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HYDRAGEL 7 ISO-LDH

Ref. 4110

HYDRAGEL 15 ISO-LDH

Ref. 4130

HYDRAGEL 30 ISO-LDH

Ref. 4136

IVD

CE

2004/11

INTENDED USE

The HYDRAGEL 7, 15 and 30 ISO-LDH kits are designed for the identification and quantification of the five lactate dehydrogenase (LD) isoenzymes in human serum by electrophoresis on alkaline buffered (pH 8.4) agarose gels. The kits are used in conjunction with the semi-automated HYDRASYS electrophoresis system to obtain gels ready for interpretation. The serum samples are electrophoresed and the separated LD isoenzymes are visualized using a specific chromogenic substrate. The dried gels are ready for visual examination and densitometry to obtain accurate relative quantification of individual zones.

Each agarose gel is intended to run:
- 7 samples in the HYDRAGEL 7 ISO-LDH kit,
- 15 samples in the HYDRAGEL 15 ISO-LDH kit,
- 30 samples in the HYDRAGEL 30 ISO-LDH kit.

For *In Vitro* Diagnostic Use.

PRINCIPLE OF THE TEST [1-4]

Each LD isoenzyme is a tetramer made up of four subunits (polypeptide chains). There are two types of these subunits designated M («muscle») and H («heart»). The five LD isoenzymes consist of the five possible combinations of M and H subunits which confer distinct electrophoretic and other properties on each LD isoenzyme. The isoenzymes are designated by their electrophoretic mobility whereby LD1 has been given to the isoenzyme with greatest anodic mobility. The following table summarizes the nomenclature, composition and the primary tissue source of LD isoenzymes:

NOMENCLATURE	COMPOSITION	TISSUE SOURCE
LD1	H_4	heart (myocardium)
LD2	H_3M	heart (myocardium)
LD3	H_2M_2	variable amounts in many tissues
LD4	HM_3	variable amounts in many tissues
LD5	M_4	skeletal muscle, liver

Diagnosis of myocardial infarction (MI) represents the major value of LD isoenzyme electrophoresis. In normal serum, LD2 is the most prevalent isoenzyme and the LD1 / LD2 ratio is generally < 1. The concentrations of LD1 and, to a lesser degree of LD2, increase after MI and the LD1 / LD2 ratio becomes > 1 (the so called LD1 / LD2 flip). The total LD concentration increases by a factor of two to three within 12 to 24 hours after MI. The LD activity reaches its maximum after two or three days and remains at a high level during about two weeks after infarction.
The LD isoenzyme analysis is generally run in tandem with CK (creatine kinase) isoenzymes and / or other early cardiac markers to confirm or rule out the diagnosis of MI, assess its severity and monitor patient's condition. Because of the rather unique distribution of LD isoenzymes in various tissues, their assay in serum aids in diagnosing tissue damage such as in pulmonary and renal infarctions, and hepatic disease.
All LD isoenzymes catalyze the same reversible reaction which is utilized in their visualization. In the HYDRAGEL 7, 15 and 30 ISO-LDH kits, the visualization of LD isoenzymes is performed according to the following reactions:

The amount of resulting formazan precipitate is proportional to the LD enzymatic activity.

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

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7, 15 AND 30 ISO-LDH KITS

ITEMS	PN 4110	PN 4130	PN 4136
Agarose Gels (ready to use)	10 gels	10 gels	10 gels
Buffered Strips (ready to use)	10 packs of 2 each	10 packs of 2 each	10 packs of 2 each
Substrate solvent (ready to use)	1 vial, 45 mL	1 vial, 45 mL	1 vial, 45 mL
ISO-LDH Substrate (freeze-dried)	10 vials	10 vials	10 vials
ISO CK/LD Blocking Solution (ready to use)	1 vial, 40 mL	1 vial, 40 mL	1 vial, 40 mL
Applicators (ready to use)	1 pack of 10	1 pack of 10	2 packs of 10
Filter Papers - Thin	1 pack of 10	1 pack of 10	1 pack of 10
Filter Papers - Thick	1 pack of 10	1 pack of 10	1 pack of 10

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 ; alkaline buffer pH 8.40 ± 0.05 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The gels contain 0.10 % sodium azide. Do not ingest ! If ingested, consult physician immediately !

Use

Support medium for lactate dehydrogenase isoenzymes 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 or the gel package label. (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 important variation 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, or
(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 8.3 ± 0.1 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The buffer in the strips contains 0.20 % sodium azide. 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.

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. SUBSTRATE SOLVENT

Preparation

Substrate solvent is ready to use. It contains: lithium lactate ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

For the preparation of the developing solution as described in paragraph 4.

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 labels.
Discard substrate solvent if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. ISO-LDH SUBSTRATE

Preparation

Each substrate vial contains: nicotinamide adenine dinucleotide (NAD), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), additives, nonhazardous at concentrations used, necessary for optimum performance.
4.1. HYDRAGEL 7 ISO-LDH: Prepare the ISO-LDH substrate solution, away from light, 10 minutes before use by adding 2.25 mL of substrate solvent to the vial. Close the vial, leave at room temperature (15 to 30 °C) for 5 minutes, then mix gently.
4.2. HYDRAGEL ISO-LDH 15/30: Prepare the ISO-LDH substrate solution, away from light, 10 minutes before use by adding 4.5 mL of substrate solvent to the vial. Close the vial, leave at room temperature (15 to 30 °C) for 5 minutes, then mix gently.

Use

For the visualization of electrophoresed lactate dehydrogenase isoenzymes.

Storage, stability and signs of deterioration

Store ISO-LDH substrate refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or substrate vial labels.
Substrate must be free of green or violet coloration.

NOTE: During transportation, ISO-LDH substrate can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

5. ISO CK/LD BLOCKING SOLUTION

Preparation

The blocking solution is ready to use and contains: acetic acid, 5 % ; citric acid, 0.5 %.

Use

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

Storage, stability and signs of deterioration

The blocking solution can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the vial.

6. APPLICATORS

Use

Precut, single use applicators for sample application onto gel.

Storage, stability and signs of deterioration
Store the applicators in a dry place at room temperature or refrigerated.

7. THIN FILTER PAPERS
Use
Precut, 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.

8. THICK FILTER PAPERS
Use
Precut, single use, thick absorbent paper pads for blotting excessive blocking solution off the gel surface before drying.

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

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. Template Guide Bar SEBIA, supplied with HYDRASYS.
5. Accessory Kit for HYDRASYS ISO-LDH, ISO-PAL & CHOL, SEBIA, PN 1261.
6. Pipettes: 10 µL, 200 µL and 5 mL.
7. Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 mm gels at 570 nm (yellow filter) or at 530/540 nm (green filter), e.g., HYRYS SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer's instructions for operation and calibration procedures.
8. Quality control materials.

SAMPLES FOR ANALYSIS
Sample collection and storage
Sera must be collected according to conventional procedures used in clinical laboratory testing. It is recommended to carry out analyses on fresh sera, or stored for up to one week at room temperature (15 to 30 °C).

Sample preparation
Use neat serum samples. Dilute serum samples with saline to achieve a total LDH activity of about 750 IU/L when this activity is > 750 IU/L. Mix and incubate for 10 minutes at room temperature.

Sample to avoid
Do not use frozen or hemolyzed samples.

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, incubation with substrate, stopping the enzymatic reaction, blotting and final drying of the gel. The manual steps include handling samples and gels, application of reagents 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 ISO CK/LD (7 samples) and HYDRAGEL ISO CK/LD 15/30 (15 samples), or two applicators for HYDRAGEL ISO CK/LD 15/30 (30 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL of 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. For later use (up to 8 hours), keep the entire chamber under refrigeration. *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 «ISO-LDH 7» migration program for HYDRAGEL 7 ISO CK/LD, «ISO-LDH 15» migration program for HYDRAGEL ISO CK/LD 15/30 (15 samples) or «ISO-LDH 30» migration program for HYDRAGEL ISO CK/LD 15/30 (30 samples) 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 ISO CK/LD, or 200 µL for HYDRAGEL ISO CK/LD 15/30, 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 from the wet chamber. Handle it by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - For 7 and 15 samples analysis, place the aplicator into position No 6 on the carrier.
   - For 30 samples analysis, place the two applicators each into position No 4 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.

    IMPORTANT: Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

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 under 10 W constant for HYDRAGEL 7 ISO CK/LD, 20 W constant for HYDRAGEL ISO CK/LD 15/30 (15 samples), at 20 °C controlled by Peltier effect until 40 Vh accumulated (for about 8 minutes) or 20 W constant for HYDRAGEL ISO CK/LD 15/30 (30 samples), at 20 °C until 30 Vh accumulated (for about 6 minutes).
- The electrode carrier rises to disconnect the electrodes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following flashing message is displayed on the screen: «APPLY SUBSTRATE».

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

II. PREPARATION FOR INCUBATION WITH LDH SUBSTRATE

1. After the migration, open the lid.
2. Remove the sample applicator and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Clean the electrodes by wiping them carefully with a soft wet tissue.
   - Leave the gel in place in the migration module.
4. Set up the reagent application **template 4** as follows (Fig. 5):
   - Position the application template guide on the anchoring clips (the guide can be left on the HYDRASYS at all times).
   - Hold the flap on the template and put the notches in the guide marks.
   - Lower the template onto the gel.
5. Apply 2 mL of ISO-LDH substrate solution for HYDRAGEL 7 ISO CK/LD, or 4 mL of ISO-LDH substrate solution for HYDRAGEL ISO CK/LD 15/30, as follows (Fig. 6):
   - Hold the pipette vertically.
   - Lightly press the tip of the pipette into the hole of the template.
   - Carefully and progressively inject the reagent without introducing air bubbles under the template.
6. Close the HYDRASYS cover.
7. Start immediately the incubation procedure by pressing the green arrow «START» key on the left side of the keyboard. The following message is displayed on the screen: «INCUBATION».

INCUBATION - DESCRIPTION OF AUTOMATIC STEPS

- Incubation at 45 °C controlled by Peltier effect, for 20 minutes.
- Cooling of the plate to 20 °C during 5 minutes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following flashing message is displayed on the screen: «REMOVE SUBSTRATE».

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

III. SUBSTRATE ELIMINATION AND APPLICATION OF BLOCKING SOLUTION

1. Open the cover.
2. Start the run by pressing the «START» key (green arrow on the left side of the keyboard).
3. Remove the remaining substrate solution during the 15 seconds countdown:
   - Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig. 6).
   - Carefully and progressively withdraw the reagent.
4. After 15 seconds have passed, a beep sounds and the following flashing message is displayed on the screen: «APPLY BLOCK. SOL.».
5. Pipet 2 mL of blocking solution for HYDRAGEL 7 ISO CK/LD or 4 mL for HYDRAGEL ISO CK/LD 15/30 into the template (Fig. 6). Do not introduce air bubbles.
6. Close the HYDRASYS cover.
7. 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 20 °C, for 10 minutes.
- A beep sounds and the cover unlocks. The following flashing message is displayed on the screen: «REMOVE BLOCK. SOL.».

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

IV. ELIMINATION OF BLOCKING SOLUTION AND FILTER PAPER APPLICATION

1. Open the cover.
2. Start the run by pressing the «START» key (green arrow on the left side of the keyboard).
3. Eliminate the blocking solution with a pipet during the 15 seconds countdown (see SUBSTRATE ELIMINATION).
4. Remove the template:
   - Grasp the flap of the template.
   - Raise the template and remove it.

5. After 15 seconds have passed, a beep sounds and the following flashing message is displayed on the screen: «APPLY PAPER».
6. 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.
7. Close the HYDRASYS cover.
8. Start the blotting sequence by pressing the «START» key (green arrow on the left side of the keyboard).
9. 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 20 °C controlled by Peltier effect, for 3 minutes. The following message is displayed on the screen: «BLOTTING».
- Temperature of plate raises to 50 °C, for 1 minute.
- A beep sounds until the operator intervenes. The following flashing message is displayed on the screen: «REMOVE PAPER».

V. DRYING OF THE GEL

1. Open the cover.
2. Remove the filter paper and leave the gel in place.
3. Close the cover of HYDRASYS.
4. Start the drying step by pressing the «START» key (green arrow on the **left** side of the keyboard).

DRYING - DESCRIPTION OF AUTOMATIC STEPS

- Drying of the gel at 50 °C, for 4 minutes.
- A beep sounds and the cover unlocks. The plate temperature remains at 50 °C until the lid is opened.

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

VI. GEL SCANNING

1. Open the cover.
2. Remove the dried gel for densitometry.
3. Clean the back side (the plastic support side) of the dried film with a damp soft paper.
4. If needed, take off any lint from the gel side with soft paper.
5. Scan using a densitometer / scanner at 570 nm (yellow filter) or at 530/540 nm (green 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.

6. The temperature of the plate decreases to 20 °C in less than 5 minutes. When 20 °C is reached, a new migration run can be started.
7. Position the sample applicator and electrode carriers in place.
8. Wipe the temperature control plate with a soft wet tissue.

RESULTS

Quality Control

It is advised to include an assayed control serum (like Enzycontrol SEBIA, PN 4790) into each run of samples.

Values

Densitometer scanning of stained electrophoregrams yields relative concentrations (percentages) of individual zones.
After electrophoresis and visualization, the different fractions are identified according to their position: LD1, the fastest fraction, is found in the albumin zone. LD5, the most cathodic is found in the gamma globulin zone.
In normal samples, LD2 is the largest fraction, followed by LD1 and LD3 ; LD4 and LD5 are the minor components.
Normal values (mean ± 2 SD) for individual zones on HYDRAGEL 7 ISO CK/LD and HYDRAGEL ISO CK/LD 15/30 have been established from a healthy population of 200 adults (men and women):

LDH1	:	16.1	-	31.5 %
LDH2	:	29.2	-	41.6 %
LDH3	:	17.0	-	26.2 %
LDH4	:	5.9	-	12.3 %
LDH5	:	3.2	-	17.3 %

It is recommended each laboratory establishes its own normal values.

Interpretation [2-4]

LD isoenzyme patterns should not be interpreted without a knowledge of the patient's clinical history. The following are examples of abnormal LD isoenzyme patterns and their interpretation:

1. Elevated LD1 and LD2 while LD1 value becomes generally greater than LD2 value (LD1 / LD2 flip):
   - myocardial infarction and surgery,
   - pernicious, hemolytic, acute sickle cell and megaloblastic anemia,
   - hemolysis of any cause,
   - Duchenne muscular dystrophy (relative increase of LD1 and LD2).
2. Elevated mid-zone fractions: LD3 and, generally also LD2 and LD4:
   - massive platelet destruction such as in pulmonary infarction,
   - lymphatic system involvement such as in infectious mononucleosis, lymphomas and lymphocytic leukemias.
3. Elevated LD5:
   - injury, and inflammatory and degenerative diseases of the skeletal muscle,
   - many types of liver injury such as cirrhosis, hepatitis and congestion,
   - congestive heart failure.

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 measurements.

Within gel and lot-to-lot reproducibility

Three different serum samples (A, B & C) were electrophoresed on HYDRAGEL ISO CK/LD 15/30 gels from two lots. Sample A was a specimen with a normal LD isoenzyme pattern, samples B and C had elevated LD5. Each sample was applied in all 15 tracks of a single gel from each lot. The electrophoregrams were evaluated by densitometry. The following table shows (a) within gel reproducibility, i.e., the means, SD's and CV's for each LD fraction, individually for each sample and gel/lot and (b) lot-to-lot reproducibility, i.e., the means, SD's and CV's calculated from pooled data for each fraction (the 15 tracks from each gel/lot were combined for each sample).

PARAMETER	LD1	LD2	LD3	LD4	LD5
Sample A: lot no. 1 / lot no. 2 / pooled data					
MEAN	28.0 - 28.9 - 28.5	34.8 - 35.2 - 35.0	19.4 - 19.1 - 19.2	8.2 - 7.7 - 8.0	9.6 - 9.0 - 9.3
SD	0.60 - 0.96 - 0.92	0.76 - 0.67 - 0.73	0.57 - 0.54 - 0.56	0.38 - 0.54 - 0.51	0.45 - 0.51 - 0.56
CV (%)	2.1 - 3.3 - 3.2	2.2 - 1.9 - 2.1	2.9 - 2.8 - 2.9	4.7 - 6.9 - 6.4	4.6 - 5.6 - 5.0
Sample B: lot no. 1 / lot no. 2 / pooled data					
MEAN	6.8 - 7.0 - 6.9	14.7 - 14.8 - 14.8	13.3 - 13.5 - 13.4	11.5 - 11.3 - 11.4	53.6 - 53.4 - 53.6
SD	0.33 - 0.34 - 0.35	0.52 - 0.53 - 0.52	0.23 - 0.18 - 0.22	0.32 - 0.28 - 0.31	1.18 - 1.22 - 1.19
CV (%)	4.9 - 4.8 - 5.0	3.5 - 3.6 - 3.5	1.8 - 1.4 - 1.7	2.8 - 2.5 - 2.7	2.2 - 2.3 - 2.2
Sample C: lot no. 1 / lot no. 2 / pooled data					
MEAN	16.5 - 16.8 - 16.7	17.1 - 17.0 - 17.0	18.3 - 18.2 - 18.3	12.9 - 13.0 - 13.0	35.1 - 35.0 - 35.1
SD	0.45 - 0.47 - 0.47	0.30 - 0.48 - 0.40	0.24 - 0.27 - 0.25	0.26 - 0.20 - 0.23	0.57 - 0.48 - 0.52
CV (%)	2.7 - 2.8 - 2.8	1.8 - 2.9 - 2.4	1.3 - 1.5 - 1.4	2.0 - 1.5 - 1.8	1.6 - 1.4 - 1.5

Accuracy - Comparative study

One hundred and twenty (120) different serum samples (normal and pathological) were analyzed using SEBIA's HYDRAGEL 15 & 30 ISO-LDH kits and another, commercially available test for electrophoretic determination of LD isoenzymes. The results of linear regression analysis of the densitometric values by both procedures are tabulated below. The LD1 / LD2 ratio was calculated for each sample and each procedure ; there was a 97.5 % agreement between the two procedures in identifying samples with LD1 / LD2 ratio < 1.0 and with LD1 / LD2 ratio > 1.0 (the so called LD1 / LD2 flip).

PARAMETER	LD1	LD2	LD3	LD4	LD5
Correlation coefficient	0.96	0.96	0.95	0.96	0.99
y-intercept	2.7	- 0.08	0.6	1.1	-0.7
Slope	0.94	0.96	1.01	0.89	0.97
Range of % values (SEBIA's test)	6.6 - 36.5	13.3 - 52.9	7.7 - 30.4	3.1 - 16.6	5.6 - 60.1

y = SEBIA values

Linearity - Sensitivity

A sample applied on the gel, with a total activity of 50 IU/L to 750 IU/L, shows a perfect linearity for the 5 fractions.
The good sensitivity shows that a mean variation of 2 IU/L per fraction can be detected.

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SCHÉMAS / FIGURES

Figure 1

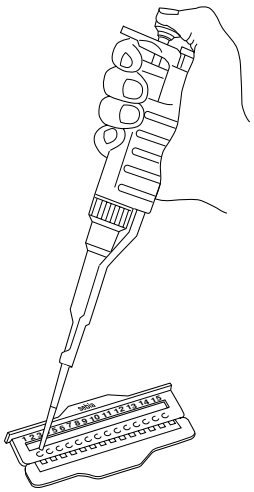


Figure 2

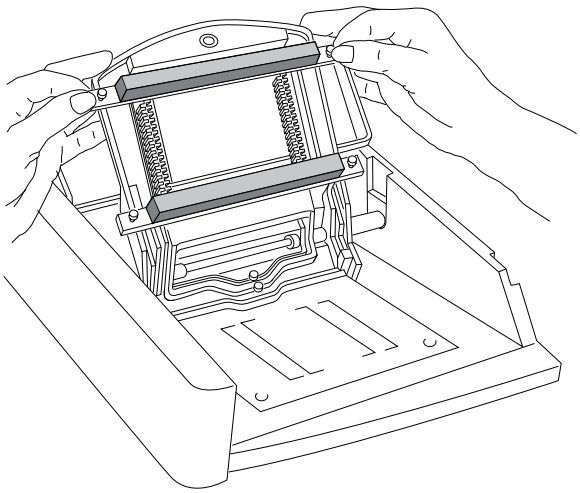


Figure 3

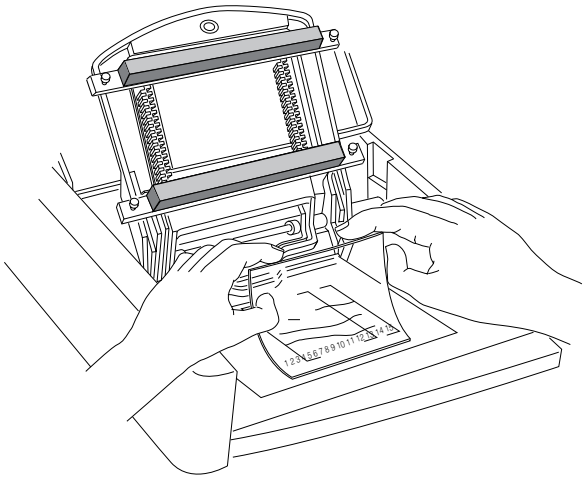


Figure 4

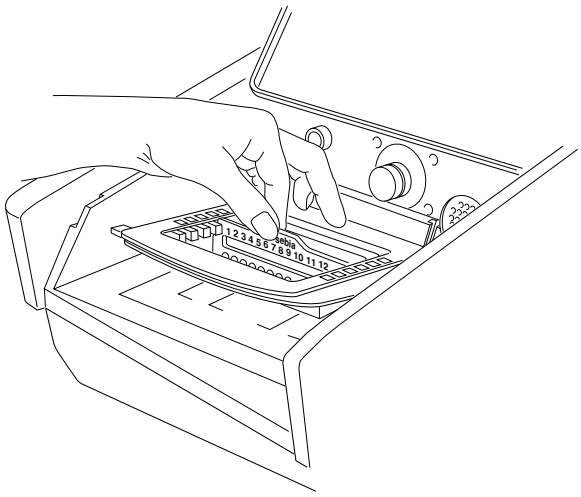


Figure 5

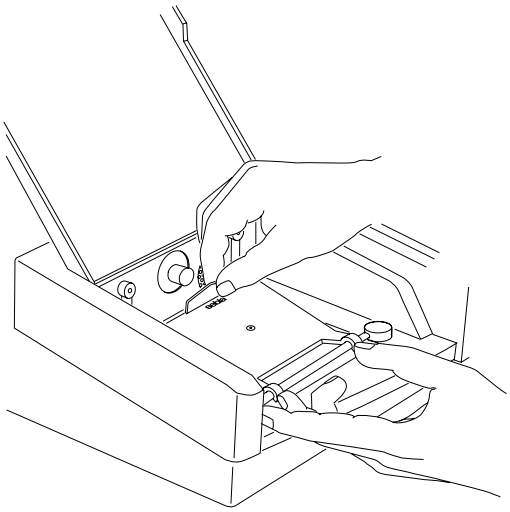
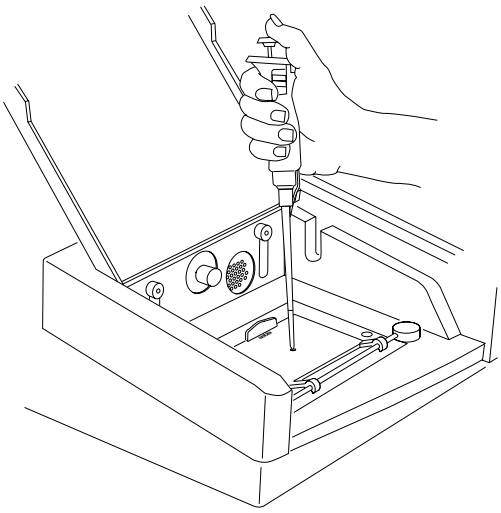


Figure 6



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