lipoprotein, which increases the relative migration rates of the fractions, especially the beta lipoprotein.

3. Titan® Lipo Plates (Cat. No. 3900, 3901)  

4. The patient should be fasting for a 12-14 hour period. Chylomicrons are present, pre-Beta normal or only slightly increased. Abetalipoproteinemia is a primary inherited defect characterized by severe deficiency of all lipoproteins of density less than 1.006. Marked increase in the Alpha lipoproteins are seen in obstructive liver disease and cirrhosis. Marked decreases are seen in obstructive liver disease and cirrhosis. These lipoproteins are decreased in biliary cirrhosis.

5. Abetalipoproteinemia is a primary inherited defect characterized by severe deficiency of all lipoproteins of density less than 1.006. Alpha lipoproteins are decreased in biliary cirrhosis.

6. The need for accurate determination of lipoprotein phenotypes resulted from the recognition that hyperlipoproteinemia is systematic of a group of disorders disimilar in clinical features, prognosis and responsiveness to treatment. Since treatments of the disorders vary widely, it is essential that the correct phenotype be established before therapy is begun. In the classification of lipoproteins and the Editors, there are 6 types, I and II have a proven relation to atherogenesis. Plasma lipids do not circulate freely in the plasma, but are transported bound to protein and can thus be classified as lipoproteins. The various fractions are made of different combinations of protein, cholesterol, glycerides, cholesterol esters, phospholipids and free fatty acids.

7. Several techniques 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 I shows the correlation of these classifications and the relative lipid and protein composition of each fraction.

8. For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.

9. For best results, fresh serum should be used.

10. The reagent is stable until the expiration date on the bottle. The stock stain is stable for 2 years at 15 to 30 °C. Signs of deterioration: Deteriorated, esp. at room temperature. The reagent is stable until the expiration date on the bottle.

11. The lipid profile is a primary inherited defect characterized by severe deficiency of all lipoproteins of density less than 1.063 (all but the Alpha lipoprotein are normal). It is a rare familial hypolipidemia. Secondary Causes: Myxedema, myasthenia, macroglobulinemia, nephrosis, liver disease, excesses in dietary cholesterol and saturated fats.

12. The need for accurate determination of lipoprotein phenotypes resulted from the recognition that hyperlipoproteinemia is systematic of a group of disorders dissimilar in clinical features, prognosis and responsiveness to treatment. Since treatments of the disorders vary widely, it is essential that the correct phenotype be established before therapy is begun. In the classification of lipoproteins and the Editors, there are 6 types, I and II have a proven relation to atherogenesis. Plasma lipids do not circulate freely in the plasma, but are transported bound to protein and can thus be classified as lipoproteins. The various fractions are made of different combinations of protein, cholesterol, glycerides, cholesterol esters, phospholipids and free fatty acids.

13. Several techniques 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 I shows the correlation of these classifications and the relative lipid and protein composition of each fraction.

14. Any high quality scanning densitometer capable of accurately scanning uncelloose acetate cellulose on a backing of 525 microns may be used. Recommended is the Helena EDC® (Cat. No. 1375) densitometer.
3. Stain the plate for 15 to 25 minutes. It is recommended that the pre-Beta Application Point 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 diabetes, there will appear to be a smear of material extending anodically from the point of application to the beta band.

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 the staining chamber. Press the button down and hold for 5 seconds. Make a second super-imposed application by repeating this step.

5. After rinsing away excess stain with water, carefully layer the buffer and drape one over each support bridge, making sure it makes contact with the buffer and that there are no air bubbles under the wicks.

6. Scan the lipoprotein bands in a scanning densitometer using the 525 nm filter. Place plate in densitometer cell and place the alpha band at the left (scanned first) instrument zeroed anode to the alpha band.

7. Treatment of Plate for Qualitative Results and Permanent Storage

The following steps should be taken if the plate is to be visually inspected for qualitative results only or if permanent storage of the plate after densitometry is desired. Plates cannot be scanned accurately after treatment with glycerine; therefore, must be scanned prior to the steps outlined below:

1. Dip the plate, cellulose acetate side up, in a mixture of 3 parts glycerine and 1 part methano.

2. Place the plate, cellulose acetate side up, on a clean blotter until dry.

3. The plate can be stored for an indefinite period of time. The glycerine solution can be used several times if kept tightly closed.

4. Store in a Titan Plastic Envelope (Cat. No. 5052 or 5053).

The following is an in-house study of apparently healthy fasting adults. Each laboratory should conduct its own normal range study because of population differences in various regions.

These values were derived from an in-house study of apparently healthy fasting adults. Each laboratory should conduct its own normal range study because of population 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.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Buffer</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>21.3-54.5%</td>
<td>10.1-53.9%</td>
<td>4.9-58.5%</td>
<td>15.1-59.3%</td>
<td>6.2-60.8%</td>
<td>8.0-62.5%</td>
<td>1.3-56.5%</td>
<td>2.9-59.3%</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>3.0-63.9%</td>
<td>3.0-63.9%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Chloromycin 0.1-9

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

INTERPRETATION OF RESULTS

LIPOPROTEIN PHENOTYPING USING THE HELENA LIPOPROTEIN EQUILIBRIUM DENSITY梯度法

A normal fasting serum can be defined as a clear serum with normal cholesterol and normal cholesterol-ester triglyceride levels. On electrophoresis, the Beta-lipoprotein appears as the major fraction, with the Pre-Beta lipoprotein being absent and the Alpha band definitive but less intense than the Beta.

Abnormal Phenotypes: Abnormal phenotypes may be described as having elevated cholesterol or triglyceride to have hyperlipoproteinemia. The elevation must be determinable by additional or secondary to metabolic disorders such as hypothyroidism, obstructive jaundice, nephrotic syndrome, dyspro- teinemias or poorly controlled insulinopenic diabetes mellitus.

Primary lipemia arises from genetically determined factors or

Calculation of the Unknown:

Figure 2: A typical lipoprotein scan produced by a Helena EDC

LIMITATIONS

Limiting Factors: Fat Red 7B, as well as the other Sudan fats, stain fat with greater affinity for triglycerides and cholesterol esters. When heparin is used since heparin alters the migration patterns of the lipoprotein fractions. For this reason, it is not recommended that relative percentages of lipoprotein bands be used to calculate the total lipid 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 with the Lipotrol procedure. It is suggested that the migration patterns of the lipoprotein 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 II hyperlipoproteinemia, a more definitive quantification of the lipoproteins such as ultracentrifugation or electrophoresis on polyacrylamide gel is essential.

REFERENCE VALUES

Normal Range: Alpha 21.3-54.5% Beta 10.1-53.9% Beta: 3.0-63.9%

Chloromycin 0.1-9
E. Staining and Evaluation of Lipoprotein Bands

1. Quickly place the plate, cellulose acetate side down, into the sample well 3 or 4 times and promptly transferring the applicator to the aligning base. Press the button down and hold it 5 seconds. Make a second super-imposed application by repeating this step.

2. 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 the aligning base. Press the button down and hold it 5 seconds. Make a second super-imposed application by repeating this step.

3. Heat the stained plate to 120°C for 30 minutes. This will help to fix the stain to the plate and prevent any further development of the bands.

4. Remove the plate from the staining dish and wash it in tap water for a few seconds. If necessary, gently agitate to remove residual precipitate.

5. The plate is to be scanned in the densitometer, quickly proceed to the next step.

6. After rinsing away excess stain with water, carefully lay the plate on a clean sheet of blotting paper and allow it to air dry.

7. Scan the lipoprotein bands in a scanning densitometer using the 525 nm filter. Place plate in densitometer cellulose anode side up, with the alpha band to the left (scanned first). Instrument zeroed to alpha band of the alpha.

8. The plate can be scanned for qualitative results and permanent storage.

F. Treatment of Plate for Qualitative Results and Permanent Storage

The following steps should be taken if the plate is to be visually inspected for qualitative results only or if permanent storage of the plate after densitometry is desired. Please cannot be properly evaluated after treatment with glycerine, therefore, must be scanned prior to the steps outlined below:

1. Dip the plate, cellulose acetate side up, in a mixture of 3 parts glycerine and 1 part methanol.

2. Dip the plate, glycerine solution and blot. If the plate seems to be completely wetted, proceed to the next step. If the plate is not wetted, rewet it and repeat this step until it is wetted.

3. Place the plate, cellulose acetate side up, on a clean sheet of blotting paper and allow it to air dry.

4. The plate can be stored for an indefinite period of time. The glycerine solution can be used several times if it is kept tightly closed.

5. Store in a Titan® Plastic Envelope (Cat. No. 5202 or 5003).

6. Stains for a Type II-a lipoprotein fraction may vary. Please refer to the manufacturer’s data sheet before attempting to classify a pattern.

7. When it becomes necessary to diagnose or rule out a Type II-a lipoprotein fraction, it is essential to determine total cholesterol and triglyceride levels before attempting to classify a pattern.

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environmental factors of unknown mechanism such as diet, alcohol intake, and drugs, especially hormones. 1 Also considered primary are those lipoproteins associated with ketosis, renal disease, 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. 2

**References:**


**Summary:**

Since Fredrickson and Lesco proposed a system for phenotyping hyperlipoproteinemia in 1965, the concept of corotary artery disease determined by the presence of lipoprotein abnormalities has become a reality. Epidemiologic studies have related diet, activity, and other environmental factors of unknown mechanism such as diet, alcohol intake, and drugs, especially hormones. Diabetes mellitus and pancreatitis can be confusing, for it is often difficult to tell whether the hyperlipoproteinemia or the disease is the causative factor. 2

**REFERENCES:**


**BIBLIOGRAPHY**


