HYDRAGEL 7 LDL/HDL CHOL Direct
Ref. 4116

HYDRAGEL 15 LDL/HDL CHOL Direct
Ref. 4135

HYDRAGEL 30 LDL/HDL CHOL Direct
Ref. 4139

HYDRAGEL 54 LDL/HDL CHOL Direct
Ref. 4147
INTENDED USE

The HYDRAGEL 7, 15, 30 and 54 LDL/HDL CHOL Direct kits are designed for quantification of the cholesterol in the lipoproteins of human serum by electrophoresis on alkaline buffered (8.9) agarose gels on the semi-automated HYDRASYS instrument. The electrophorograms can be evaluated visually for qualitative assessment of the patterns and by densitometry to obtain relative quantification of the individual zones. The system is intended for measurement of the cholesterol of the high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions. The cholesterol values of these two fractions are indicated as an aid in the diagnosis and treatment of lipid disorders.

Each agarose gel is intended to run:
• 7 samples in the HYDRAGEL 7 LDL/HDL CHOL Direct kit,
• 15 samples in the HYDRAGEL 15 LDL/HDL CHOL Direct kit,
• 30 samples in the HYDRAGEL 30 LDL/HDL CHOL Direct kit,
• 54 samples in the HYDRAGEL 54 LDL/HDL CHOL Direct kit.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

The screening and management of dyslipoproteinemias are usually based upon the total serum cholesterol and triglyceride levels\(^1,4\). Analysis of lipoproteins provides further diagnostic information.

It has been well documented that the risk of cardiovascular diseases increases with the increase of the percentage of low density lipoproteins, i.e., VLDL (Very Low Density Lipoproteins), IDL (Intermediary Density Lipoproteins) and LDL (Low Density Lipoproteins) in the serum\(^5,7,10,13\). The high density lipoproteins (HDL) on the other hand, protect against atherosclerosis by removing cholesterol from the tissues back to the liver which is the only organ able to catabolize and excrete it\(^8,9\). A lipid constituent, such as cholesterol, can play different roles depending on the lipoprotein that transports it\(^6\). Thus, to evaluate the heart disease risk of a dyslipoproteinemia, it is necessary to quantify the cholesterol in the lipoprotein fractions, primarily LDL and HDL\(^1,12,14,15,17,19\).

HYDRAGEL LDL/HDL CHOL Direct procedure is carried out in two stages:
• electrophoresis on agarose gel to separate the LDL, VLDL and HDL as well as chylomicrons ; Lp(a) when present comigrates with LDL,
• visualization of lipoprotein fractions based on a sensitive and cholesterol-specific enzymatic method, according to the following reactions:

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{free cholesterol} + \text{fatty acids} \\
\text{Free cholesterol} + \text{NAD} \xrightarrow{\text{Cholesterol dehydrogenase}} \text{cholestenone} + \text{NADH} + \text{H}^+ \\
\text{NADH} + \text{PMS} \xrightarrow{\text{Reduced PMS} + \text{NAD}} \text{Reduced PMS} + \text{NBT} \\
\text{Reduced PMS} + \text{NBT} \xrightarrow{\text{Precipitate of formazan (blue)}} \text{PMS} + \text{reduced NBT}
\]

The amount of resulting formazan precipitate is proportional to the cholesterol concentration.

ABBREVIATIONS:
NAD: Nicotinamide Adenine Dinucleotide
PMS: Phenazine Methosulfate
NBT: Nitro Blue Tetrazolium

The quantitation of cholesterol in LDL and HDL fractions is not affected by the presence of triglycerides (TG) for the following reasons:
• The exogenous TG are associated with chylomicrons and the endogenous TG with VLDL ; both chylomicrons and VLDL are clearly separated from other lipoprotein fractions - the chylomicrons stay at the application point and VLDL migrate between LDL and HDL.
• It is the cholesterol not the lipids which are assayed in each fraction.
• The absence of interference has been experimentally verified.

Recent studies at the National Institute of Health (NIH Bethesda, USA) have shown that the risk of developing a coronary disease with atherosclerosis is function of the LDL cholesterol / HDL cholesterol ratio: the higher the ratio, the greater the risk\(^1,3,18\). In the case of subnormal cholesterolemia, it appears to be essential to investigate the cholesterol distribution in the different lipoproteins before determining any therapy\(^16\).

HYDRAGEL LDL/HDL CHOL Direct procedure allows a precise and direct measurement of LDL, VLDL and HDL cholesterol level regardless the triglyceride level.

ABBREVIATIONS:
VLDL: Very Low Density Lipoproteins
LDL: Low Density Lipoproteins
IDL: Intermediary Density Lipoproteins
HDL: High Density Lipoproteins
REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7, 15, 30 & 54 LDL/HDL CHOL Direct KITS

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4116</th>
<th>PN 4135</th>
<th>PN 4139</th>
<th>PN 4147</th>
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<tr>
<td>Agarose Gels (ready to use)</td>
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<td>10 gels</td>
<td>10 gels</td>
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<tr>
<td>Buffered Strips (ready to use)</td>
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<td>Enzymes (freeze dried)</td>
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<td>20 vials</td>
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<td>1 pack of 10 (15 teeth)</td>
<td>2 packs of 10 (15 teeth)</td>
<td>3 packs of 10 (18 teeth)</td>
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<tr>
<td>Filter Papers - Thin</td>
<td>1 pack of 10</td>
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<tr>
<td>Filter Papers - Thick</td>
<td>1 pack of 10</td>
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</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, 0.65 g/dL; alkaline buffer pH 8.9 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for lipoproteins 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). They are stable until the expiration date indicated on the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards). Avoid storage close to a window or to a heat source. Avoid significant variations of temperature during storage. DO NOT FREEZE.

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: alkaline buffer pH 9.0 ± 0.1; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

**WARNING**: The buffer in the strips contains 0.30 % 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 preferentially refrigerated (2 to 8 °C). (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. SUBSTRATE SOLVENT
Preparation
Substrate solvent is ready to use. It contains: NaCl, additives nonhazardous at concentrations used, necessary for optimum performance.

Use
For the preparation of the developing solution as described in paragraph 5.

Storage, stability and signs of deterioration
Store substrate solvent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or substrate solvent vial label. Discard substrate solvent if it changes its appearance, e.g., becomes cloudy due to microbial contamination. Substrate solvent must be free of precipitate.

4. CHROMOGEN
Preparation
Chromogen is ready to use. It contains: Nitroblue Tetrazolium and Phenazine Methosulfate.

Use
For the preparation of the developing solution as described in paragraph 5.

Storage, stability and signs of deterioration
Store chromogen at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or chromogen vial label. Discard chromogen if it changes its appearance, e.g., becomes cloudy due to microbial contamination. Chromogen must be free of precipitate.
5. ENZYMES

Preparation
Each enzymes vial contains: Cholesterol Esterase, Cholesterol Dehydrogenase, Nicotinamide Adenine Dinucleotide, additives nonhazardous at concentrations used, necessary for optimum performance.
Prepare the developing solution 10 minutes before use.

5.1. HYDRAGEL 7 LDL/HDL CHOL Direct gels - 7 samples/gel: Add 1 mL substrate solvent to one enzymes (1/2) vial and 5 minutes later, mix gently. Just before use, add 0.25 mL chromogen and mix.

5.2. HYDRAGEL LDL/HDL CHOL Direct 15/30 gels - 15 samples/gel: Add 2 mL substrate solvent to one enzymes vial and 5 minutes later, mix gently. Just before use, add 0.5 mL chromogen and mix.

5.3. HYDRAGEL LDL/HDL CHOL Direct 15/30 gels - 30 samples/gel: Add 2 mL substrate solvent to each of the 2 enzymes vials and 5 minutes later, mix gently. Just before use, add 0.5 mL chromogen to each vial and mix.

5.4. HYDRAGEL 54 LDL/HDL CHOL Direct – 54 samples/gel: Add 2 mL substrate solvent to each of the 2 enzymes vials and 5 minutes later, mix gently. Just before use, add 0.5 mL chromogen to each vial and mix.

Use
As a component of developing solution for enzymatic visualization of cholesterol.

Storage, stability and signs of deterioration
Store enzymes refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or enzymes vial label.

NOTE: During transportation, the enzymes may be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

6. HYDRAGEL LDL/HDL CHOL Direct BLOCKING SOLUTION

Preparation
The blocking solution is ready to use and contains: buffer; additives nonhazardous at concentrations used, necessary for optimum performance.

Use
To stop the enzymatic reaction with the substrate after incubating the gels for specified time.

Storage, stability and signs of deterioration
Store the blocking solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or blocking solution vial label.

NOTE: When stored between 2 and 8 °C, the blocking solution may develop a crystalline precipitate without any adverse effects on its performance; then, leave the blocking solution vial at room temperature for at least 10 minutes before use to re-dissolve the crystals.

7. APPLICATORS

Use
Precut, single use applicators for sample application.

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

8. FILTER PAPERS - THIN

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

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

9. FILTER PAPERS - THICK

Use
Precut, single use, thick absorbent paper pads for blotting excessive blocking solution off the gel surface before washing.

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

REAGENTS REQUIRED BUT NOT SUPPLIED

1. FLUIDIL

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

Use
To dilute viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel.

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.

2. DESTAINING SOLUTION

Preparation
Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials 100 mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container. After dilution, the working destaining solution contains: citric acid, 0.05 g/dL.

Use
For washing the gels and the staining chamber after cleaning it with wash solution. To neutralize the acidity of the destaining solution, pour 15 mL of a 50 % solution of sodium hydroxide, into the empty waste container.
Storage, stability and signs of deterioration

Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the packaging or destaining solution vial labels. Working destaining solution is stable for one week at room temperature in a closed bottle; to prevent microbial growth for longer storage, add 5 µL/dL of ProClin 300. Do not add any sodium azide.

Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination. Working destaining solution with ProClin is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

3. HYDRASYS WASH SOLUTION

Preparation

Each vial of stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3; sodium azide.

WARNING: The stock wash solution contains 0.625 % sodium azide. Do not ingest! If ingested, consult physician immediately! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acids, lead or copper. Always flush with a large quantity of water when disposing.

Use

For cleaning of the HYDRASYS Staining Compartment before processing the HYDRAGEL LDL/HDL CHOL Direct gels. See the wash solution package insert for directions to use.

Storage, stability and signs of deterioration

Store the stock solution and working wash solution at room temperature or refrigerated. They are stable until the expiration date indicated on the kit package or wash solution vial labels.

Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. HYDRASYS System SEBIA, PN 1210 or PN 1211.
2. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216 or HYDRAPLUS 2 SEBIA, PN 1217, for an alternative way of loading the sample applicators.
3. Wet Storage Chamber supplied with HYDRASYS, PN 1270.
4. Container Kit supplied with HYDRASYS.
5. Accessory Kit for HYDRASYS ISO-LDH, ISO-PAL, CHOL, SEBIA, PN 1261, containing templates ENZ 2 mL and ENZ 4 mL.
6. Template Guide Bar SEBIA, supplied with HYDRASYS.
7. Template Guide Bar specific for ENZ 4 mL template used in the analysis of 54 sample gels, SEBIA, PN 1272.
8. Pipettes: 10 µL, 200 µL, 1 mL and 5 mL.
9. Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 mm gels at 570 nm or with a yellow filter, e.g., HYRYS SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer’s instructions for operation and calibration procedures.
10. Gel holder for half gels (7 sample gel) SEBIA, PN 1278.

SAMPLES FOR ANALYSIS

Sample collection and storage

Fresh serum samples are recommended for analysis. It is recommended to collect samples from individuals fasting for at least 12 hours. Samples must be collected according to established procedures used in clinical laboratory testing. Store samples at 2 to 8 °C as soon as possible after collection and for three days maximum.

Do not freeze the samples.

Do not use samples collected on heparin.

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 Fluidi 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, enzymatic development, washing and drying. The manual steps include handling samples and gels, application of the developing reagent 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 LDL/HDL CHOL Direct (7 samples) and HYDRAGEL LDL/HDL CHOL Direct 15/30 (15 samples), two applicators for HYDRAGEL LDL/HDL CHOL Direct 15/30 (30 samples), or three applicators for HYDRAGEL 54 LDL/HDL CHOL Direct (54 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL 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 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 LDL/HDL" migration program for HYDRAGEL 7 LDL/HDL CHOL Direct, "15/30 LDL/HDL" migration program for HYDRAGEL LDL/HDL CHOL Direct 15/30 or "54 LDL/HDL" for HYDRAGEL 54 LDL/HDL CHOL Direct, 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 agarose gel 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 a too long contact with the gel to avoid its dehydration.
   - Pool 120 µL distilled or deionized water for HYDRAGEL 7 LDL/HDL CHOL Direct or 200 µL for HYDRAGEL 15/30 and 54 LDL/HDL CHOL Direct, on the lower third of the frame printed on the temperature control plate of the migration module.
   - Place the gel plate (the 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(s) teeth's protection frame.
   - Place the applicator(s) on the carrier:
     - With HYDRAGEL 7 LDL/HDL CHOL Direct or HYDRAGEL LDL/HDL CHOL Direct 15/30 (15 samples) : into position No. 3,
     - With HYDRAGEL LDL/HDL CHOL Direct 15/30 (30 samples) : into position No. 1 and 7,
     - With HYDRAGEL 54 LDL/HDL CHOL Direct : into positions No. 1, 5 and 9.
   
   **IMPORTANT:** The numbers printed on the applicator 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.
   - During migration, wash the Processing / Staining module. See paragraph II below.
   
   **IMPORTANT:** Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

11. After the migration step is terminated, proceed to paragraph III.

**MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS**

- The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out at 20 °C controlled by Peltier effect under constant current : 10 W for HYDRAGEL 7 LDL/HDL CHOL Direct and 20 W for HYDRAGEL LDL/HDL CHOL Direct 15/30, until 32 Vh accumulated (for about 8 minutes) and 20 W until 23 Vh accumulated (for about 7 minutes) for HYDRAGEL 54 LDL/HDL CHOL Direct.
- The electrode carrier rises to disconnect the electrodes.
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: "*δ* REAG.*.

**NOTE:** The migration module lid remains closed during all migration steps.

**II. WASH OF 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 wash container contains at least 400 mL of wash 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 "LDL/HDL" washing program from the instrument menu. Start the run by pressing the "START" key (green arrow on the right side of the keyboard).

**WASH - DESCRIPTION OF THE AUTOMATED STEPS**

- Washing step: wash solution circulates for 10 minutes through the compartment.
- After wash, an audible beep signals that the compartment unlocks.
- Remove the gel holder from the Processing / Staining module.

**III. VISUALIZATION**

1. Open the migration module lid. The following flashing message stops flashing : "*δ* REAG.*.

2. Remove the sample applicator(s) and discard.

3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Wipe electrodes with soft wet tissue.
   - Leave the gel in place in the migration module.

4. Set up the reagent application template, template ENZ 2 mL for 7 or 15 samples and template ENZ 4 mL for 30 and 54 samples, as follows (Fig. 5) :
   - HYDRAGEL 7 & 15/30 LDL/HDL CHOL Direct : Position the standard application template guide bar on the lower two anchoring pins (the guide may stay in the migration module all the time).
   - HYDRAGEL 54 LDL/HDL CHOL Direct : Position the application template guide bar, specific for ENZ 4 mL template used in the analysis of 54 samples, on the lower two anchoring pins. It is recommended to remove this bar after the LDL/HDL CHOL Direct procedure to avoid any mistake during further manipulations.
   - Hold the template by the flap and insert it into the template guide (the right clip on the notch).
   - Lower the template onto the gel.

5. Apply immediately visualization solution : 1.25 mL (HYDRAGEL 7 LDL/HDL CHOL Direct), 2.5 mL (HYDRAGEL LDL/HDL CHOL Direct 15/30 for 15 samples) or 5 mL (2 vials) (HYDRAGEL LDL/HDL CHOL Direct 15/30 for 30 samples and HYDRAGEL 54 LDL/HDL CHOL Direct) (Fig. 6).
   - Take reagents without trapping any air bubbles into the pipette tip.
   - Hold pipette vertically and rest its tip lightly at the bottom of the well.
   - Inject reagent so it spreads through the trough without trapping any bubbles.
6. Close the lid of the migration module.
7. Start the procedure immediately by pressing the green arrow “START” key on the left side of the keyboard. A message “[INCUBATION]” appears on the screen.

**VISUALIZATION - DESCRIPTION OF THE AUTOMATED STEPS**

- Incubation at 30 °C for 30 minutes controlled by Peltier effect.
- An audible beep signals that the migration module lid unlocks. This signal remains until the operator intervenes. The following message is displayed on the screen: " φ REAG. + φ BLOC."

**NOTE:** The migration module lid remains locked during incubation.

**IV. SUBSTRATE ELIMINATION AND APPLICATION OF BLOCKING SOLUTION**

1. Open the cover. The following flashing message stops flashing: " φ REAG. + φ BLOC."
2. Remove the remaining substrate solution:
   - Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig. 6).
   - Carefully and progressively withdraw the reagent.
3. Pipet blocking solution into the template (Fig. 6): 1 mL for HYDRAGEL 7 LDL/HDL CHOL Direct, 2 mL for HYDRAGEL LDL/HDL CHOL Direct 15/30 (15 samples) or 4 mL for HYDRAGEL LDL/HDL CHOL Direct 15/30 (30 samples) and HYDRAGEL 54 LDL/HDL CHOL Direct. Do not introduce air bubbles.
4. Close the HYDRASYS cover.
5. Start the run by pressing the “START” key (green arrow on the left side of the keyboard). The following message is displayed on the screen: "[BLOCKING]"

**BLOCKING - DESCRIPTION OF AUTOMATIC STEPS**

- Incubation at 30 °C, for 10 minutes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following flashing message is displayed on the screen: " φ BLOC. + φ PAP."

**NOTE:** During the blocking step, the cover of the migration module remains locked.

**V. ELIMINATION OF BLOCKING SOLUTION AND FILTER PAPER APPLICATION**

1. Open the cover.
2. Remove the blocking solution with a pipet (see SUBSTRATE ELIMINATION).
3. Remove the template:
   - Grasp the flap of the template.
   - Raise the template and remove it.
4. Apply one thick filter paper on the gel:
   - Slope the filter paper at about 45°.
   - Align the lower side of the filter paper with the edge of the gel.
   - Lower the filter paper onto the gel.
   - Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
5. Close the HYDRASYS cover.
6. Start the blotting sequence by pressing the “START” key (green arrow on the left side of the keyboard). The following message is displayed on the screen: "[BLOTTING]"
7. Rinse the template with distilled water or alcohol and dry it thoroughly with soft absorbent paper. Prior to re-use, ensure the template is completely dry.

**BLOTTING - DESCRIPTION OF AUTOMATIC STEPS**

- Blotting at 30 °C and at 50 °C controlled by Peltier effect, for 5 minutes.
- A beep sounds until the operator intervenes. The following flashing message is displayed on the screen: " φ PAP. + STOP"

**NOTE:** During the blotting step, the cover of the migration module remains locked.

**VI. WASH OF THE GEL**

1. Remove the gel plate for further processing.
2. Open the gel holder. Lay it flat and position the 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. 7).
3. Place the gel holder into the gel Processing / Staining module.

**IMPORTANT:** Before starting the gel processing / staining program, check the following:
- the destaining solution container contains at least 800 mL of destaining solution;
- the waste container is empty.
4. Start the run by pressing the “START” key (green arrow on the right side of the keyboard).
   - During washing 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).
5. Remove the gel holder from the compartment; open the clips and remove the dried gel.
VII. GEL SCANNING

1. If needed, clean the back side (the plastic support side) of the dried film with a damp soft tissue paper.
2. Scan using a densitometer / scanner at 570 nm or with a yellow filter.
   
   NOTE: The lengths of electrophoretic migrations may be slightly different with gels containing 2 or 3 analysis rows, without any adverse effects on performance.

NOTE:
The temperature of the HYDRASYS migration module keeps decreasing until it reaches 20 °C (in less than 5 minutes) after which a new migration run can be started.
Wipe the temperature control plate with a soft wet tissue.
Put the sample applicator and electrode carriers back in place.

RESULTS

Quality Control
Include a cholesterol control into each run according to applicable regulations. Establish own control intervals and preferably, use a two level control material traceable to Cholesterol Reference Method Laboratory Network (CRMLN).

Values
Densitometer scanning with a yellow filter (570 nm) yields relative concentrations (percentages) of individual zones. The profiles may differ from one another. This depends on the presence or absence of VLDL (see the migration patterns).

When quantitating HDL and LDL, make sure that the minima delimiting the HDL and LDL fractions are situated at the feet of the HDL and LDL peaks in the densitometric profile.
Calculate the HDL cholesterol and LDL cholesterol concentrations with the following formulas:

\[
\text{HDL cholesterol (HYRYS)} = \frac{\text{Total cholesterol (mg/dL or mmol/L)}}{100} \times \text{HDL percentage}
\]

\[
\text{LDL cholesterol (HYRYS)} = \frac{\text{Total cholesterol (mg/dL or mmol/L)}}{100} \times \text{LDL percentage}
\]

Normal values (mean ± 2 SD) for LDL and HDL fractions with HYDRAGEL 7, 15, 30 & 54 LDL/HDL CHOL Direct procedure have been established from a healthy population of 256 adults from France (124 men and 132 women) with HYRYS densitometer and PHORESIS scanner:

<table>
<thead>
<tr>
<th>Total Cholesterol</th>
<th>HDL cholesterol (HYRYS)</th>
<th>LDL cholesterol (HYRYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td>≤ 2.00 g/L or ≤ 5.17 mmol/L</td>
<td>0.40 - 1.00 g/L or 1.03 - 2.59 mmol/L</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>≤ 2.00 g/L or ≤ 5.17 mmol/L</td>
<td>0.33 - 0.84 g/L or 0.84 - 2.17 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cholesterol total</th>
<th>HDL cholesterol (PHORESIS)</th>
<th>LDL cholesterol (PHORESIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td>≤ 2.00 g/L or ≤ 5.17 mmol/L</td>
<td>0.41 - 1.04 g/L or 1.05 - 2.69 mmol/L</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>≤ 2.00 g/L or ≤ 5.17 mmol/L</td>
<td>0.30 - 0.88 g/L or 0.77 - 2.27 mmol/L</td>
</tr>
</tbody>
</table>

[N.C.E.P. recommends LDL cholesterol value to be lower than 160 mg/dL (4.14 mmol/L)].
It is recommended each laboratory establish its own normal values.

Decreased levels of HDL cholesterol are associated with increased risk of coronary heart disease (CHD)\(^{16-20}\). HDL cholesterol is considered high risk at ≤ 33 mg/dL (0.84 mmol/L) and protective at ≥ 84 mg/dL (2.17 mmol/L) (values obtained with HYRYS densitometer).
The other risk factors include age, family history of premature CHD, hypertension, diabetes and smoking\(^{16-20}\).

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

![Migration patterns diagram]

Lp(a) when present co-migrates with LDL; chylomicrons stay at the application point.

Interference and Limitations
• Do not use samples collected on heparin, or which were frozen or stored over 3 days.
• The HYDRAGEL 7, 15, 30 and 54 LDL/HDL CHOL Direct procedures have not been standardized by the CRMLN system.
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 information on waste products disposal are available from the Technical Service of the supplier.

PERFORMANCE DATA

SEBIA’s HYRYS densitometer and PHORESIS scanner were used for all densitometric measurements.

Reproducibility
The individual values, means, SD and CV are shown below. In overall, the results indicate a very good reproducibility for all the aspects tested for LDL and HDL cholesterol quantification : 1.9 % being the mean CV value.

Within gel reproducibility
Four different serum samples (A, B, C & D) were electrophoresed using HYDRAGEL 15, 30 & 54 LDL/HDL CHOL Direct procedure on gels from three lots (lot No. 1 : 15 samples per gel, lot No. 2 : 30 samples per gel and lot No. 3 : 54 samples per gel). The electrophoreograms were evaluated by densitometry. The following tables show within gel reproducibility, i.e., the means, SD’s and CV’s for each HDL and LDL fraction, individually for each sample and lot.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>HDL (HYRYS)</th>
<th>LDL (HYRYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample A: lot No. 1 / lot No. 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>42.7 - 41.8</td>
<td>56.5 - 57.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.7 - 0.7</td>
<td>0.7 - 0.7</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.6 - 1.7</td>
<td>1.2 - 1.2</td>
</tr>
<tr>
<td><strong>Sample B: lot No. 1 / lot No. 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>15.1 - 14.6</td>
<td>63.1 - 63.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.5 - 0.5</td>
<td>0.8 - 0.9</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.2 - 3.7</td>
<td>1.2 - 1.4</td>
</tr>
<tr>
<td><strong>Sample C: lot No. 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>42.7</td>
<td>54.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Sample D: lot No. 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>13.5</td>
<td>47.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>HDL (PHORESIS)</th>
<th>LDL (PHORESIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample A: lot No. 1 / lot No. 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>45.5 - 44.3</td>
<td>53.9 - 55.3</td>
</tr>
<tr>
<td>SD</td>
<td>1.0 - 1.0</td>
<td>1.0 - 1.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.1 - 2.2</td>
<td>1.8 - 1.8</td>
</tr>
<tr>
<td><strong>Sample B: lot No. 1 / lot No. 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>13.7 - 13.2</td>
<td>62.7 - 63.1</td>
</tr>
<tr>
<td>SD</td>
<td>0.9 - 0.9</td>
<td>1.4 - 1.3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.9 - 6.9</td>
<td>2.2 - 2.0</td>
</tr>
<tr>
<td><strong>Sample C: lot No. 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>42.5</td>
<td>55.0</td>
</tr>
<tr>
<td>SD</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Sample D: lot No. 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN (%)</td>
<td>12.3</td>
<td>48.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Between gels reproducibility
Fifteen (15) different serum samples were each electrophoresed using HYDRAGEL 15 & 30 LDL/HDL CHOL Direct procedure on 10 gels from the same lot (lot No. 1 : 15 samples per gel ; lot No. 2 : 30 samples per gel with 2 analysis per sample on each gel).
Eighteen (18) different serum samples were each electrophoresed using HYDRAGEL 54 LDL/HDL CHOL Direct procedure on 10 gels from the same lot (lot No. 3 : 54 samples per gel with 3 analysis per sample on each gel).
The electrophoregrams were evaluated by densitometry. The following tables show between gels reproducibility, i.e., the range of mean values, SD's and CV's for each HDL and LDL fraction and the mean CV representing all samples (n = 15 or 18).

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>lot No.</th>
<th>HDL (HYRYS)</th>
<th>LDL (HYRYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (%)</td>
<td>1</td>
<td>17.5 - 51.0</td>
<td>46.7 - 73.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.9 - 50.9</td>
<td>47.3 - 73.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.7 - 50.6</td>
<td>48.3 - 80.3</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>0.5 - 0.8</td>
<td>0.5 - 1.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4 - 1.1</td>
<td>0.5 - 1.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5 - 1.0</td>
<td>0.5 - 1.4</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1</td>
<td>1.5 - 3.9</td>
<td>0.7 - 1.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.1 - 3.3</td>
<td>0.8 - 1.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.2 - 4.4</td>
<td>0.9 - 2.1</td>
</tr>
<tr>
<td>MEAN CV (%)</td>
<td>1</td>
<td>2.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>lot No.</th>
<th>HDL (PHORESIS)</th>
<th>LDL (PHORESIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (%)</td>
<td>1</td>
<td>16.4 - 54.3</td>
<td>44.6 - 78.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.9 - 53.3</td>
<td>45.6 - 77.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13.9 - 49.7</td>
<td>49.4 - 82.4</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>0.6 - 1.1</td>
<td>0.6 - 1.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.9 - 1.7</td>
<td>0.8 - 1.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.6 - 1.4</td>
<td>0.7 - 1.6</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1</td>
<td>1.1 - 5.4</td>
<td>1.0 - 2.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.7 - 7.6</td>
<td>1.7 - 3.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.0 - 5.6</td>
<td>1.2 - 2.3</td>
</tr>
<tr>
<td>MEAN CV (%)</td>
<td>1</td>
<td>2.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.2</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Accuracy - Comparative study

Study No. 1
One hundred and forty (140) different serum samples (normal and pathological) were analyzed using SEBIA’s HYDRAGEL 15 & 30 LDL/HDL CHOL Direct kits and another, commercially available test for quantification of HDL cholesterol. LDL quantification was performed by calculation from HDL quantification (Friedewald procedure).

The results of linear regression analysis of the densitometric values by both procedures are tabulated below. Similar results were obtained with HYDRAGEL 54 LDL/HDL CHOL Direct procedure.

**HYRYS**:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-intercept*</th>
<th>Slope</th>
<th>Range of cholesterol c° values** (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0.976</td>
<td>-0.049</td>
<td>0.950</td>
<td>0.57 - 4.37</td>
</tr>
<tr>
<td>LDL</td>
<td>0.977</td>
<td>0.573</td>
<td>0.951</td>
<td>1.40 - 6.53</td>
</tr>
</tbody>
</table>

* y = SEBIA test  
** measured by the HYDRAGEL 15 & 30 LDL/HDL CHOL Direct test and HYRYS densitometer

**PHORESIS**:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-intercept*</th>
<th>Slope</th>
<th>Range of cholesterol c° values** (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0.965</td>
<td>-0.215</td>
<td>1.056</td>
<td>0.40 - 4.44</td>
</tr>
<tr>
<td>LDL</td>
<td>0.969</td>
<td>0.552</td>
<td>0.985</td>
<td>1.37 - 6.61</td>
</tr>
</tbody>
</table>

* y = SEBIA test  
** measured by the HYDRAGEL 15 & 30 LDL/HDL CHOL Direct test and PHORESIS scanner

Study No. 2
Ninety (90) different serum samples (normal and pathological) were analyzed using SEBIA’s HYDRAGEL 15 & 30 LDL/HDL CHOL Direct kits and another, commercially available test for quantification of HDL and LDL cholesterol. The results of linear regression analysis of the densitometric values by both procedures are tabulated below. Similar results were obtained with HYDRAGEL 54 LDL/HDL CHOL Direct procedure.
**HYDRAGEL 7, 15, 30 & 54 LDL/HDL CHOL Direct - 2005/05**

### HYRYS:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-intercept*</th>
<th>Slope</th>
<th>Range of cholesterol c° values** (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0.954</td>
<td>-0.051</td>
<td>0.997</td>
<td>0.37 - 2.71</td>
</tr>
<tr>
<td>LDL</td>
<td>0.984</td>
<td>0.454</td>
<td>0.943</td>
<td>0.88 - 5.74</td>
</tr>
</tbody>
</table>

*y = SEBIA test

**measured by the HYDRAGEL 15 & 30 LDL/HDL CHOL Direct test and HYRYS densitometer**

### PHORESIS:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-intercept*</th>
<th>Slope</th>
<th>Range of cholesterol c° values** (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0.961</td>
<td>-0.263</td>
<td>1.126</td>
<td>0.22 - 2.82</td>
</tr>
<tr>
<td>LDL</td>
<td>0.978</td>
<td>0.434</td>
<td>0.981</td>
<td>0.91 - 5.96</td>
</tr>
</tbody>
</table>

*y = SEBIA test

**measured by the HYDRAGEL 15 & 30 LDL/HDL CHOL Direct test and PHORESIS scanner**

### Linearity - Sensitivity

The linearity of the HYDRAGEL 15 LDL/HDL CHOL Direct test was determined by assaying mixtures of two samples (total cholesterol : 6.1 mmol/L). The modified LDL/HDL cholesterol assays were found linear in the entire range studied, e.g., HYDRAGEL 15 LDL/HDL CHOL Direct test : HDL, 1.03 – 4.34 mmol/L ; LDL, 1.79 – 5.09 mmol/L.

The minimum mean variation of the LDL and HDL fractions detectable by the test was 5.4 %.

### BIBLIOGRAPHY


Figure 1

Figure 2

Figure 3

Figure 4