sub>/HDL<sub>3</sub> Quantitation Method Based on Precipitation with Polyethylene Glycol. Clinica Chimica Acta 1985; 148: 139-147.
7. Ubbink JB, Serfontein WJ, deVillers LS: The Direct Quantification in Whole Serum of HDL Subfractions. Clinica Chimica Acta 1982; 125: 165-175.
8. Ballantyne FC, Clark RS, Simpson HS, Ballatine D: High Density and Low Density Lipoprotein Subfraction in Survivors of Myocardial Infarction and in Control Subjects. Metabolism 1982; 31,5: 433-437.
9. Patsch J, Karlin JB, Scott LW, Smoth LC, Gotto AM Jr: Inverse Relationship Between Blood Levels of High Density Lipoprotein Subfraction 2 and Magnitude of Postprandial Lipemia. Proc. Natl. Acad. Sci. 1986; 80: 1449-1453.
10. Nichols AV, Blanche PJ, Gong EL: Gradient Gel Electrophoresis of Human Plasma High Density Lipoproteins: CRC Handbook of Electrophoresis Vol. 3; CRC Press, Boca Raton, FL 1983; Lewis L, Oppl J, eds.; pp.29-47.
11. Muniz N: Measurement of Plasma Lipoproteins by Electrophoresis on Polyacrylamide Gel. Clin Chem 1977; 23: 1826-1833.
12. Alger V, Malon D, Bertiera MD, Diaya F, Girard-Globa A: Cholesterol Distribution Between High Density Lipoprotein Subfractions HDL<sub>2</sub> and HDL<sub>3</sub> Determined in Serum by Discontinuous Gradient Gel Electrophoresis J. Clin Chem 1991; 37, 7: 1149-1152.
13. Musliner TA, Krauss RM: Lipoprotein Subspecies and Risk of Coronary Diseases. J. Clin. Chem. 1988; 34, 8 (B): B78-B83.
14. Chang LBF, Hopkins GJ, Barter PJ: Particle Size Distribution of High Density Lipoproteins as a Function of Plasma Triglyceride Concentration in Human Subjects. Atherosclerosis 1985; 56: 61-70.

## TECHNICAL SUPPORT

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