

**INTENDED USE**

The SPIFE Vis Cholesterol electrophoresis system is intended for use in the quantitative determination of cholesterol and cholesterol esters in the lipoproteins of serum using the SPIFE 2000/3000 agarose electrophoresis system. The system is intended for the assessment of the cholesterol content of the high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), and Lp(a)-C, when present in concentrations greater than 2.5 mg/dL. However, in some patients Lp(a)-C may not be present at concentrations that are detectable by electrophoresis.

**SUMMARY**

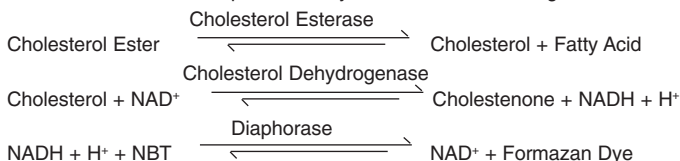
The relationship of HDL Cholesterol to coronary heart disease (CHD) was reported by Barr et al., 1951<sup>1</sup> and by Miller and Miller in 1975<sup>2</sup>. The work of Castelli et al.,<sup>3-6</sup> focused attention on HDL cholesterol assessment as the definitive laboratory test in determining the risk of coronary heart disease. The cholesterol content of the lipoprotein fractions has been determined by ultracentrifugation<sup>7</sup>, selective precipitation<sup>8</sup>, and electrophoresis on several media<sup>9</sup>. Clinical laboratory measurement of the serum lipoproteins is primarily due to their predictive association with risk of coronary heart disease (CHD). Current practice guiding laboratory measurement of total serum cholesterol, triglycerides, HDL cholesterol and LDL cholesterol is derived from recommendations of expert panels convened by the National Cholesterol Education Program (NCEP). The expert panels considered epidemiological, clinical and intervention studies in developing the recommendations for treatment decision cutpoints and recommended workup sequences for adults and children. The clinical recommendations from the NCEP panels direct clinical laboratories to perform measurements of total, HDL and LDL, cholesterol and triglycerides. The triglycerides are primarily associated with chylomicrons, very low density (VLDL) and intermediate density (IDL) lipoproteins thought to be atherogenic, but the association of triglycerides with risk of coronary heart disease in epidemiological studies is ambiguous.

LDL, as the validated atherogenic lipoprotein based on its cholesterol content, is the primary basis for treatment decisions in the NCEP clinical guidelines<sup>10</sup>. The major protein component of LDL is apolipoprotein B100 (apoB) which has been measured previously by immunoassay. The common research method for accurate LDL cholesterol quantitation and the basis for the reference method is designated beta-quantification, beta referring to the electrophoretic term for LDL. The beta-quantification technique involves a combination of ultracentrifugation and chemical precipitation<sup>11,12</sup>. The beta-quantification method gives a so-called "broad cut" LDL which includes the Lp(a)-C lipoprotein<sup>13,14</sup>, often referred to as "lipoprotein little a".

The NCEP panel concluded that alternative methods are needed for routine diagnostic use, preferably ones which directly separate LDL for cholesterol quantitation<sup>15</sup>. One such direct method involves electrophoresis. Electrophoretic methods (reviewed in Lewis and Oppit<sup>16,17</sup>) have a long history of use in qualitative and quantitative analysis of lipoproteins. Electrophoresis not only allows separation and quantitation of major lipoprotein classes, but also provides a visual display useful in detecting unusual or variant patterns. Agarose has been the preferred media for separation of whole lipoproteins, providing a clear background and convenience<sup>18-21</sup>. Early electrophoretic methods were, in general, considered useful for qualitative analysis but less than desirable for lipoprotein quantitation because of poor precision and large systematic biases compared to other methods<sup>22</sup>. Recent improvements to the Helena SPIFE automated electrophoresis system demonstrate that electrophoretic quantitation can be precise and accurate. Evaluations demonstrate good separation of the major lipoprotein classes with precise and accurate quantitation of HDL, LDL, and VLDL cholesterol and Lp(a)-C in comparisons with the reference methods<sup>23</sup>.

**PRINCIPLE**

The SPIFE system separates the major lipoprotein classes using agarose electrophoresis. The lipoprotein bands are stained with enzymic reagent and their cholesterol content quantitated by densitometric scanning.



The alpha band which migrates the farthest toward the anode corresponds to HDL. The next band, pre-beta, corresponds to VLDL, and the slowest moving

beta band corresponds approximately to LDL. If a band appears between alpha and pre-beta, it should be quantitated as the Lp(a)-C band. This band may not be observed in every specimen. Chylomicrons, if present, remain at the origin. The amount of formazan dye produced is directly proportional to the amount of cholesterol and cholesterol esters originally present in the sample. The relative percent cholesterol in each fraction is obtained by scanning in a densitometer equipped with 570 nm filter or with the Quick Scan 2000 Scanner.

**REAGENTS****1. SPIFE Vis Cholesterol Gel**

**Ingredients:** Each gel contains agarose in a sodium barbital buffer with EDTA, guanidine hydrochloride, bovine albumin and magnesium chloride. Sodium azide and other preservatives have been added.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.** The gel contains barbital which, in sufficient quantities, can be toxic. To prevent the formation of toxic vapors, this product should not be mixed with acidic solutions. When discarding this reagent always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

**Preparation for Use:** The gels are ready for use as packaged.

**Storage and Stability:** The gels should be stored horizontally at room temperature (15 to 30°C), in the protective packaging, and are stable until the expiration date indicated on the package. DO NOT REFRIGERATE OR FREEZE THE GELS.

**Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of gel blocks.

**2. SPIFE Vis Cholesterol Reagent**

**Ingredients:** When reconstituted as directed, the concentration of the reactive ingredients is as follows:

Cholesterol Esterase ( <i>Pseudomonas</i> sp.)	5.4 U/mL
Cholesterol Dehydrogenase ( <i>Nocardia</i> sp.)	1.1 U/mL
Diaphorase ( <i>Clostridium kluyveri</i> )	75.0 U/mL
NAD	35.3 mM
NBT	2.3 mM

**Preparation for Use:** Reconstitute each vial of SPIFE Vis Cholesterol Reagent with 2.5 mL SPIFE Vis Cholesterol Diluent. Swirl gently to dissolve. Do not shake. Be sure the reagent is completely dissolved before using.

**Storage and Stability:** Cholesterol Reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial. The reconstituted reagent is stable for 6 hours at 2 to 8°C.

**Signs of Deterioration:** The unreconstituted reagent should be uniformly pale or light yellow. The reconstituted reagent is a clear to light yellow solution.

**3. SPIFE Vis Cholesterol Diluent**

**Ingredients:** Cholesterol Diluent contains 100 mM Hepes Buffer

**Preparation for Use:** The diluent is ready for use as packaged.

**Storage and Stability:** The diluent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

**Signs of Deterioration:** Discard the diluent if it shows signs of bacterial growth.

**4. Citric Acid Destain**

**Ingredients:** After dissolution, the destain contains 0.3% (w/v) citric acid.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. - IRRITANT - DO NOT INGEST.**

**Preparation for Use:** Pour 11 L. of deionized water into the Destain vat. Add the entire package of Destain. Mix well until completely dissolved.

**Storage and Stability:** Store the Destain at 15 to 30°C. It is stable until the expiration date on the package.

**Signs of Deterioration:** Discard if solution becomes cloudy.

**INSTRUMENTS**

A SPIFE 2000 or 3000 must be used to electrophorese the gel. The gel can be scanned on a densitometer such as the Quick Scan 2000 (Cat. No. 1660). Refer to the appropriate Operator's Manual for detailed operating instructions.

**SPECIMEN COLLECTION AND HANDLING**

**Specimen:** Serum samples are the specimen of choice.

**Patient Preparation:** The cholesterol content of the Alpha (HDL) and Beta (LDL) and Lp(a)-C lipoproteins is not materially affected by recent meals.<sup>3</sup> Therefore, if the HDL cholesterol is the only parameter of interest, the patient need not be fasting.

### Interfering Substances:

- Heparin administered I.V. causes activation of lipoprotein lipase, which tends to increase the relative migration rate of the fractions, especially the Beta lipoprotein<sup>24</sup>.
- For effects of various drugs, refer to Young et al<sup>25</sup>.

**Specimen Storage:** For best separation of the various lipoproteins, fresh serum should be used. If testing cannot be performed immediately, the sample should be stored at 2 to 8°C no longer than 4 days. The specimen should never be stored frozen. Freezing may irreversibly alter the lipoprotein separation<sup>26</sup>. No additives or preservatives are necessary.

### PROCEDURE

**Materials Provided:** The following materials are provided in the SPIFE Vis Cholesterol Kits. Individual items are not available.

- SPIFE Vis Cholesterol Gels (10)
- SPIFE Vis Cholesterol Reagent (10 x 2.5 mL)
- SPIFE Vis Cholesterol Diluent (1 x 25 mL)
- Citric Acid Destain (1 pkg)
- REP Blotter C (10)
- Electrode Blotter (20)
- Applicator Blade Assembly - 20 Sample

### Materials provided by Helena but not contained in the kit:

	Cat. No.
SPIFE 3000	1088
SPIFE 2000	1130
SPIFE Reagent Spreader	3386
Quick Scan 2000	1660
Cholesterol Profile Control	3218
REP Prep	3100
Gel Block Remover	1115
SPIFE 3000/REP 3 Reagent Spreaders	3706
SPIFE 2000/3000 Disposable Cups (deep well)	3360
SPIFE 2000/3000 20 -100 Disposable Cup Tray	3366
SPIFE Disposable Stainless Steel Electrodes	3388
100-Sample Overlay	3417

### STEP BY STEP METHOD

**NOTE:** If a SPIFE procedure requiring a stain has been run prior to running the cholesterol gels, the stainer unit must be cleaned/washed before washing the cholesterol gel.

#### SPIFE 3000

The SPIFE has an automatic wash cycle prompted by initiation of a test which does not use the stainer unit for staining when the previous test did use the stainer for staining. To avoid delays after electrophoresis, this wash cycle should be initiated at least seven (7) minutes prior to the end of the run. To verify the status, press the **TEST SELECT/CONTINUE** button on the stainer until the appropriate test is selected. Place an empty Gel Holder in the stainer unit. If cleaning is required, the "Wash 1" prompt will appear, followed by "Plate out, Holder in" prompts. Press "Continue" to begin the stainer wash. The cleaning process will complete automatically in about 7 minutes. The unit is then ready to process the gel after electrophoresis.

#### SPIFE 2000

If utilizing the unit for both stained and non-stained gels, log usage to determine when cleaning is necessary. Create a program to clean the unit as a "User Test" according to the following:

#### User Test

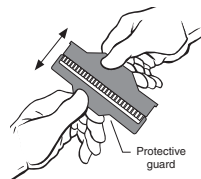
- |              |      |          |          |
|--------------|------|----------|----------|
| 1) No Prompt |      |          |          |
| Wash 1       | 1:00 | REC = ON | VALVE =7 |
| 2) No Prompt |      |          |          |
| Wash 2       | 1:00 | REC = ON | VALVE =7 |
| 3) No Prompt |      |          |          |
| Wash 3       | 1:00 | REC = ON | VALVE =7 |
| 4) No Prompt |      |          |          |
| Wash 4       | 1:00 | REC = ON | VALVE =7 |
| 5) No Prompt |      |          |          |
| END OF TEST  |      |          |          |

#### I. Preparation of Reagent

- Reconstitute the SPIFE Vis Cholesterol Reagent with 2.5 mL SPIFE Vis Cholesterol Diluent. Mix well by inversion.

#### II. Sample Preparation

- If testing 81 to 100 samples, remove five disposable Applicator Blades from the packaging. If testing fewer samples, remove the appropriate number of Applicator Blades from the packaging. Remove the protective guards from the blades by gently bending the protective piece back and forth until it breaks free.

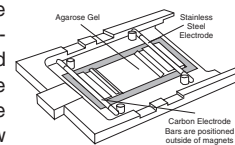


- Place the five Applicator Blades into the vertical slots in the Applicator Assembly identified as 2, A, 9, 13 and 16. Press on the end of each blade so that it slides to the back of the slot. If using fewer Applicator Blades, place them into any of the five slots noted above. **Please note that the blade assembly will only fit into the slots one way; do not try to force the blade assembly into the slots.**

- Slide cup strips into appropriate cup tray.
- Pipette 75 to 80 µL of patient serum or control into Disposable Sample Cups. If testing less than 81 samples, pipette samples into the row of cups that corresponds with applicator placement. Cover the tray until ready to use.

### III. Gel Preparation

- Remove the gel from the protective packaging and discard overlay. Using a REP Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
- Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
- Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
- Thoroughly wash the electrodes with deionized water before and after each use. Wipe the carbon electrode with a lint-free tissue. The Disposable Electrode must be patted dry because of the rough surface. Ensure that the endcaps are screwed on tightly. The Disposable Electrode must be replaced after use on 50 gels. Unscrew the endcaps from the old electrode and screw them tightly onto the new electrode.
- Place a carbon electrode on the outside ledge of the cathode gel block (left side of the gel) outside the magnetic posts.
- Place a Disposable Stainless Steel Electrode on the outside ledge of the anode gel block (right side of the gel) outside the magnetic posts.
- For SPIFE 3000, place a glass rod on each inner gel block, inside the magnetic posts.
- Place an Electrode Blotter directly above and below the cathode end of the gel. Slide the blotters under the ends of the carbon electrode so that they touch the gel block ends.
- Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the instrument until **CHOLESTEROL** option appears on the display.



### IV. Sample Application/Electrophoresis

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

#### A. SPIFE 3000

	<b>Electrophoresis Unit</b>			
1) No Prompt				
Load Sample1	00:30	20°C	SPD6	
2) No Prompt				
Apply Sample1	1:00	20°C	SPD6	LOC1
3) No Prompt				
Electrophoresis 1	20:00	16°C	400 V	150mA
4) Remove blotter, (continue)				
Apply Reagent 1		30°C	8 cycles	
5) No Prompt				
Incubate 1	15:00	30°C		
6) No prompt				
END OF TEST				

#### Stainer Unit

1) No Prompt				
Wash 1	5:00	REC = REV	VALVE = 2	
2) Prompt				
Wash 2	5:00	REC = REV	VALVE =7	
3) No Prompt				
Dry 1	20:00	70°C		
4) No Prompt				
END OF TEST				

- Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
- Place a reconstituted vial of reagent in the center hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.
- With **CHOLESTEROL** on the display, press the **START/STOP** button.

An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The SPIFE 3000 will apply the samples, electrophorese and beep.

- Open the chamber lid, remove and dispose of Electrode Blotters. Dispose of blades as biohazardous waste.
- Close the chamber lid and press the **TEST SELECT/CONTINUE** button to pour, spread reagent and start the incubation timer.
- At the end of the incubation, remove the gel from the chamber and place it on a blotter, agarose side up. Using a blade or straight edge, completely remove and discard the two gel blocks from the gel. The gel blocks interfere with washing.

#### B. SPIFE 2000

Electrophoresis Unit				
1) No Prompt				
Load Sample 1	00:30	20°C	SPD.= 6	
2) No Prompt				
Apply Sample 1	1:00	20°C	SPD.= 6	
3) No Prompt				
Electrophoresis 1	20:00	16°C	400 V	
4) Apply/Spread Reagent, (continue)				
Incubate 1	15:00	30°C		
5) No prompt				
END OF TEST				
Stainer Unit				
1) No Prompt				
Wash	5:00	CIR = ON	VALVE = 2	
2) No Prompt				
Wash 2	5:00	CIR = ON	VALVE = 7	
3) No Prompt				
Dry 1	25:00	70°C		
4) No Prompt				
END OF TEST				

- Place the Cup Tray with samples on the SPIFE 2000. Align the holes in the tray with the pins on the instrument. Close the chamber lid
- With **CHOLESTEROL** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The SPIFE 2000 will apply the samples, electrophorese and beep when completed. Dispose of blades as biohazardous waste.
- Open the chamber lid, remove and dispose of Electrode Blotters.
- Pour the contents of the SPIFE Vis Cholesterol Reagent vial across the anode end of the gel. Use the Reagent Spreader to work the reagent into the gel. Set the ends of the Reagent Spreader on the inner ledge of the electrophoresis chamber with the rod laying on the gel inside the gel blocks.
- Slowly slide the spreader across the gel. Tilt the spreader toward the gel block so the reagent relocates along the rod.
- Repeat this until the reagent has been spread eight times in each direction. On the last spread across the gel, continue to drag the rod up over the anode gel block to dispose of excess reagent. Close the chamber lid.
- Press the **TEST SELECT/CONTINUE** button to start the incubation timer.
- At the end of the incubation, remove the gel from the chamber and place it on a blotter, agarose side up. Using a blade or straight edge, completely remove and discard the two gel blocks from the gel. The gel blocks interfere with washing.

#### V. Washing

- Attach the gel to the holder by placing the round hole in the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.
- Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
- With **CHOLESTEROL** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The instrument will wash and dry the gel.
- When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer and you can scan the bands.

#### Evaluation of Fractions

For quantitation of the lipoprotein cholesterol fractions, scan the gel, agarose side up, in the Quick Scan 2000 using Slit 5.

#### Stability of End Product:

For best results, scan the SPIFE Vis Cholesterol Gel within 5 minutes.

#### Calibration:

A calibration curve is not necessary as relative density of the fractions is the only parameter determined.

**Quality Control:** Quantitation of HDL Cholesterol values should be monitored using the Cholesterol Profile Control (Cat. No. 3218). This control verifies all

phases of the procedure and should be used on each gel run. Refer to the package insert provided with the control for detailed information and assay values.

#### REFERENCE VALUES

Lipoprotein cholesterol values vary according to age and sex<sup>26</sup>, and wide variations among different geographical locations and races have been reported<sup>6</sup>. Therefore, it is essential that each laboratory establish its own expected range for its particular population. A total of 60 patients with normal total cholesterol (total cholesterol  $\leq$  200 mg/dL) were tested using the SPIFE Vis Cholesterol system. These patients have not been differentiated by age, race or sex.

	Range ( $\bar{x} \pm 2$ SD)
HDL (%)	10.7 - 37.7
Lp(a)-C%	0.0 - 10.3
VLDL (%)	0.0 - 33.6
LDL (%)	45.8 - 80.4

These values should only serve as guidelines. Each laboratory should establish its own range for age, sex and race.

#### RESULTS

The SPIFE Vis Cholesterol system separates the major lipoprotein classes. The alpha band which migrates the farthest toward the anode corresponds to HDL. The next band, pre-beta, corresponds to VLDL. If a band appears between alpha and pre-beta, it is the Lp(a)-C band and should be added to the LDL quantitation when reporting the total LDL value.<sup>27</sup> It does not appear in every sample at measurable concentrations. The slowest moving beta band corresponds approximately to LDL. Chylomicrons, if present, remain at the origin.

#### Calculations

Helena densitometers will automatically calculate and print the relative percent and the absolute values for each band when the specimen total cholesterol is entered. Refer to the Operator's Manual provided with the instrument.

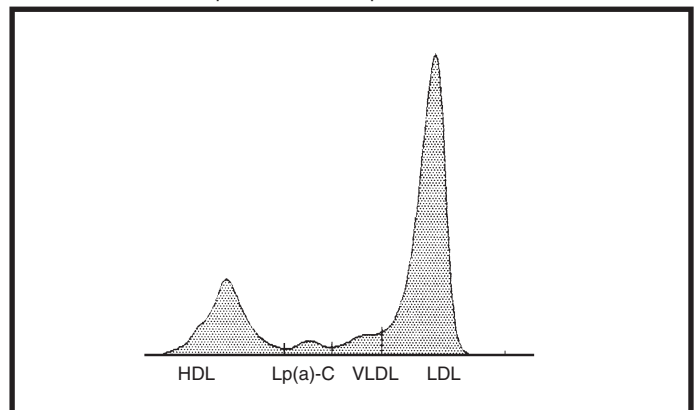


Figure 1: A scan of a SPIFE Vis Cholesterol pattern.

#### LIMITATIONS

This method is intended for the separation and quantitation of lipoprotein classes. Refer to the SPECIMEN COLLECTION AND HANDLING section of this procedure for interfering factors.

The system is linear to 400 mg/dL total cholesterol, with sensitivity to 2.5 mg/dL per band. Patient sample quantitations which exceed the linearity of the system should be diluted with deionized water and retested.

Lp(a)-C below the threshold level of 2.5 mg/dL may not be seen using this method, even if Lp(a)-C is present in the sample. To quantitate patients who have an Lp(a)-C below 2.5 mg/dL it is recommended that an alternative method be used.

#### INTERPRETATION OF RESULTS

Treatment decisions in the NCEP guidelines are based primarily on LDL cholesterol levels<sup>10</sup>. The risk factors considered in the classification scheme are age (males equal to or older than 45 years and females equal to or older than 55), family history of premature CHD, smoking, hypertension, and diabetes. Treatment is appropriate when LDL cholesterol is at or above the following cut points: all patients at or above 160 mg/dL, with two or more risk factors a value above 130 mg/dL and with symptoms of CHD a value above 100 mg/dL.

HDL cholesterol is considered high risk at or below 35 mg/dL and counted as one of the risk factors in the classification scheme. An HDL cholesterol value above 60 mg/dL is considered protective and subtracts one from the total number of risk factors.

#### Treatment Decision Cut-Points<sup>10</sup>

Total Cholesterol	
Desirable Blood Cholesterol	< 200 mg/dL
Borderline-High Blood Cholesterol	200-239 mg/dL
High Blood Cholesterol	$\geq$ 240 mg/dL
HDL Cholesterol	
Low HDL Cholesterol	< 40 mg/dL
Protective HDL Cholesterol	$\geq$ 60 mg/dL

Triglycerides	
Desirable	< 150 mg/dL
Borderline	150-199 mg/dL
Elevated	200-499 mg/dL
Very elevated	≥ 500 mg/dL

LDL Cholesterol	<u>Initiation</u>	
Dietary Therapy	<u>Level</u>	<u>LDL Goal</u>
Without CHD and fewer than 2 risk factors	≥ 160 mg/dL	< 160 mg/dL
Without CHD and with 2 or more risk factors	≥ 130 mg/dL	< 130 mg/dL
With CHD	> 100 mg/dL	≤ 100 mg/dL

LDL Cholesterol	<u>Initiation</u>	
Drug Treatment	<u>Level</u>	<u>LDL Goal</u>
Without CHD and fewer than 2 risk factors	≥ 190 mg/dL	< 160 mg/dL
Without CHD and with 2 or more risk factors	≥ 160 mg/dL	< 130 mg/dL
With CHD	≥ 130 mg/dL	< 100 mg/dL

## PERFORMANCE CHARACTERISTICS

### PRECISION

#### SPIFE 2000

**Within Run** - A single patient sample was run 100 times on one gel.

N = 100

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	18.2	5.8	15.2	60.8
SD	1.1	0.3	1.0	1.6
CV	5.9%	5.7%	6.5%	2.7%

**Run to Run** - A single patient sample was run 100 times on six gels.

N = 600

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	17.8	5.8	14.5	6.2
SD	1.2	0.4	1.1	1.8
CV	7.0%	7.1%	7.3%	2.9%

#### SPIFE 3000

### Within Run

A patient sample was run 100 times on 1 gel of SPIFE Vis Cholesterol.

N = 100

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	17.5	5.6	15.3	61.5
SD	0.9	0.2	0.5	0.9
CV	5.3%	4.3%	3.2%	1.5%

### Run to Run

A patient sample was run 100 times on 6 gels of SPIFE Vis Cholesterol.

N = 600

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	18.0	5.7	14.8	61.6
SD	1.1	0.5	1	1.4
CV	5.8%	7.8%	6.6%	2.3%

### LINEARITY AND SENSITIVITY

Serial dilutions of an elevated cholesterol sample were made and tested by this system. The linearity study showed that the system is linear to 400 mg/dL total cholesterol and that the system is sensitive to 2.5 mg/dL per band.

**CORRELATION STUDIES** A total of 131 patient samples, 72 normal (total cholesterol ≤ 200 mg/dL) and 59 abnormal (total cholesterol > 200 mg/dL), were run using SPIFE Vis Cholesterol as the reference method. The following is the correlation data produced.

N = 131

Observations = 524

#### SPIFE 2000

R = 0.997

Y = 1.018X - 0.458

X = SPIFE Vis Cholesterol, 60 sample, acetic acid wash

Y = SPIFE Vis Cholesterol, 100 sample, citric acid wash

#### SPIFE 3000

R = 0.996

Y = 1.026X - 0.623

X = SPIFE Vis Cholesterol, 60 sample, acetic acid wash

Y = SPIFE Vis Cholesterol, 100 sample, citric acid wash

### BIBLIOGRAPHY

- Barr, D.P. et al., Protein-lipid Relationships in Human Plasma, *Am J Med*, 11:480-493, 1951.
- Miller, G.J. and Miller, N.E., Plasma-High Density-Lipoprotein Concentration and Development of Ischemic Heart Disease, *Lancet*, 1:16-19, 1976.
- Kannel, W.B. et al., Serum Cholesterol, Lipoproteins, and the Risk of Coronary Heart Disease, *Ann Intern Med*, 74(1):1-12, 1971.
- Gordon, T. et al., High Density Lipoprotein As a Protective Factor Against Coronary Heart Disease. The Framingham Study. *Am J Med* 62:707-714, 1977.

- Galen, R.S., HDL Cholesterol, How Good a Risk Factor, *Diag Med*, 39-58, Nov/Dec, 1979.
- Castelli, W.P. et al., HDL Cholesterol and Other Lipids in Coronary Heart Disease - The Cooperative Lipoprotein Phenotyping Study. *Circulation*, 55(5):767-772, 1977.
- Delalla, O.F. and Gofman, J.W., Ultracentrifugal Analysis of Serum Lipoprotein, in *Methods of Biochemical Analysis*, Vol. 1, Edited by D. Glick, New York, Interscience, 459-478, 1954.
- Burstein, M. and Scholnick, H.R., Precipitation of chylomicrons and very low density lipoproteins from human serum with sodium lauryl sulfate. *Life Sci* 11:177-184, 1972.
- Cobb, S.A. and Sanders, J.L. Enzymic Determination of Cholesterol in Serum Lipoproteins Separated by Electrophoresis, *Clin Chem* 24(7):1116-1120, 1978.
- National Cholesterol Education Program, Third Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, 285(19): 2486-2497, 2001.
- U.S. Department of Health and Human Services, Lipid Research Clinics Program. In: Hainline Jr., A., Karon, J., Lippel, K., eds. *Manual of Laboratory Operations 1983*. Second Edition, NIH Publication.
- Belcher, J.D., McNamara, J.R., Grinstead, G.F., Rifai, N., Warnick, G.R., Bachorik P., Frantz Jr. I. Measurement of low density lipoprotein cholesterol concentration. In: Rifai, N., Warnick, G.R., eds. *Methods for Clinical Laboratory Measurement of Lipid and Lipoprotein Risk Factors*. Washington D.C.:AACC Press, 1991:75-86.
- Utermann, G. The mysteries of lipoprotein(a). *Science* 246:904-910, 1989.
- Loscalzo, J. Lipoprotein(a) a unique risk factor for atherothrombotic disease. *Arteriosclerosis* 10:672-679, 1990.
- National Cholesterol Education Program Lipoprotein Measurement Working Group. Recommendations for measurement of low density lipoprotein cholesterol. NIH Publication In Press.
- Lewis, L.A., Opplitt, J.J. *CRC Handbook of Electrophoresis*. Volume 1. Boca Raton: CRC Press, Inc., 1980.
- Lewis L.A., Opplitt, J.J. *CRC Handbook of Electrophoresis*. Volume 2. Boca Raton: CRC Press, Inc., 1980.
- Noble, R.P. Electrophoretic separation of plasma lipoproteins in agarose gel. *J Lipid Res* 9:693, 1968.
- Lindgren, F.T., Silvers, J., Jutagir, R., et al. A comparison of simplified methods for lipoprotein quantitation using the analytic ultracentrifuge as a standard. *Lipids* 12:278, 1977.
- Conlon, D., Blankstein, L.A., Pasakarnis, P.A. Quantitative determination of high-density lipoprotein cholesterol by agarose gel electrophoresis updated. *Clin Chem*. 24:227, 1979.
- Papadopoulos, N.M. Hyperlipoproteinemia phenotype determination by agarose gel electrophoresis updated. *Clin. Chem.* 24:227-229, 1978.
- Warnick, G.R., Nguyen, T., Bergelin, R.O., Wahl, P.W., Albers, J.J. quantification: An electrophoretic method compared with the lipid research clinics method. *Clin Chem* 28:2116-20, 1982.
- Warnick, G.R., Leary E.T., Goetsch, J. Electrophoretic quantification of LDL-cholesterol using the Helena REP. Abstract 0011, *Clin. Chem.* 39:1122, 1993.
- Houtsmuller, A.J. Heparin-induced Post Beta Lipoprotein, *Lancet* 7470, II, 976, 1966.
- Young, D.S. et al., *Effects of Drugs on Clinical Laboratory Tests*. 3rd ed., AACC Press, Washington, D.C., 1990.
- Fredrickson, S.D. et al., Fat Transport in Lipoproteins-An Integrated Approach to Mechanisms and Disorders, *New Eng J Med*, 276(1);34-43, 276(2);94-103, 276(3):148-156, 276(4): 215-225, 276(5):273-281, 1967.
- Warnick, Russell G., Lipoprotein (a) Is Included in Low-Density Lipoprotein by NCEP Definition, *Clin Chem* 40(11):2115-2116, 1994.

### SPIFE Vis Cholesterol System

**Cat. No. 3440, 3441, 3442, 3439, 3438**

SPIFE Vis Cholesterol Gels (10)

SPIFE Vis Cholesterol Reagent (10 x 2.5 mL)

SPIFE Cholesterol Diluent (1 x 25 mL)

Citric Acid Destain (1 pkg)

REP Blotter C (10)

Electrode Blotter (20)

Applicator Blade Assembly - 20 Sample

### Other Supplies and Equipment

The following items, needed for the performance of the SPIFE Vis Cholesterol Kit, must be ordered individually.

	Cat. No.
SPIFE 3000	1088
SPIFE 2000	1130
SPIFE Reagent Spreader	3386
Quick Scan 2000	1660
Cholesterol Profile Control	3218
REP Prep	3100
Gel Block Remover	1115
SPIFE 3000/REP 3 Reagent Spreaders	3706
SPIFE Disposable Cups (deep well)	3360
SPIFE 3000 20-100 Disposable Cup Tray	3366
SPIFE Disposable Stainless Steel Electrodes	3388
100-Sample Overlay	3417

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

Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena's liability under this contract or otherwise shall be limited to replacement or refund of any amount not to exceed the purchase price attributable to the goods as to which such claim is made. These alternatives shall be buyer's exclusive remedies. In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damages.

The foregoing warranties are in lieu of all warranties expressed or implied including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

Shading indicated that text has been modified, added or deleted.