INTENDED USE

The SPIFE Touch Vis Cholesterol electrophoresis procedure is intended for use in the quantitative determination of cholesterol esters in lipoprotein classes of the lipoprotein profile using the SPIFE Touch System. This testing system is intended for use in laboratories utilizing lipid quantitation procedures. 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 mmol/L. 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. in 1951, and by Miller and Miller in 1970. The work of Castelli et al. in 1972 and related studies confirmed the association between low HDL cholesterol and increased risk for CHD.

The relationship between HDL cholesterol and CHD is thought to be due to its protective role in the atherosclerotic process. HDL cholesterol is known to reverse atherosclerotic lesions. It is thought that HDL cholesterol may prevent atherosclerotic lesions by preventing the formation of LDL from HDL, thereby preventing the formation of atherosclerotic plaque.

The major protein component of HDL is apo A-I, which is a 30-kDa protein that is present in all lipoproteins. The major protein component of LDL is apo B-100, which is a 52-kDa protein that is present in all lipoproteins.

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2. Place a REP Blotter C on the gel with the longer end parallel with the gel blocks.

4. Slide cup strips into appropriate cup tray.

NOTE:

1. Reconstitute the SPIFE Vis Cholesterol Reagent with 2.5 mL SPIFE Vis

I. Preparation of Reagent

II. Sterile Method

NOTE: If the staining chamber was last used to stain a gel, the SPIFE Touch software has an automatic wash cycle prompted by the initiation of the SPIFE Vis Cholesterol test. To verify the status of the stainer chamber, use the arrows under the STAINER UNIT to select the appropriate test. Press START and choose an operation to proceed. The instrument will wash and dry the gel.

V. Washing

1. Attach the gel holder by placing the round hole on the gel over the pins on the instrument. Turn the gel holder clockwise until it clicks. Press RETRY button to pour, spread reagent and wash the gel.

2. Place an Applicator Blade on the SPERE over the blades as biohazardous waste.

4. The system is linear to 400 mg/dL total cholesterol, with sensitivity to 2.5 mg/dL per band. Palpate sample punctuations which exceed the system’s ability to be diluted with deionized water and rejected.

5. Polyethylene glycol (PEG) ≤ 2.5 mg/dL is recommended that an alternative method be used.

6. 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. Palpate sample punctuations which exceed the system’s ability to be diluted with deionized water and rejected.

Total HDL Cholesterol

Without HDL and fewer than 2 risk factors > 160 mg/dL ≥ 160 mg/dL

Without HDL and with 2 or more risk factors ≥ 130 mg/dL ≥ 130 mg/dL

With HDL ≥ 100 mg/dL

LDL Cholesterol

Dietary Therapy

Without HDL and fewer than 2 risk factors > 160 mg/dL ≥ 160 mg/dL

Without HDL and with 2 or more risk factors ≥ 130 mg/dL ≥ 130 mg/dL

With HDL ≥ 100 mg/dL

PERFORMANCE CHARACTERISTICS

Precision

Within Run

A control sample was run 100 times on 1 gel of SPIFE Vis Cholesterol Touch. N = 100

HDL (%)

LDL (%)

CV

Mean

± 2 SD

CV

± 2 SD

0.6 ± 0.8

5.0 ± 4.0

2.9

2.5

3.3

5.4

7.7

2.9

0.9

3.4

Dietary Therapy

LDL Cholesterol

Without HDL and fewer than 2 risk factors > 160 mg/dL ≥ 160 mg/dL

Without HDL and with 2 or more risk factors ≥ 130 mg/dL ≥ 130 mg/dL

With HDL ≥ 100 mg/dL

Comparative Results

HDL Cholesterol

Interpretation

Low

LDL Goal

Without HDL and fewer than 2 risk factors > 160 mg/dL ≥ 160 mg/dL

Without HDL and with 2 or more risk factors ≥ 130 mg/dL ≥ 130 mg/dL

With HDL ≥ 100 mg/dL

Treatment Decision Cut-Points

Total Cholesterol

Desirable Blood Cholesterol Borderline-High Blood Cholesterol

200-239 mg/dL

240 mg/dL or more

High Blood Cholesterol

HDL Cholesterol

Low HDL Cholesterol

Protective HDL Cholesterol

60 mg/dL or less

60-80 mg/dL

80-100 mg/dL

100 mg/dL or more

Very elevated

Individuals with triglyceride levels greater than 300 mg/dL or individuals with a history of triglycerides 150-199 mg/dL are considered to be at increased risk for developing CHD. Individuals with triglyceride levels 200-399 mg/dL are considered to be at greater risk for developing CHD. Individuals with triglyceride levels ≥ 400 mg/dL are considered to be at very high risk for developing CHD.
5. Thoroughly wash the electrodes with deionized water before and after each use. Wire the carbon electrode with a lint-free tissue. The Disposable Stainless Steel electrode must be replaced after use on 50 gels. Unscrew the end-caps from the gel electrode and screw them tightly onto the new electrode.

6. Place a carbon electrode on the outside ledge of the cathode block (left side of the gel) outside the magnetic posts. Improper contact between the electrode and cathode may cause arcing.

7. Place a Disposable Stainless Steel Electrode on the outside ledge of the anode gel block (right side of the gel) outside the magnetic posts.

8. Place a glass rod on each inner gel block, inside the magnetic posts.

9. Place an Electrode Blotter directly above and below the cathode end of the gel. Slice blotters under the gel so that the carbon electrode so that they touch the gel-block ends. Close the chamber lid.

10. Use the arrows under SEPARATOR UNIT to select the appropriate test. To check if the electrophoresis is complete, wash the gel and choose an operation to proceed. The instrument will wash and dry the gel. If 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 Results

For quantification of the lipoprotein cholesterol fractions, scan the gel, gel image side up, in the Quick Scan Touch2000 on the acid violet setting. A slit size of f/14 is recommended. Autocut is used with the test.

Linearity and Sensitivity

The sensitivity of this method is intended for the separation and quantitation of lipoprotein classes. Refer to 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. Patients on medications which exceed the system’s linearity should be diluted with distilled water and retested.

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

INTRODUCTION OF RESULTS

Treatment decisions in the NCEP guidelines are based primarily on LDL cholesterol levels. 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 150 mg/dL, and with symptoms of CHD 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.

Drug Treatment

Level Drug Treatment

Without CHD and fewer than 2 risk factors

Without CHD and with 2 or more risk factors

With CHD

LDL Cholesterol

Lp(a)-Cholesterol

Drug Treatment

Without CHD and fewer than 2 risk factors

Without CHD and with 2 or more risk factors

With CHD

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run

A control sample was run 100 times on 1 gel of SPIFE Vis Cholesterol Touch. N = 100

<table>
<thead>
<tr>
<th>HDL %</th>
<th>Lp(a)-C %</th>
<th>VLDL %</th>
<th>LDL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>27.2</td>
<td>5.6</td>
<td>18.2</td>
</tr>
<tr>
<td>CV</td>
<td>3.3%</td>
<td>5.4%</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

Run to Run

A control sample was run 100 times on 9 gels of SPIFE Vis Cholesterol Touch. N = 300

<table>
<thead>
<tr>
<th>HDL %</th>
<th>Lp(a)-C %</th>
<th>VLDL %</th>
<th>LDL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>20.5</td>
<td>5.5</td>
<td>17.3</td>
</tr>
<tr>
<td>CV</td>
<td>3.8%</td>
<td>9.9%</td>
<td>6.4%</td>
</tr>
</tbody>
</table>

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.
INTENDED USE

The SPIFE Touch VCs Cholesterol electrophoresis procedure is intended for use in the quantitative determination of high density, low density, and chylomicron cholesterol in the lipoproteins in the serum using the SPIFE Touch 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 chylomicrons (CM), when present in concentrations greater than 2.5 mg/dL. However, in some patients Lip(a)-CM 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 Bayliss in 1919 and by Miller and Miller in 1979. The work of Castelli et al.1,11,13 has focused on LDL cholesterol as the major target for therapy and the major contributor to the risk for developing CHD.

The NCEP panel concluded that alternative methods are needed for routine diagnostic purposes. The NCEP panel recommended the following: the beta-quantification technique involves a combination of ultracentrifugation and chemical precipitation16. The beta-quantification method gives a so-called "bzcut" of LDL which includes the Lp(a)-C lipoprotein16,17, often referred to as "lipoprotein little a".

The NCEP panel concluded that alternative methods are needed for routine diagnostic use, preferably with separate LDL, for chylomicrons and for HDL. The major protein component of LDL is apolipoprotein B-100 (apoB), which has been measured previously by immunocytochemistry. The common research method for accurate LDL cholesterol quantitation in the SPIFE Touch agarose electrophoresis system is alpha-quantitation, in which the reference method is designated beta-quantitation, beta referring to the electrophoretic term for LDL. This beta-quantitation technique involves a combination of ultracentrifugation and chemical precipitation16. The beta-quantification method gives a so-called "bzcut" of LDL which includes the Lp(a)-C lipoprotein16,17, often referred to as "lipoprotein little a".

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The SPIFE Touch agarose electrophoresis system demonstrates that electrophoretic quantitation can be precise and accurate. Evaluations demonstrate good separation of the major lipoprotein classes with precise and accurate quantification of HDL, LDL and VLDL cholesterol, chylomicrons and Lp(a)-C in comparisons with the reference methods14.

PRINCIPLE

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

Cholesterol Esterase

Cholesterol + NADH + H+ Formazan Dye

Cholesterol Dehydrogenase

Cholesterol + CoQ

Diaphorase

NAD+ + H2O \rightarrow NADH + Proton

The formazan dye, which migrates the farthest toward the anode, corresponds to LDL. 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 by the Lp(a)-C band. This band may also be observed in elderly patients. Chylomicrons, if present, remain at the origin. The amount of formazan dye produced is directly proportional to the amount of cholesterol and chylomicron cholesterol in the serum using the SPIFE Touch 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 chylomicrons (CM) when present in concentrations greater than 2.5 mg/dL. However, in some patients Lip(a)-CM may not be present at concentrations that are detectable by electrophoresis.

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