HYDRAGEL 7 LIPO + Lp(a)
Ref. 4104

HYDRAGEL 15 LIPO + Lp(a)
Ref. 4124

HYDRAGEL 30 LIPO + Lp(a)
Ref. 4144
INTENDED USE

The HYDRAGEL 7, 15 and 30 LIPO + Lp(a) kits are designed for determination of lipoprotein profiles and for screening for Lp(a) in human serum.

Each agarose gel is intended to run:
• 7 samples in the HYDRAGEL 7 LIPO + Lp(a) kit,
• 15 samples in the HYDRAGEL 15 LIPO + Lp(a) kit,
• 30 samples in the HYDRAGEL 30 LIPO + Lp(a) kit.

For In Vitro Diagnostic Use. FDA clearance for Lp(a) application in the USA is pending.

PRINCIPLE OF THE TEST 1-14

The analysis is performed by electrophoresis on buffered (pH 7.5) agarose gels on the semi-automated HYDRASYS instrument. The separated lipoproteins are stained with a lipid specific Sudan black stain. The excess of stain is removed with an alcoholic solution. The resulting electrophoregrams can be evaluated visually for pattern abnormalities or by densitometry to obtain relative quantification of individual zones.

The lipoproteins are circulating complexes constituted of lipids and proteins. The classification of lipoproteins is based on their properties that lend themselves to the techniques for their separation:
• their density (ultracentrifugation),
• their charge (zone electrophoresis),
• their size (molecular filtration by polyacrylamide gel electrophoresis).

Hyperlipemia classification is based on zone electrophoresis according to Fredrickson’s classification 16. On agarose gels, lipoproteins separate into the following fractions (in the order of increasing mobility):
• the chylomicrons: these are very large molecules with high triglycerides content, present as small particles in the serum and responsible for the opalescence of the serum. They normally remain at the application point.
• the beta lipoproteins or Low Density Lipoproteins (LDL): they normally migrate in beta-2 globulins position.
• the pre-beta lipoproteins or Very Low Density Lipoproteins (VLDL): they have molecular weight higher and density lower than LDL. They are more mobile than LDL and migrate in beta-1 globulins position.
• the fast pre-beta lipoproteins: this fraction is composed of lipoprotein (a) or Lp(a) which is similar in size and composition to LDL. Lp(a) when present at a sufficiently high concentration, can be seen migrating between VLDL and HDL.
• the alpha lipoproteins or High Density Lipoproteins (HDL): they are the fastest fraction of lipoproteins. They migrate in alpha-2 globulins position.

Electrophoresis is a simple and useful technique for the assessment of lipoprotein abnormalities and coronary disease risk factors in serum. The differentiation of lipoproteins according to their electrophoretic mobility is the basis of the Fredrickson classification. This classification readily allows application of dietary measures or therapeutic treatment.

Electrophoresis on HYDRAGEL 7 LIPO + Lp(a) and HYDRAGEL LIPO + Lp(a) 15/30 gels presents a particular advantage: it permits simultaneous determination of the lipoprotein profile and the fast pre-beta or Lp(a) fraction.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7, 15 AND 30 LIPO + Lp(a) KITS

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4104</th>
<th>PN 4124</th>
<th>PN 4144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
<td>10 gels</td>
<td>10 gels</td>
</tr>
<tr>
<td>Buffered Strips (ready to use)</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
</tr>
<tr>
<td>Sudan Black Stain (stock solution)</td>
<td>1 vial, 20 mL</td>
<td>1 vial, 20 mL</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10 (7 teeth)</td>
<td>1 pack of 10 (15 teeth)</td>
<td>2 packs of 10 (15 teeth)</td>
</tr>
<tr>
<td>Filter Papers - Thin</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
</tr>
</tbody>
</table>

FOR OPTIMAL RESULTS

All reagents from the same kit must be always used together and according to the package insert instructions.

PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. AGAROSE GELS

Preparation

Agarose gels are ready to use. Each gel contains: agarose, 0.8 g/dL; buffer pH 7.5 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

Support medium for lipoprotein electrophoresis.

Storage, stability and signs of deterioration

Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). (The arrow on the front of the kit box must be pointing upwards). DO NOT FREEZE. Avoid obvious temperature fluctuations during storage (e.g., do not store close to a window or to a heat source). The gels are stable until the expiration date indicated on the kit package or the gel package labels. Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal quantity of liquid is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).
2. BUFFERED STRIPS

Preparation
Buffered sponge strips are ready to use. Each contains: buffer pH 7.5 ± 0.1; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

**WARNING:** The buffer in the strips contains 0.3 % sodium azide. Harmful if swallowed. Do not ingest! If ingested consult physician immediately! When disposing, prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide. After contact with skin, wash immediately with plenty of water.

Use
Buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. (The arrow on the front of the kit box must be pointing upwards). They are stable until the expiration date indicated on the kit package or buffered strips package label.

**DO NOT FREEZE.**

Discard buffered strips if the package is opened and the strips dry out.

3. SUDAN BLACK STAIN

Preparation
Prepare the working Sudan black stain solution at least 30 minutes before use. Add exact volumes of the individual components in the following order, under gentle stirring:

1) Pure ethanol (96 %), 120 mL; Sudan Black Stain, stock solution (6.6 g/dL in dimethylformamide), 1.45 mL. Wait until the Sudan Black is completely dissolved and then add distilled or deionized water, 100 mL. Stir for 30 minutes minimum.

or

2) Pure isopropanol (100 %), 100 mL; Sudan Black Stain, stock solution (6.6 g/dL in dimethylformamide), 1.45 mL. Wait until the Sudan Black is completely dissolved and then add distilled or deionized water, 120 mL. Stir for 30 minutes minimum.

Keep these solutions away from any source of heat. Discard after each staining procedure.

**IMPORTANT:** The use of another alcohol or denatured ethanol, may lead to atypical results. If lower concentrations of pure alcohol are used, adjust accordingly the volumes of alcohol and water.

**WARNING:** The vial of sudan black contains dimethylformamide. Harmful by inhalation. In case of insufficient ventilation, wear suitable respiratory equipment. Do not ingest! If ingested, consult physician immediately! After contact with eyes or skin, rinse immediately with plenty of water and seek medical advice.

Use
For staining gels with electrophoretic lipoprotein separations.

Storage, stability and signs of deterioration
Store the stock stain solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or stain vial labels. The working stain solution is stable for 12 hours maximum at room temperature in a closed container to prevent evaporation.

4. APPLICATORS

Use
Precut, single use applicators for sample application.

Storage
Store applicators in a dry place at room temperature or refrigerated.

5. FILTER PAPERS - THIN

Use
Single use absorbent pads for blotting excessive moisture from off the gel surface before sample application.

Storage
Store the thin filter papers in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED

1. DESTAINING SOLUTION (& WASH SOLUTION No. 1)

Preparation
At least 15 minutes before use, prepare 440 mL of solution containing (vol./vol.):

1) 45 % pure ethanol and 55 % distilled or deionized water,

or,

2) 30 % pure isopropanol and 70 % distilled or deionized water.

**IMPORTANT:** The use of another alcohol or denatured ethanol, may lead to atypical results. If lower concentrations of pure alcohol are used, adjust accordingly the volumes of alcohol and water.

Use
It serves for cleaning of the HYDRASYS Staining Compartment before staining the HYDRAGEL 7 LIPO + Lp(a) and HYDRAGEL LIPO + Lp(a) 15/30 gels. For destaining, i.e., removal of excess and background stain from the gels.

Storage, stability and signs of deterioration
Store destaining solution at room temperature tightly capped to prevent evaporation. It is stable for one month at room temperature. Discard destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
2. WASH SOLUTION No. 2

Preparation
Prepare 1 liter of a solution containing (vol./vol.):
1) 75% pure ethanol and 25% distilled or deionized water, or,
2) 70% pure isopropanol and 30% distilled or deionized water.
Denatured ethanol may also be used.

Use
For washing of the HYDRASYS staining compartment after staining the gels.

Storage, stability and signs of deterioration
Store wash solution No. 2 at room temperature tightly capped to prevent evaporation. It is stable for three months at room temperature.
Discard destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

3. FLUIDIL

Preparation
Fluidil (SEBIA, PN 4587, 1 vial, 5 mL) is ready to use.

Use
To dilute viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel (turbidity may be induced by cryoglobulin for instance but not by triglycerides).

Storage, stability and signs of deterioration
Store at room temperature or refrigerated. It is stable until the expiration date indicated on the Fluidil vial label.
Fluidil must be free of precipitate.

EQUIPMENT AND ACCESSORIES REQUIRED
1. HYDRASYS System SEBIA, PN 1210 or PN 1211.
2. Micropipettor, either manual or automated, such as HYDRAplus SEBIA, PN 1215, for an alternative way of loading the sample applicators.
3. Wet Storage Chamber PN 1270 supplied with HYDRASYS.
4. Accessory Kit HYDRASYS LIPOPROTEINS SEBIA, PN 1263.
5. Pipettes: 10 µL, 200 µL, and 1000 µL.
6. HYDRASYS Gel holder for 82 x 51 mm gels, SEBIA, PN 10043110.
7. Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 mm gel plates at 570 nm or with a yellow filter: HYRYS SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer’s instructions for operation and calibration procedures.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh serum samples are recommended for analysis. It is recommended to collect samples on fasting patients for at least 12 hours. They must be collected according to established procedures used in clinical laboratory testing.
Store samples refrigerated (2 to 8 °C) as soon as possible after collection and for 3 days maximum.
Do not freeze the samples.
Do not use samples collected on heparin.
Storage causes decreasing of pre-beta lipoprotein mobility, and therefore, undervaluation of the corresponding fraction. The fast pre-beta (or Lp(a)) lipoprotein mobility will not be affected by storage.

Sample preparation
Use undiluted serum samples.
Upon storage at 2 to 8 °C, some samples (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. Such samples might present application problems due to hindered diffusion through the sample applicator teeth. In such case, add 25 µL Fluidil to 75 µL serum and vortex for 15 seconds. Then follow the standard procedure.

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, drying, staining, destaining and final drying. The manual steps include handling samples and gels, and setting up the instrument for operation.
READ CAREFULLY HYDRASYS INSTRUCTION MANUAL.

I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator for HYDRAGEL 7 LIPO + Lp(a) (7 samples) and HYDRAGEL LIPO + Lp(a) 15/30 (15 samples), or two applicators for HYDRAGEL LIPO + Lp(a) 15/30 (30 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL neat sample in each well. Load each applicator within 2 minutes.
   - Place the applicator(s) into the wet storage chamber with the teeth up. Handle it (them) by the plastic tooth protection frame. Let the samples diffuse into the teeth for 5 minutes after the last sample application.
   See wet chamber package insert for further details.
3. Open the lid of the Migration Module and raise the electrode and applicator carriers.
   WARNING: Never close the lid while the carriers are raised!
4. Select «7 LIPO + Lp(a)» migration program for HYDRAGEL 7 LIPO + Lp(a) or «15/30 LIPO + Lp(a)» migration program for HYDRAGEL LIPO + Lp(a) 15/30 from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package; handle them by the plastic ends. Engage the punched ends of the strip’s plastic backing to the pins on the electrode carrier; the strip’s plastic backing must face the carrier (Fig. 2).
6. Unpack the HYDRAGEL plate.
   - Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately.

   **WARNING:** Do not leave the filter paper for too long contact with the gel to avoid its dehydration.
   - Pool 120 µL distilled or deionized water for HYDRAGEL 7 LIPO + Lp(a) gel, or 200 µL for HYDRAGEL LIPO + Lp(a) 15/30 gel, on the lower third of the frame printed on the Temperature Control Plate of the migration module.
   - Place the gel plate (gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 3).
   - Bend the gel and ease it down onto the water pool (Fig. 3). Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.

7. Lower both carriers down. In this position, the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.

8. Remove the applicator(s) from the wet chamber. Handle it (them) by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - For 7 and 15 samples analysis, place the applicator into position No 4 on the carrier.
   - For 30 samples analysis, place the two applicators each into position No 1 and 7.

   **IMPORTANT:** The numbers printed on the applicator(s) must face the operator (Fig. 4).

9. Close the lid of the migration module.

10. Start the procedure immediately by pressing the green arrow «START» key on the left side of the keyboard.

   **IMPORTANT:** After starting the washing program, check the following:
   - the destaining container contains at least 220 mL of destaining solution;
   - the waste container is empty.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines**).

   **IMPORTANT:** Do not forget to block up the unused lines.

   **II. WASH No. 1: THE PROCESSING / STAINING MODULE**

   1. Place the empty Gel Holder into the gel Processing / Staining Module.

   **IMPORTANT:** Before starting the washing program, check the following:
   - the staining container contains at least 220 mL of staining solution;
   - the waste container is empty.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines**).

   **IMPORTANT:** Do not forget to block up the unused lines.

   2. Select the «LIPO + Lp(a) & LIPO» staining program from the instrument menu. Start the run by pressing the «START» key (green arrow on the right side of the keyboard).

   During washing step, the compartment remains locked.

   After wash, an audible beep signals that the compartment unlocks. Remove the gel holder from the processing / staining module.

   **III. GEL PROCESSING SET-UP**

   1. Open the lid.
   2. Remove the applicator(s) and discard.
   3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   4. When the gel is dried, remove it immediately for further processing.
   5. After each use, wipe the electrodes and the temperature control plate with a soft wet tissue.
   6. Open the gel holder. Lay it flat and position the dried gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder (Fig. 5).
   7. Place the gel holder into the gel processing / staining module.

   **IMPORTANT:** Before starting the gel processing / staining program, check the following:
   - the staining container is filled with 220 mL of staining solution;
   - the destaining container contains at least 220 mL of destaining solution.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines**).

   **IMPORTANT:** Do not forget to block up the unused lines.

   8. Start the run by pressing the «START» key (green arrow on the right side of the keyboard).

   During gel staining, destaining, rinsing and drying steps, the compartment remains locked.

   After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).

   **IV. GEL PROCESSING COMPLETION**

   1. Remove the gel holder from the compartment; open the clips and remove the dried gel film.
   2. Scan using a densitometer / scanner with a yellow filter or at 570 nm, make the background zero at the lowest point.

   **NOTE:** The lengths of electrophoretic migrations may be slightly different with gels containing 2 or 3 analysis rows, without any adverse effects on performance.
V. WASH No. 2: THE STAINING COMPARTMENT

1. Place the empty gel holder into the gel processing / staining module.

   **IMPORTANT:** Before starting the washing program, check the following:
   - the wash container No. 2 contains at least 250 mL of wash solution No. 2;
   - the waste container is empty.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: **Reagent Lines**).

   **IMPORTANT:** Do not forget to block up the unused lines.

2. Start the run by pressing the «START» key (green arrow on the right side of the keyboard).

   During washing step, the compartment remains locked.

   After drying, an audible beep signals that the compartment unlocks.

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RESULTS

**Quality Control**

It is advised to include an assayed serum with normal triglycerides and cholesterol levels into each run of samples.

**Values**

Densitometry of stained electrophoregrams at 570 nm yields relative concentrations (percentages) of each fraction. The ranges of normal values (mean ± 2 SD) for individual zones on HYDRAGEL 7 LIPO + Lp(a) and HYDRAGEL LIPO + Lp(a) 15/30 gels have been established from a healthy population of 200 adults (men and women):

- Beta lipoproteins (LDL): 38.6 - 69.4 %
- Pre-beta lipoproteins (VLDL): 4.4 - 23.1 %
- Alpha lipoproteins (HDL): 22.3 - 53.3 %

The fast pre-beta lipoproteins, Lp(a), can be seen on lipidograms of normal individuals.

*It is recommended each laboratory establishes its own normal values.*

**Migration pattern**

Depending on sample composition, one of the following patterns can be observed:

- HDL
- VLDL
- LDL
- Application point

**Interpretation of lipoprotein patterns**

The lipoprotein pattern of a clinical sample must be interpreted visually by comparing it with a control or a normal serum pattern. Densitometry may be useful for the follow up of the patient to see which fraction will increase or decrease. Values can not be used to type hyperlipemia. Densitometry provides accurate relative percentage of individual lipoprotein fractions. Qualitative (presence of abnormal or absence of normal fractions) or semi-quantitative (relative increase or decrease of fractions) abnormalities necessitate further lipoprotein analyses. Further aid in the interpretation of lipoprotein patterns is the Fredrickson classification of lipoproteins.

**Lipoprotein electrophoresis interpretation - Fredrickson classification**

<table>
<thead>
<tr>
<th>TYPE OF HYPERLIPEMIA</th>
<th>TYPE I</th>
<th>TYPE II a</th>
<th>TYPE II b</th>
<th>TYPE III</th>
<th>TYPE IV</th>
<th>TYPE V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL CHOLESTEROL</td>
<td>2 - 4</td>
<td>3 - 10</td>
<td>2.8 - 3.5</td>
<td>3 - 5</td>
<td>&lt; 2.7</td>
<td>≤ 5</td>
</tr>
<tr>
<td>g/L</td>
<td>5.2 - 10.4</td>
<td>7.8 - 26</td>
<td>7.3 - 9.1</td>
<td>7.8 - 13</td>
<td>&lt; 7</td>
<td>≤ 13</td>
</tr>
<tr>
<td>mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRIGLYCERIDES</td>
<td>30 - 70</td>
<td>&lt; 1.6</td>
<td>2 - 5</td>
<td>2 - 9</td>
<td>2 - 10</td>
<td>≤ 30</td>
</tr>
<tr>
<td>g/L</td>
<td>34 - 79</td>
<td>&lt; 2</td>
<td>2.3 - 5.6</td>
<td>2.3 - 10.2</td>
<td>2.3 - 11.3</td>
<td>≤ 34</td>
</tr>
<tr>
<td>mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERUM APPEARANCE</td>
<td>milky</td>
<td>clear</td>
<td>clear to slightly turbid</td>
<td>clear to slightly turbid</td>
<td>turbid</td>
<td>milky</td>
</tr>
<tr>
<td>CHYLOMICRONS</td>
<td>++++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++++</td>
</tr>
<tr>
<td>LDL</td>
<td>- -</td>
<td>+++</td>
<td>++</td>
<td>++ coupled</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VLDL</td>
<td>normal to - -</td>
<td>normal</td>
<td>++</td>
<td>++++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>- -</td>
<td>normal to -</td>
<td>normal to -</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Lp(a) screening**

An additional fraction located between alpha and pre-beta fractions corresponds to Lp(a). Its mobility can vary slightly according to Lp(a) phenotypes. The minimal level of detection varies from about 0.03 g/dL. When this fraction is evidenced on the lipidogram, it is recommended to quantitate Lp(a) by a suitable assay, such as nephelometry or electroimmunodiffusion procedure.°
Particular cases

• When the pre-beta fraction (VLDL) is particularly heavy and anodic (in case of type IV or V), it may hide the Lp(a) fraction. In this case, let the serum stand for 24 to 48 hours at room temperature (which slows down VLDL mobility) and repeat the electrophoresis. If in doubt, quantify the Lp(a) by any suitable means.

• In the absence of chylomicrons, a fraction with a cathodic mobility indicates a special lipoprotein called LP X. It is found in icteric sera and in case of cholestasis and biliary disease.

Interference and limitations

• Do not use frozen samples.

• Storage causes decreasing of pre-beta mobility, and therefore, undervaluation of the corresponding fraction (VLDL). The mobility of Lp(a) is not affected by storage.

• Albumin migrates 8 mm ahead of the alpha lipoprotein band. This fraction may be very lightly stained with Sudan black. It must not be included in the lipoprotein analysis.

• In some sera, a more or less diffuse trail may appear in front of alpha lipoprotein fraction (HDL). This zone corresponds to partially delipidated HDL and it has to be included in the HDL percentage.

• The procedure is qualitative; detected changes necessitate further analysis.

Troubleshooting

Call Technical Service of the supplier when the test fails to perform while the instruction for the preparation and storage of materials, and for the procedure were carefully followed.

Kit reagent Safety Data Sheets and informations on waste products elimination are available from the Technical Service of the supplier.

PERFORMANCE DATA

SEBIA’s HYRYS densitometer was used for all densitometric evaluations. All electrophoregrams were also interpreted visually.

Reproducibility

In all reproducibility studies, the electrophoregrams were scanned and relative per cent concentration of each lipoprotein fraction recorded. Means, SD and CV were calculated with respect to the parameters examined. Upon visual examination of the electrophoregrams no differences were observed among the repeats. Good reproducibility (precision) was obtained under all circumstances. The tables show representative examples.

Within gel reproducibility

Three pathological samples with Lp(a) fraction were analyzed using HYDRAGEL 15 & 30 LIPO + Lp(a) procedure. Each sample was applied in all 15 tracks of a single gel from two different lots. The table shows for the three samples, the within gel data (one gel/lot example).

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>HDL (%)</th>
<th>Lp(a) (%)</th>
<th>VLDL (%)</th>
<th>LDL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>38.2 - 23.4 - 27.0</td>
<td>4.6 - 6.4 - 9.7</td>
<td>13.0 - 23.8 - 8.7</td>
<td>44.2 - 46.4 - 54.7</td>
</tr>
<tr>
<td>SD</td>
<td>0.8 - 0.6 - 0.6</td>
<td>0.2 - 0.3 - 0.3</td>
<td>0.3 - 0.3 - 0.3</td>
<td>0.8 - 0.6 - 0.6</td>
</tr>
<tr>
<td>CV %</td>
<td>2.1 - 2.8 - 2.2</td>
<td>4.2 - 4.2 - 3.5</td>
<td>2.7 - 1.2 - 3.4</td>
<td>1.7 - 1.2 - 1.1</td>
</tr>
</tbody>
</table>

Gel-to-gel reproducibility

Fifteen different serum samples were analyzed using HYDRAGEL 15 & 30 LIPO + Lp(a) procedure. Each sample was applied in one track in each of the 10 gels used from two different lots. Results obtained with one representative sample are shown.

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>HDL (%)</th>
<th>Lp(a) (%)</th>
<th>VLDL (%)</th>
<th>LDL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>30.5</td>
<td>4.3</td>
<td>10.8</td>
<td>54.4</td>
</tr>
<tr>
<td>SD</td>
<td>0.8</td>
<td>0.1</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>CV %</td>
<td>2.7</td>
<td>3.5</td>
<td>2.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Linearity and Sensitivity

Relative concentrations of lipoprotein fractions were determined in serial dilutions of a serum sample. The densitometric concentrations were found independent of the dilution factor. The highest dilution (1 : 4) providing a scannable pattern (i.e., essentially the same as obtained with the neat serum sample).

Concentration of Lp(a) was determined in serum samples by electroimmunodiffusion. The detection limit was about 20 mg/dL (from 10 to ≤ 30 mg/dL).

Accuracy

Densitometric, relative concentrations of individual lipoprotein fractions were measured in normal and abnormal serum samples (n = 56); 16 samples were with Lp(a). All samples were analyzed using the HYDRAGEL 15 & 30 LIPO + Lp(a) test and an equivalent electrophoretic test. No differences in visual interpretation of the electrophoregrams were observed. The densitometric data were analyzed using a linear regression statistical procedure:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>Y-Intercept</th>
<th>Slope</th>
<th>Range of % values of samples used*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0.985</td>
<td>-1.860</td>
<td>1.035</td>
<td>5.1 - 53.5</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.988</td>
<td>-0.168</td>
<td>1.013</td>
<td>2.4 - 18.4</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.994</td>
<td>-0.037</td>
<td>1.044</td>
<td>3.1 - 44.5</td>
</tr>
<tr>
<td>LDL</td>
<td>0.985</td>
<td>-0.731</td>
<td>1.016</td>
<td>32.2 - 92.0</td>
</tr>
</tbody>
</table>

* The % values are as determined in the SEBIA system.

Identification of the fast pre-beta fraction as Lp(a)

Immunofixation and immunosubtraction techniques were used to demonstrate that Lp(a) migrates as the "fast pre-beta" fraction as the only detectable component and that it is absent in LDL, VLDL and HDL fractions.
BIBLIOGRAPHY
