Type IV have an abnormal glucose tolerance. Probably the most common type of hyperlipoproteinemia reflects an imbalance in synthesis and clearance of endogenous triglycerides.

Type V: Mixed Triglyceridemia (carbohydrate and fat included)

Criteria: Increased serum triglycerides (i.e., >500 mg/dL) and cholesterol, increased triglycerides, increased cholesterol, increased LDL, increased HDL, increased VLDL. Most cases of Type V are due to a dysfunction in the lipoprotein lipase (LPL) system. LPL is responsible for the conversion of triglycerides to free fatty acids, which are then taken up by muscle cells. In Type V hyperlipoproteinemia, LPL activity is reduced, leading to an accumulation of triglyceride-rich lipoproteins in the plasma.

The ALPHA LIPOPROTEINS IN DISEASE

Marked increases in the Alpha lipoproteins are seen in obstructive liver disease. Only Types II, III, and IV have been correlated to vascular disease.

DECREASES IN THE BETA LIPOPROTEINS

Type V: Mixed Triglyceridemia

Lipoprotein electrophoresis is an abnormal lipoprotein often seen in patients with liver disease. It consists of unclassified (free) cholesterol, phospholipids, and protein. It migrates slower than LDL because of its particular lipid content. It stains poorly or not at all with the usual lipid stains and is so is not usually detected by standard lipoprotein electrophoretic methods.

PERFORMANCE CHARACTERISTICS

The SPIFE Lipoprotein Electrophoresis System is intended for the separation and quantification of plasma lipoproteins by agarose gel electrophoresis using the SPIFE 3000 system.

PRECAUTIONS

When using the SPIFE Lipoprotein Electrophoresis System, it is important to follow the manufacturer’s instructions for proper use and handling of the system and its components.

The SPIFE Lipoprotein Electrophoresis System is intended for the separation and quantification of plasma lipoproteins by agarose gel electrophoresis using the SPIFE 3000 system.

SUMMARY

Since Fredrickson and Less proposed a system for phenotyping hyperlipoproteinemias, the need for accurate determination of lipoprotein phenotypes resulted from the recognition that hyperlipoproteinemia is symptomatic of a group of disorders that may present a risk to the patient. Selective treatment of the disorders varies with the different phenotypes. It is absolutely necessary that the correct phenotype be established before therapy is begun. The classification system proposed by Fredrickson and Lees, only Types II, III, and IV have a proven relationship to atherosclerosis. Plasma lipoprotein electrophoresis is a rapid and simple test, but are transported bound to protein and can be classified as lipoproteins. These various fractions are made of different combinations of protein, cholesterol, glycerides, cholesterol esters, phospholipids and free fatty acids. Yeast have been employed to separate the plasma lipoproteins, including ultracentrifugation, thin layer chromatography, immunological techniques, and electrophoresis. Electrophoresis and ultracentrifugation are two of the most widely used methods and each has given rise to its own terminology. Table 1 shows the correlation of these classifications and the relative lipid and protein composition of each fraction.

Table 1: Classification and Composition of Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Lipoprotein Fraction</th>
<th>Composition - % in Each Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha HDL*</td>
<td>Cholesterol (45%+), Protein (35%), Glycerides (10%), Phospholipids (5%)</td>
</tr>
<tr>
<td>Beta LDL*</td>
<td>Cholesterol (35%+), Protein (45%), Glycerides (10%), Phospholipids (10%)</td>
</tr>
<tr>
<td>Pre-Beta</td>
<td>Cholesterol (15%+), Protein (35%), Glycerides (10%), Phospholipids (8%)</td>
</tr>
<tr>
<td>Type V</td>
<td>Cholesterol (10%+), Protein (20%), Glycerides (10%), Phospholipids (9%)</td>
</tr>
</tbody>
</table>

Note: All standard abbreviations: LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein).

The stain should be stored at 15 to 30°C and is stable in the refrigerator. The stain is stored in a screw-top container at 15 to 30°C.

The dilution of the stain (one drop of stain per 1 mL of water) is necessary to define hyperlipoproteinemia.

For the most accurate phenotyping of lipoprotein patterns, the following precautions should be observed before sampling:

1. Discontinue all drugs, if possible, for 3-4 weeks.
2. The patient should be fasting for a 12-14 hour period. Chylomicrons normally appear in the blood 2-10 hours after a meal; therefore, a 12-14 hour fast is necessary to define hyperlipoproteinemia.

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Figure 2 shows a typical lipoprotein scan produced by a SPIFE Lipoprotein Electrophoresis System. The relative percent of each band is computed and printed automatically by the densitometer. The calculated percent of the Lipoprotein fraction bands must be added to the percent of the pre-Beta band for a total pre-Beta value.

Calculating the mg/dl of total lipids from the relative percent values obtained is not recommended. It has been shown that the lipoprotein electrophoresis is the causative factor.

**INTERPRETATION OF RESULTS**

**Lipoprotein Electrophoresis Method:**

**Normal Range:** The normal fasting serum can be defined as a clear serum with negligible chylomicrons and normal cholesterol and triglyceride levels. On electrophoresis, a typical fasted serum appears with a prominent fraction near the origin of the pre-Beta fraction. The pre-Beta band and the alpha band are well defined and separate. It is recommended that the pre-Beta band be quantified with the pre-Beta band.

**Abnormal Sedimentation:**

A abnormally increased cholesterol or triglyceride level is caused by abnormalities in lipoprotein electrophoresis. Abnormal lipoprotein electrophoresis is not recommended to determine the presence of Type IV hyperlipoproteinemia. Abnormal lipoprotein electrophoresis is not diagnosed due to primary or secondary abnormality, following the removal of noninvasive tests, which are subjective in nature. Hyperlipoproteinemia is defined by a variety of causes or factors of an abnormal lipoprotein level, such as diet, alcohol intake, and drugs, especially estrogen or steroid hormones. Also considered primary the lipoprotein electrophoresis is, any abnormal lipoprotein electrophoresis, cholesterol and triglyceride levels must be determined in order to complete the test.

**LIMITATIONS**

**Limiting Factors:**

Type A and B bands, as well as high density lipoprotein bands of the lipoprotein fraction. Type A and B bands are used to determine the abnormal lipoprotein fraction. Any abnormal lipoprotein fraction must be determined by a definitive abnormality of the abnormal lipoprotein electrophoresis.

**Primer Lipoprotein Electrophoresis:**

**Type I Hyperlipoproteinemia:**

Crite...
I. Sample Preparation

1. If testing 61-80 samples, remove four disposable Applicator Blade Assemblies from the Dispenser. If testing fewer samples, remove the appropriate number of Applicator Assemblies from the Dispenser. Reassemble the pre-Beta lipoprotein bands by gently bending the protective piece back and forth until it breaks free.

2. Place the Applicator Blades into the slots in the Applicator Assembly identified as 2, 6, 11 and 15. If using fewer Applicator Blades, place each into one of the four slots noted above.

Please note that the blade assembly will only fit into the slots one way and will force the blade assembly into the slots.

3. Slide the Applicator Blade into the slots in the Applicator Assembly.

4. Pipette 75-80 µL of patient serum or control into each well of the SPIFE 2000/3000 20-80 Disposable Cup Tray 3364.

II. Gel Preparation

1. Remove the gel from the protective packaging and discard outer packaging. Using a REP Bottle C, gently loosen the gel by using slightly differential pressures on the bottom. Remove the bottle.

2. Disassemble approximately 2/3 of the REP Prep onto the left side of the electrophoresis chamber.

3. Place the right edge of the gel over the REP Prep aligning the right edge of the gel over the bottom of the chamber. Use force just enough to push the gel against the side of the chamber, especially next to electrode posts, to remove excess bubbles. Make sure no bubbles remain under the gel.

4. The electrode wells with electrodes containing deionized water before and after each use. Wipe the carbon electrodes with a lint-free tissue. The electrode must be partly covered by the rough surface. Ensure that the electrodes are soaked on tightly. The Dispenser (REP Prep) slide is then returned to the Applicator Holder. After use, rinse electrodes in distilled water. Unplug the electrodes from the old electrowire and screw tightly onto the new electrowire.

5. Place a carbon electrode on the outside edge of the cathode gel block (left side of the gel) outside the magnetic pots.

6. Place an Electrode Blotter directly above and below the cathode end of the SPIFE Dispenser. Displace the gel block to the bottom of the cathode so that they touch the gel block edges. Close the chamber lid.

7. Press the TEST SELECT/CONTINUE button located on the Electrode Blotter to advance the instrument to the next test sequence.

III. Electrophoresis

Using the instructions provided in the Operator's Manual, set up the parameters for the following tests for the SPIFE 2000 or the SPIFE 3000.

A. SPIFE 3000

1. Place a Disposable Stainless Steel Electrode on the outside ledge of the gel block.

2. Place the left edge of the gel over the REP Prep aligning the round hole in the electrode with the hole in the gel block. Surround the edges of the plastic gel backing, starting from the left side and ending on the right side of the gel. The Alpha-lipoprotein (HDL) band is usually 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 cathodally to the point of application. The Pre-Beta-lipoprotein (VLDL) band migrates between Alpha and Beta lipoprotein. The mobility of the pre-Beta fraction varies with the degree of resolution obtained, the type of pre-Beta present, and the percent of Beta lipoprotein. The malacia bands arise from genetic factors or environmental factors of unknown mechanism such as diet, alcohol intake, and drugs, especially estrogen or steroid hormones. As an alternative homeostatic mechanism, hyperlipoproteinemia can be the result of secondary to metabolic disorders such as hypothyroidism, obstructive jaundice, nephrotic syndromes, dysproteinemias, or poorly controlled insulin-dependent diabetes mellitus. Primary dysproteinemias are typically caused by mutations in the genes that encode apolipoprotein C-III, E, or other apolipoproteins. Chylomicrons and very low-density lipoprotein are rarely seen with exogenous triglyceride intake. Ninety percent of persons with familial Type I: Hyperlipoproteinemia

Criteria: Chylomicronemia - concentration of triglyceride is slightly elevated. alpha and Beta decreased, often markedly so. Standing plasma with marked creamy appearance. Type II: Hyperbetalipoproteinemia

Criteria: Increased total cholesterol due to an increased Beta-lipoprotein cholesteryl ester storage. Typical clinical picture is that of a familial pattern of hypercholesterolemia. Type III: “Broad Beta” - Abnormal Lipoprotein

Criteria: Presence of triglyceride burdened lipoprotein of abnormal composition and density. Chylomicronemia, nephrosis, liver disease, excesses in dietary cholesterol and saturated fats. Type IV: Mixed Lipoprotein

Criteria: Presence of triglyceride burdened lipoprotein of abnormal composition and density. Chylomicronemia, nephrosis, liver disease, excesses in dietary cholesterol and saturated fats. Type V: Hyperlipoproteinemia

Criteria: Increased total cholesterol due to an increased Beta-lipoprotein cholesteryl ester storage. Typical clinical picture is that of a familial pattern of hypercholesterolemia. Type I: Hyperlipoproteinemia

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Criteria: Increased total cholesterol due to an increased Beta-lipoprotein cholesteryl ester storage. Typical clinical picture is that of a familial pattern of hypercholesterolemia.
The gels should be stored horizontally at room temperature in a cool area. For best results, fresh serum should be used. Storage at 2 to 8 °C may cause moderate elevations in the Alpha lipoproteins. Heterozygotes exhibit decreased levels of Alpha lipoproteins.

BIBLIOGRAPHY

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