SPIFE® Vis Cholesterol

**INTENDED USE**

The SPIFE® Vis Cholesterol electrophoresis system is intended for use in the quantitation of lipoprotein subclasses. The beta band which migrates the farthest toward the anode corresponds to LDL cholesterol. If a band appears between alpha and beta bands, the band may not be resolved in every specimen. Cholesterol, if present in esters, is released in the form of free cholesterol. The amount of formic acid produced is directly proportional to the amount of cholesterol and cholesteryl ester hydrolysis. The percent cholesterol in each fraction is obtained by scanning in a densitometer equipped with 570 nm filter or with the Quick Scan 2000 Scanner.

**REFERENCES**

1. **SPIFE® Vis Cholesterol Gel**
   - Ingredients: Each gel contains agarose in a barbital barbital buffer with EDTA, guanidine hydrogen sulfate, acetic acid, and magnesium chloride. Sodium acid and other preservatives have been added. When gel is activated, it will turn yellow. CHD (coronary heart disease) is indicated by a single patient sample was run 100 times on one gel. When gel is activated, it will turn yellow. CHD (coronary heart disease) is indicated by a single patient sample was run 100 times on one gel. When gel is activated, it will turn yellow.

2. **SPIFE® Vis Cholesterol Reagent**
   - Preparation for Use: Reagent should be stored at 2°C to 8°C and is stable until the expiration date indicated on the vial. The reconstituted reagent is stable for 6 hours at 30°C.

3. **SPIFE® Vis Cholesterol Diluent**
   - Ingredients: Cholesterol Ester (Pseudomonas sp.) 4.5 U/mL
   - Storage and Stability: The diluent should be stored at 2°C to 8°C until the expiration date indicated on the vial. Sodium azide may cause discoloration. Do not use.

4. **CHD (coronary heart disease) is indicated by a single patient sample was run 100 times on one gel. When gel is activated, it will turn yellow. CHD (coronary heart disease) is indicated by a single patient sample was run 100 times on one gel. When gel is activated, it will turn yellow.

5. **SPIFE® Vis Cholesterol Linear Density (LDL) cholesterol” is the primary basis for total cholesterol measurement in CHD (coronary heart disease) is indicated by a single patient sample was run 100 times on one gel. When gel is activated, it will turn yellow.
2) No Prompt
mine when cleaning is necessary. Create a program to clean the unit as a
If utilizing the unit for both stained and non-stained gels, log usage to deter
The cleaning process will complete automatically in about 7 minutes. The unit
until the appropriate test is selected. Place an empty Gel Holder in the stainer
the cholesterol gel.
NOTE:
STEP BY STEP METHOD
Electrode Blotter (20)
REP Blotter C (10)
SPIFE Vis Cholesterol Diluent (1 x 25 mL)
PROCEDURE
should be stored at 2 to 8°C no longer than 4 days. The specimen should
serum should be used. If testing cannot be performed immediately, the sample
Interfering Substances:
the following materials are provided in the SPIFE Vis
Wash 1 5:00 REC = REV VALVE = 2
Wash 2 5:00 REC = REV VALVE = 2
Wash 3 5:00 REC = REV VALVE = 2
Wash 4 5:00 REC = REV VALVE = 2
Wash 5 5:00 REC = REV VALVE = 2
END OF TEST
Stainer Unit
1. Place the Cup Tray with samples on the SPIFE 2000. Align the holes in
2. Place the Electrode Blotters into the Ethanol chamber of the instrument.
3. Open the chamber lid, remove and dispose of Electrode Blotters.
4. Using a REP Blotter C, gently blot the entire gel using slight fingertip
5. Using the Cholesterol Profile Control (Cat. No. 3218). This control verifies all
6. Place a Disposable Stainless Steel Electrode on the outside ledge of the
7. Dry 1 25:00 70°C
8) No Prompt
5) No Prompt
Press
1) No Prompt
2) No Prompt on the display, press the
3) No Prompt
4) No Prompt
9. Preset the TEST SELECT/CONTINUE button located on the Electrophoresis and Stainer sides of the instrument unit
CHOLESTEROL:
Figure 1: A scan of a SPIFE Vis Cholesterol pattern.
LIMITATIONS
The system is linear to 400 mg/dL total cholesterol, with sensitivity to 2.5
This method is intended for the separation and quantitation of lipoprotein
This method is not intended for the detection of Lp(a)-C in the sample. To quantify patients who
INTERPRETATION OF RESULTS
Treatment decisions in the NCEP guidelines are based primarily on LDL cho
have an Lp(a)-C below 2.5 mg/dL it is recommended that an alternative
It does not appear in every sample
Figure 1: A scan of a SPIFE Vis Cholesterol pattern.
HDL (%)
32 - 50
−6 - 8.6
VLDL (%)
0 - 10.3
−6 - 8.6
LDL
HDL
Lp(a)-C
200 mg/dL
 Borderline-High Cholesterol
> 200 mg/dL
High Blood Cholesterol
40 mg/dL
Protective HDL Cholesterol
60 mg/dL
Desirable Blood Cholesterol
< 200 mg/dL.
This page contains instructions and information related to a laboratory procedure, specifically the SPIFE Vis Cholesterol testing method. The page includes steps for sample preparation, electrophoresis, staining, and analysis of results. Key sections include:

- **Preparation of Reagents**
- **Sample Preparation**
- **Electrophoresis**
- **Staining**
- **Calculations**

The instructions are detailed and include specific steps for handling samples, operating the SPIFE unit, and interpreting results. The page also references the SPIFE Operating Manual and SPIFE Vis Cholesterol Control 3218 for additional guidance.

**Calculations**

Helenas dentifermia 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.

**RESULTS**

The SPIFE Vis Cholesterol system separates the major lipoprotein classes. The alpha band which migrates the fastest toward the anode constitutes to HDL. The next band, pre-beta, corresponds to LDL. If this band appears between alpha and pre-beta, the sample must be discarded. The LDL band should be added to the calculation when reporting the total LDL value. It does not appear in every sample at measurable concentrations. The oldest moving beta band corresponds approximately to LDL. Chylomicrons, if present, remain at the origin.

**Limitations**

The LDL value is intended for the separation and quantification of lipoprotein classes. Refer to the SPECIMEN COLLECTION AND HANDLING section of the procedure for interfering factors. The system is linear for 200 mg/dL total cholesterol, with sensitivity to 2.5 mg/dL per band. Patient sample quantities which exceed the linearity of the system should be diluted with deionized water and retested.

**Interfering Substances**

- Impurities in the patient's serum may interfere with the assay results, especially the Beta lipoprotein.
- For effects of various drugs, refer to Young et al.**3**

**Sample Storage**

Best results are obtained for the following lipoproteins, fresh serum should be used. If testing cannot be performed immediately, the sample should be stored at or below 4°C no longer than 4 days. The specimen may need to be frozen if storing may reasonably alter the lipoprotein separa-

**Procedure**

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

- SPIFE Vis Cholesterol Control 3218
- SPIFE Disposable Stainless Steel Electrodes 3388
- SPIFE Disposable Stainless Steel Electrodes 3388
- SPIFE Disposable Stainless Steel Electrodes 3388
- SPIFE Disposable Stainless Steel Electrodes 3388

**Materials Provided**

- Stainless Steel Electrodes
- SPIFE Disposable Stainless Steel Electrodes
- SPIFE Disposable Stainless Steel Electrodes

A. SPIFE 3000

1. 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 bending the protective piece back and forth until it breaks free.

2. Place five Disposable Applicator Blades into the vertical slots in the Applicator Unit. 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 five Applicator Blades into the vertical slots in the Applicator Unit. The cleaning process will complete automatically in about 7 minutes. The unit will automatically cycle through the wash cycles and electrophoresis. If cleaning is required, the "Wash 1" prompt will appear, followed by an option to either begin the test or skip the operation. The SPIFE Vis 3000 will apply the samples, electrophoreses and beep. Open the chamber lid, remove and dispose of Electrode Blotters. Close the chamber lid and press the TEST SELECT/CONTINUE button.

3. If the anode gel band is not seen on tightly. The Disposable Electrode must be replaced after use on 50 gels. Unscrew the blador from the electrode and screw them tightly onto the new one.

4. Place a SPIFE Disposable Stainless Steel Electrode on the outside of the cathode gel block (right side of the gel) outside the magnetic posts. The gel holder from the stainer chamber.

5. Place the five Applicator Blades into the vertical slots in the Applicator Unit. For 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 bending the protective piece back and forth until it breaks free.

6. Apply Sample 1 1:00 20°C SPD=6 LOC1

7. For SPIFE 3000, place a glass rod on each inner gel block, inside the electrophoresis chamber. If a SPIFE procedure requiring a stain has been run prior to running an electrophoresis chamber.

8. Place the five Applicator Blades into the vertical slots in the Applicator Unit. Allow the gel to dry. Press on the display, press the START/STOP button. An option to either begin the test or skip the operation will be presented. Press the START/STOP button. The SPIFE Vis 3000 will apply the samples, electrophoreses and beep. Open the chamber lid, remove and dispose of Electrode Blotters. Close the chamber lid and press the TEST SELECT/CONTINUE button.

9. Place the Gel Holder with the attached gel facing backwards into the gel holder. Place the Gel Holder with the attached gel facing backwards into the gel holder. Place the Gel Holder with the attached gel facing backwards into the gel holder.

10. With CHOLESTEROL on the display, press the START/STOP button.
The gels should be stored horizontally at room temperature. Laboratories - Pro 185 The diluent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

PERFORMANCE CHARACTERISTICS

Signs of Deterioration: The diluted reagent should be uniformly pale or light yellow. The reconstituted reagent is considered useful for qualitative analysis but less than desirable for lipoprotein quantitation using the SPIFE 2000/3000 agarose electrophoresis system. quantitation using the analytic ultracentrifuge as a standard. Lipids 12:278, 1977. Lipid Research Clinics Program: III. Samples. Lipids 12:278, 1977. The preferred media for separation of whole lipoproteins, providing a clear background and convenience as well as precise, reproducible results. Use of agarose gel electrophoresis for accurate LDL cholesterol quantitation and the basis for the reference method for measurement of low density lipoprotein cholesterol. NIH Publication In Press.

The following items, needed for the performance of the SPIFE Vis Cholesterol System, are provided:


Cholesterol Diluent contains 100 mM Hepes Buffer (pH 7.4), 0.9% NaCl, sucrose 0.5%, and 0.05% sodium azide. Serum samples are the specimen of choice.

Inhibitors:


The main items for the performance of the SPIFE Vis Cholesterol Kit should be individually marked. See Table 1. for details. the primary basis for treatment decisions in the NCEP clinical guidelines. The major protein component of LDL is apolipoprotein B100 (apoB) which has been measured previously by immunoassay. The common research method for determination of LDL cholesterol is quantitation using the analytic ultracentrifuge as a standard. Lipids 12:278, 1977. For accurate LDL cholesterol quantitation and the basis for the reference method for measurement of low density lipoprotein cholesterol. NIH Publication In Press.

The beta-lipidation method gives a so-called "cutoff" LDL which includes the Lp(a)-C lipoprotein. The NCEP panel concluded that alternative methods are needed for routine diagnostic use. Other Suppliers

INSTRUMENTS

Other Supplies and Equipment

The major risk factor for coronary heart disease (CHD) is hypercholesterolemia. Increased serum cholesterol levels are the result of enhanced cholesterol absorption from the diet and impaired bile acid recycling, leading to hypercholesterolemia


...Unusual forms of high density lipoprotein (HDL) and low density lipoprotein (LDL) have been described. However, the HDL particles found in the serum represent a mixture of small HDL and large HDL subspecies, while LDL is a single discrete class. There are...