environmental factors of unknown mechanism such as diet, alcohol intake, and obesity, are identified. Such patients usually are obese and have type II diabetes mellitus. 

**Lipoproteins**

Lipoproteins are composed of different combinations of protein, cholesterol, triglycerides, phospholipids, and free fatty acids. These lipoproteins are classified by subfraction and composition. There are three main subfractions: alpha lipoprotein (HDL), beta lipoprotein (LDL), and pre-Beta lipoprotein (VLDL). HDL is responsible for cholesterol removal from the arterial wall, while LDL is associated with atherosclerosis.

**Type II Lipoproteinemia**

Type II lipoproteinemia is characterized by an increase in the pre-beta lipoprotein fraction. This condition is usually linked with a genetic disorder and is associated with a high risk of coronary artery disease.

**Type III Lipoproteinemia**

Type III lipoproteinemia is characterized by an increase in the very low density lipoprotein (VLDL) fraction. This condition is often associated with hypertriglyceridemia and an increased risk of pancreatitis.

**Type IV Lipoproteinemia**

Type IV lipoproteinemia is characterized by an increase in the low density lipoprotein (LDL) fraction. This condition is associated with a high risk of coronary artery disease.

**Type V Lipoproteinemia**

Type V lipoproteinemia is characterized by an increase in the high density lipoprotein (HDL) fraction. This condition is associated with a lower risk of coronary artery disease.

**REFERENCES**

1. Electra® HR Buffer (Cat. No. 5805) 
2. Storage and Stability: The buffer is stable for a minimum of 30 days at room temperature (15°C to 30°C).

**INSTRUMENTS**

Any high quality scanning densitometer capable of accurately scanning unaclored cellulose acetate onto a backing of 1.5 mm may be used. A recommended is the Helena EDOC® (Cat. No. 1375) densitometer.

**SPECIMEN COLLECTION AND HANDLING**

Specimen: Serum or plasma samples collected in EDTA may be used. Do not use plasma collected in heparin.

**Principle of Detection:** The principles of detection for lipoprotein electrophoresis are similar to those for protein electrophoresis. The stained bands may be visually inspected for qualitative results or may be quantitated in a scanning densitometer using a 525 nm filter.

**BIBLIOGRAPHY**

1. Electra® HR Buffer (Cat. No. 5805) 
2. Helena Lipoprotein Electrophoresis Procedure is intended for separating and quantitation of plasma lipoproteins by cellulose acetate electrophoresis.

**SUMMARY**

Since Fredrickson and Lesko proposed a system for phenotyping hyperlipoproteinemia in 1965, the concept of coronary artery disease determined by lipoprotein analysis has become a reality. Electrophoresis techniques have related dietary intake of fats, especially cholesterol, and elevated blood levels of lipids with the incidence of atherosclerotic disease. A large number of the different lipoprotein electrophoresis techniques have been developed. Some of the newest techniques are the scanning densitometer and the electrophoretic paper strip method. Yields satisfactory results. Protracted storage increases the migration rate of the pre-beta fraction. Do not freeze.
3. Stain the plate for 15 to 25 minutes. It is recommended that the acetate side up.

**Figure 1:** A typical lipoprotein scan produced by a Helena EDC.

**LIMITATIONS**

Limiting Factors: Fat Red 7B, as well as the other Sudan fat dyes, have a greater affinity for triglycerides and cholesterol esters than for free cholesterol and phospholipids. Bands seen after staining with the Sudan dyes do not reflect a true quantitation of the total lipoprotein content of the sample.

For this reason, it is not recommended that relative percentages of lipoprotein bands be used to calculate the total protein content of each fraction based on a total plasma lipid value. Since most laboratories routinely offer total cholesterol and triglyceride levels, this information is unnecessary.

Interfering Factors: Specimens collected in heparin should not be used. Heparin interacts with the migration patterns of the lipoproteins fractions.

Further Testing Required: Since the lipid composition of each lipoprotein fraction is variable, it is essential to determine total cholesterol and triglyceride levels before attempting to classify a pattern.  

When it becomes necessary to diagnose or rule out a Type III hyperlipoproteinemia, a more definitive quantitation of the lipoproteins such as ultracentrifugation or electrophoresis on polyacrylamide gel is essential.

**REFERENCE VALUES**

Normal Range:  
- Alpha: 31.5-54.5%  
- Beta: 10.5-35.5%  
- Gamma: 30.6-53.9%  
- Chylomicrons 0-1.9%

These values were derived from an in-house study of apparently healthy fasting adults. Each laboratory should conduit its own normal range study because of variation differences in various regions.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Precision**

Within Run: A study was performed using a patient sample in replicate on one plate with the following variations.

- **CV**: 5.0%
- **Mean ± SD**: 1.6

Run to Run: A patient sample was tested in replicate on 5 plates with the following data.

- **CV**: 5.0%
- **Mean ± SD**: 2.0

**INTERPRETATION OF RESULTS**

**LIPOPROTEIN PHENOTYPING USING THE HELENA LIPOPROTEIN ELECTROPHORESIS METHOD**

Normal Pattern: A normal fasting serum can be identified by the presence of a single major fraction, with the pre-Beta lipoprotein faint or absent and the Alpha band definite but less intense than the Beta.

Abnormal Patterns: A pattern may indicate the presence of elevated cholesterol or triglyceride to have hyperlipoproteinemia. The elevation must be determined whether secondary or secondary to metabolic disorders such as hypothyroidism, obstructive jaundice, nephrotic syndrome, dyspro- teinemias or poorly controlled insulin dependent diabetes mellitus.

Primary lipemia arises from genetically determined factors or
3. Stain the plate for 15 to 25 minutes. It is recommended that the

Application Point

Alpha       Beta           Chylomicrons

3. Soak the required number of plates in Electra

Type II-a

on the glossy, hard side with amarker. It is suggested that theidentification mark be placed in onecorner so that it is always alignedwith sample No. 1.

A patient must have an elevated cholesterol or

serum with negligible chylomicrons and normal cholesterol andtriglyceride levels. On electrophoresis, the...major fraction, with the pre-Beta lipoprotein faint or absent and theAlpha band definite but less intense than the Beta.

of Titan® III Lipo Plates by marking

LIPOPROTEIN PHENOTYPING USING THE HELENA LIPOPROTEINELECTROPHORESIS METHOD

Normal Pattern:

A Lipoprotein Plate showing the relative positions of the bands

1. Prepare the working staining

acetate side down

STEP-BY-STEP METHOD

1. Dissolve one package of Electra HR

(+)

E. Staining and Evaluation of Lipoprotein Bands

C. Sample Application to prevent evaporative diffusion. Discard electrophoresis buffer and wicks after use.

3. Remove the wetted Titan® II Lipo Plate from the buffer with the fingerpicks and blot once firmly between two blotters. Before placing the plate in the aligning base, place a drop of water or buffer on the center of the aligning base. This prevents the plate from sticking to the base during the application. Place the plate in the aligning base, cellulose acetate side up, aligning the top edge of the plate with the black scribe mark (CAT Scanningdensitometer). The identification mark should be aligned with sample No. 1.

4. Apply the sample to the plate by depressing the applicator tips into the sample well 3 or 4 times and promptly transferring the applicator tips to blotter paper. Press the button down and hold it 5 seconds. Make a second super-imposed application by repeating this step.

D. Electrophoresis

1. Quickly place the plate, cellulose acetate side down, in the electrolysis chamber. The applicator point should be closer to the cathode than to the anode (scanned first). Instrument settings will be 100 V (Cathode), 400 V (Anode), and 1mA (Current) for 20 minutes. Allow the plate to drain dry for several seconds. If necessary, gently wipe off residual precipitate. If the plate is to be scanned in the densitometer, quickly proceed to the next step.

2. Place the plate on the metal stand so that the sample side faces the cathode and orientate the plate so that the bands are scanned sequentially in one direction only. The plate should be scanned as soon as possible. Plates to be visually evaluated only may be kept an indefinite period of time after being processed with glycerine and as outlined above.

RESULTS

The Beta-lipoprotein (HDL) band is the fastest moving fraction and is located closest to the anode. The Beta-lipoprotein (LDL) band is usually the most prominent fraction and is near the origin, migrating usually only slightly anode to the point of application. The pre-beta lipoprotein (VLDL) band migrates between Alpha and Beta-lipoprotein. The mobility of pre-beta lipoprotein varies with the degree of resolution obtained, the type of pre-beta test, and the present of Beta lipoprotein. Sometimes pre-beta will be absent or smear (jet ahead) for a few fraction other times it may be split into two or more fractions or may be negligible or precipitate forms. The pre-beta fraction should be scanned before the sample plate.

Chylomicrons, when present, stay at the point of application. In samples with very high levels of chylomicrons, there will appear to be a smear of material extending anodically from the point of applications to the bands.

INTERPRETATION OF RESULTS

LIPOPROTEIN PHENOTYPING USING THE HELENA LIPOPROTEIN ELECTROPHORESIS METHOD

Normal Pattern: A normal fasting serum can be defined as a clear serum, without chylomicrons and normal cholesterol and triglyceride levels. On electrophoresis, the Beta-lipoprotein appears as the major fraction, with the pre-beta lipoprotein absent and the Alpha band definite but less intense than the Beta.

Abnormal Patterns: At least one of the following abnormalities should be noted. Hypercholesterolemia and/or hypoalphalipoproteinemia. The elevation must be determined to be primary or secondary to metabolic disorders such as hypercholesterolemia, obstructive jaundice, nephrotic syndrome, dysproteinemia or poorly controlled insulin-dependent diabetes mellitus. Primary lipemia arises from genetically determined factors or

Calculation of the Unknown:

Figure 1 is a typical lipoprotein scan produced by a Helena EDC

LIMITATIONS

Limiting Factors: Fat Red 7B, as well as the other Sudan fat stains, have the greatest affinity for triglycerides and cholesterol esters. It has its own childhood and chlorophyll impurities. Bands seen after staining with the Sudan dyes do not reflect a true density of the lipoprotein fraction.

For this reason, it is not recommended that relative percentages of lipoprotein bands be used to calculate the total weight percent of each fraction.

Further Testing Required: Since the lipid composition of each lipoprotein fraction is variable, it is essential to determine total cholesterol and triglyceride levels before attempting to classify a pattern. When it becomes necessary to diagnose or rule out a Type II hyperlipoproteinemia, a more definitive quantitation of the lipoproteins such as ultracentrifugation or electrophoresis on polyacrylamide gel is essential.

REFERENCE VALUES

Normal Range:     Alpha:              23.1-54.5%

Beta:               30.6-53.9%

Chylomicrons 0- 1.9%

Pre-Beta:           10.1-35.9%

Beta:               30.6-53.9%

Chylomicrons 0- 1.9%

Pre-Beta:           10.1-35.9%

Type II

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environmental factors of unknown mechanism such as diet, alcohol intake, and drugs, especially hormones. 2. Also, considered primary are those lipoproteins associated with ketosis-resistant diabetes, pancreatitis, and obesity. Diabetes mellitus and pancreatitis can be confusing, for it is often difficult to tell whether the hyperlipoproteinemia or the disease is the causative factor.3

PRINCIPAL LIPOPROTEIN ELECTROPHORESIS

The Friedrickson Classification

TYPE I: Hypoalphalipoproteinemia

Criteria: Chylomicrons present, pre-Beta normal or only slightly elevated. Serum is milky and remains so on standing. Plasma clear to slightly turbid with normal free fatty acid levels.

Secondary Causes: Myxedema, myxedema, macrodysobleminemia, nephrosis, liver disease, excesses in dietary cholesterol and saturated fats.

TYPE II: "Broad Beta" - Abnormal Lipoprotein

Criteria: Decreased or absent pre-Beta, increased Beta and Alpha lipoproteins. Plasma light yellow to yellowish green.

Secondary Causes: Decreased levels of lipoprotein in fasting state. Plasma contains abnormal lipoprotein composition and density. Cholesterol and triglycerides elevated. The abnormal material has broad beta electrophoretic mobility but separates with VDL in the ultracentrifuge. Plasma is turbid to cloudy. The abnormal lipoprotein is also known as "floating BAA." The condition is rare.

Confirmation: Polyacrylamide gel electrophoresis or ultracentrifugation studies to demonstrate abnormal lipoprotein mobility.

TYPE IV: Carbohydrate Induced and Endogenous
type lipoproteinemia

Criteria: Increased pre-Beta, increased triglycerides, normal or slightly increased cholesterol. Alpha and Beta lipoprotein usually normal. (An increased pre-Beta with normal triglyceride level is seen with the normal variant "selecting for Beta." Such samples belong to Type IV.)

Secondary causes: Nephrotic syndrome, diabetes mellitus, pancreatitis, cystic fibrosis, obesity, and other acute and chronic abnormalities. The cause of hyperlipoproteinemia is to separate into a broad "Beta" and an abnormal "Alpha" lipoprotein.

TYPE V: Mixed Triglyceridemia (Carbohydrate and fat induced)

Criteria: Increased triglycerides and cholesterol. Lipoprotein X is present.

Secondary causes: Nephrosis, myxedema, myxedema acidosis, diabetes mellitus, pancreatitis, glycogen storage disease, and other acute metabolic processes.

Note: Only Types II, IV and V have been correlated to vascular disease.

THE ALPHA LIPOPROTEINS IN DISEASE

Marked increase in the Alpha lipoprotein is seen in obstructive liver disease and cirrhosis. It also occurs in primary and secondary hyperlipoproteinemia (Type V). These changes are seen in parenchymal liver disease. Tanger's disease is a rare congenital disorder characterized by an absence of the normal Alpha lipoprotein. Heterozygotes exhibit decreased levels of Alpha. It should be noted that the Alpha lipoprotein is an important antiinflammatory and anticoagulant protein (Q-factor) and may cause moderate elevations in the Alpha lipoprotein.

DECREASES IN THE BETA LIPOPROTEINS

A significant inherited defect characterized by severe deficiency of all lipoproteins of density less than 1.036 (all but the Alpha lipoprotein are present) is considered primary. A few cases of familial hypoalphalipoproteinemia have been reported. There is a strong indication that this is different from that producing Abetalipoproteinemia.

LIPPROTEIN-X

Lipprotein-X is the abnormal lipoprotein often seen in patients with obstructive liver disease. It consists of unesterified cholesterol, cholesteryl esters, phosphoglycerides and protein. In most reported cases, Lipprotein-X is a spheroidal particle with a density between 1.019 and 1.063. Reverse cholesterol transport occurs primarily in the intestinal tract, liver, and possibly adipose tissue. Reverse cholesterol transport is important for the maintenance of lipid homeostasis and is thought to be mediated by the SR-BI receptor.