QuickGel® Cholesterol Procedure

Cat. No. 3443, 3543T

INTENDED USE

The QuickGel Cholesterol electrophoresis system is for use in the quantitative determination of cholesterol and cholesterol esters in serum lipoproteins using the SPIFE 3000 or the QuickGel chamber. 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.

Cat. No. 3443 - for use with SPIFE 2000/3000.

Cat. No. 3543T - for use with QuickGel Chamber.

For In Vitro Diagnostic use.

Rx Only

SUMMARY

The relationship of HDL Cholesterol to coronary heart disease (CHD) was reported by Barr et al., 1951[1] and by Miller and Miller in 1975 [2]. The work of Castelli et al.,[3-6] 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[7], selective precipitation[8], and electrophoresis on several media[9].

Clinical laboratory measurement of the serum lipoproteins is primarily due to their predictive association with risk of 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[10]. 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[11,12]. The beta-quantification method gives a so-called “broad cut” LDL which includes the Lp(a)-C lipoprotein[13,14], 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.[15] One such direct method involves electrophoresis. Electrophoretic methods (reviewed in Lewis and Opplt[16,17]) 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[18-21]. 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[22]. The Helena QuickGel electrophoresis system demonstrates 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[23].

PRINCIPLE

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

$$\text{Cholesterol Ester} \xrightleftharpoons{\text{Cholesterol Esterase}} \text{Cholesterol + Fatty Acid}$$

$$\text{Cholesterol + NAD}^+ \xrightleftharpoons{\text{Cholesterol Dehydrogenase}} \text{Cholestenone + NADH + H}^+$$

$$\text{NADH + H}^+ \text{ + NBT} \xrightleftharpoons{\text{Diaphorase}} \text{NAD}^+ \text{ + Formazan Dye}$$

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 QuickScan Touch Plus Scanner.

REAGENTS

1. QuickGel Cholesterol Gel

Ingredients: Each gel contains agarose in a sodium barbital buffer with EDTA, guanidine hydrochloride, bovine albumin and magnesium chloride. Sodium azide has been added as a preservative.

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 Cholesterol Reagent

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

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

Preparation for Use: Reconstitute each vial of SPIFE Cholesterol Reagent with 2.5 mL SPIFE 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 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 3000 or the QuickGel Chamber must be used to electrophorese the gel. The gel can be scanned on a densitometer such as the QuickScan Touch Plus (Cat. No. 1640). 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.[3] Therefore, if the HDL cholesterol is the only parameter of interest, the patient need not be fasting.
Interfering Substances:

1. Heparin administered I.V. causes activation of lipoprotein lipase, which tends to increase the relative migration rate of the fractions, especially the Beta lipoprotein[24].
2. For effects of various drugs, refer to Young et al[25].

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[26]. No additives or preservatives are necessary.

PROCEDURE FOR SPIFE 2000/3000

Materials Provided: The following materials are provided in the QuickGel Cholesterol Kit for SPIFE (Cat. No. 3443). Individual items are not available.

QuickGel Cholesterol Gels (10)
SPIFE Cholesterol Reagent (10 x 2.5 mL)
SPIFE Cholesterol Diluent (1 x 25 mL)
Citric Acid Destain (1 pkg)
QuickGel Blotter C (10)
QuickGel Blotter X (20)
Applicator Blade Assembly - 20 Samples (10)

Materials provided by Helena but not contained in the kit:

		Cat. No.
SPIFE 3000		1088
QuickScan Touch Plus		1640
Cholesterol Profile Control		3218
REP Prep		3100
SPIFE Gel Block Remover		1115
SPIFE Disposable Cups (Deep Well)		3360
QuickGel Dispo Cup Tray		3353
SPIFE QuickGel Electrodes		1111
SPIFE QuickGel Gel Holder		3358
QuickGel Chamber Alignment Guide	86541003	
SPIFE 3000/Rep 3 Reagent Spreaders		3706
SPIFE QuickGel Disposable Stainless Steel Electrodes		3357

STEP BY STEP METHOD

I. Chamber Preparation

1. The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement if the chamber floor has not been marked previously. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
2. Remove the contact sheet & clean the chamber floor according to instructions in the Operator's Manual.
3. Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
4. Using an indelible marker, outline the rectangular open area onto the copper floor. Allow marking to dry, and apply another contact sheet.

II. Stainer Preparation

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.

The SPIFE 3000 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.

III. Preparation of Reagent

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

IV. Sample Preparation

1. Remove one disposable Applicator Blade Assembly from the packaging. Remove the protective guards from the blade by gently bending the protective piece back and forth until it breaks free.
2. Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly.

NOTE: The blade assembly will only fit into the slots one way; do not try to force the blade assembly into the slots.

3. Slide a strip of Disposable Cups into top row numbered 1 to 10 of the appropriate Cup Tray.
4. Pipette 75 to 80 µL of patient serum or control into cups numbered 1 to 5 and 6 to 10. Cover the samples until ready to use.

V. Gel Preparation

1. Carefully cut open the end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
2. Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
3. Dispense approximately 1 mL of REP Prep onto the left side of the rectangle on the electrophoresis chamber floor.
4. Place the edge of the gel next to the left edge of the marked rectangle. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Make sure no bubbles remain under the gel.
5. Use lint-free tissue to wipe around the edges of the plastic gel backing to remove excess REP Prep.
6. Thoroughly wash electrodes with deionized water before and after each use. Wipe the QuickGel Electrode with a lint-free tissue. The Stainless Steel Electrode must be patted dry because of the rough surface. Ensure that the endcaps are screwed on tightly. The Stainless Steel Electrode must be replaced after use on 50 gels. Unscrew the endcaps from the old electrode and screw them tightly onto the new electrode.
7. Place a QuickGel Electrode on the outside ledge of the left gel block (cathode end) inside the magnetic posts.
8. Place a QuickGel Stainless Steel Electrode on the outside ledge of the right gel block (anode end) of the gel inside the magnetic posts. Close the chamber lid.
9. Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the instrument until the appropriate test name appears on the display.

VI. Sample Application/Electrophoresis

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

Electrophoresis Unit

1)	No Prompt				
	Load Sample 1	00:30	20°C	SPD6	
2)	No Prompt				
	Apply Sample 1	1:00	20°C	SPD6	LOC1
3)	To Continue, (continue)				
	Electrophoresis 1	25:00	16°C	220 V	60 mA
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)	No Prompt			
	Wash 2	5:00	REC = REV	VALVE = 7
3)	No Prompt			
	Dry 1	15:00	70°C	
4)	No Prompt			
	END OF TEST			

1. Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
2. 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.
3. With **QG-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 and beep.
4. Remove the two QuickGel Blotter Xs from the package. Slide the blotters under the ends of the carbon electrodes, directly above and below the cathode end of the gel, so that they touch the gel block ends.
5. Close the chamber lid and press **CONTINUE**. The SPIFE will electrophorese and beep when completed.
6. Open the chamber lid, remove electrodes and dispose of Blotter Xs. Dispose of blades as biohazardous waste. Using a SPIFE Gel Block Remover, completely remove and discard the gel blocks on each end of the gel.
7. Wipe the excess buffer and moisture from around the gel and chamber floor using a lint-free tissue.
8. Place a SPIFE 3000 Reagent Spreader (glass rod) inside the magnetic posts at each end of the chamber.
9. Close the chamber lid and press the **TEST SELECT/CONTINUE** button to pour, spread reagent and start the incubation timer.
10. At the end of the incubation, remove the glass rods and the gel from the chamber.

V. Washing

1. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
2. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
3. With the appropriate test name 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.
4. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer and you can scan the bands.

PROCEDURE FOR QuickGel® CHAMBER

The following instructions are for using the QuickGel Chamber (Cat. No. 1284) for electrophoresis.

Materials Provided: The following materials needed for the procedure are contained in the QuickGel Cholesterol Kit (Cat. No. 3543T). Individual items are not available.

QuickGel Cholesterol Gels (10)
SPIFE Cholesterol Reagent (10 x 2.5 mL)
SPIFE Cholesterol Diluent (1 x 25 mL)
QuickGel Blotter C (10)
Citric Acid Destain (1 pkg)
QuickGel Templates (10)
QuickGel Blotter A (10)
QuickGel Blotter X (20)

Materials provided but not contained in the kit:

Item	Cat. No.
QuickScan Touch Plus	1640
QuickGel Chamber	1284
Titan Blotter Pads	5037
Staining Dish	4061
Incubation Chamber	4062
REP Prep	3100
QuickGel Gel Block Remover	1262
Cholesterol Profile Control	3218

Materials needed but not provided:

5 mL serological pipette
Power Supply capable of providing at least 220 Volts.
Incubator capable of maintaining 30°C

STEP BY STEP METHOD

I. Chamber Preparation

1. The QuickGel Chamber must be plugged into a power supply. Set a timer for *23:00 minutes and the power at 220 Volts. *An electrophoresis time of 22:30 to 23:30 minutes is acceptable.
2. Snap the Electrophoresis Lid into place on the chamber.
3. Ensure that the chamber floor is cool (room temperature) before starting the test.

II. Preparation of the Incubation Chamber

1. Place a Titan Blotter Pad in the bottom of the incubation chamber.
2. Wet the blotter completely with water and then pour off excess.
3. Close the chamber and place it in a laboratory incubator at 30°C.
4. Allow the blotter to equilibrate to 30°C while performing the electrophoresis steps.

III. Preparation of Reagent

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

IV. Sample Template Application

1. Carefully cut open one end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the remaining gel. Remove the gel to be used from the plastic mold and discard the mold.
2. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left notch of the gel so that it fits the left pin of the chamber floor and gently roll the gel to the right side fitting the right notch to the right pin of the chamber floor. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess REP Prep. Make sure that no bubbles remain under the gel.
4. Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
5. Remove one QuickGel Template from the package. Hold the template so that the small hole in the corner is toward the front right side of the chamber.
6. Carefully place the template on the gel aligning the template slits with the marks on each side of the gel backing. The center hole in the template should align with the indention in the center of the gel.
7. Apply slight fingertip pressure to the template making sure there are no bubbles under it. **NOTE: If wearing rubber gloves to perform this step, place a QuickGel Blotter A over the template and then apply fingertip pressure to the template. Remove the blotter. Powder from the gloves can produce gel artifacts.**
8. Apply 3 µL of the appropriate sample to the template slits. After the last sample application, wait 5 minutes to allow time for the proper absorption.
9. Use the QuickGel Blotter A to gently blot the excess sample from the template. Carefully remove the template.

10. Place two Blotter X's horizontally along the top and bottom sides of the gel backing. They should be positioned along the edges (not touching the gel) so that, when the lid closes, the Blotter X's do not interfere with the electrodes.
11. Close the lid, press the power switch to turn on the chamber and start the power supply.

V. Electrophoresis and Incubation

1. Electrophorese the gel for *23:00 minutes at 220 Volts.
2. Turn off the power supply and the QuickGel Chamber.
3. Open the lid and remove the blotters.
4. Using the QuickGel Gel Block Remover, remove the two gel blocks from the gel. Again, use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture.
5. Remove the gel from the chamber and place it, agarose side up, on a clean nonporous surface with the cathode edge (top of gel) away from you.
6. Pour the contents of the vial of reagent along the cathode edge of the gel.
7. Lay a 5 mL serological pipette lengthwise along the cathode edge of the gel. Gently spread the reagent by slowly pulling the pipette across the agarose to the anode edge, being careful not to roll the reagent off the gel. Wait for 15 seconds. Then pull the pipette across the gel from anode to the cathode edge in the same manner. Wait 15 seconds. Then pull the pipette across the gel from cathode to anode and roll the excess off the gel.
8. Place the gel into the preheated (30°C) Incubation Chamber.
9. Place the Incubation Chamber in a laboratory incubator at 30°C for 15 minutes.
10. Two containers are needed: one for the Destain solution and one for the deionized water wash. The size of the container should be able to accommodate a gel laid flat. Pour a sufficient amount of Destain into one container to cover the gel. Pour the same amount of deionized water into the other container.
11. At the end of incubation, remove the gel from the incubator. Place the gel in the destain solution using a gentle alternately rocking and swirling technique. Allow the gel to remain in the destain solution for 5 minutes. Remove the gel and tap it to remove excess destain.
12. Place the gel in the container of deionized water for 5 minutes. Use the same technique as in Step 11. Tap the gel to remove the excess water.
13. Ensure the chamber floor is clean, and place the Drying Lid on the chamber. Replace the gel onto the chamber floor. Close the Drying Lid and turn on the chamber. Dry the gel for 20 minutes or until dry. Remove the gel when drying is completed, and turn off the chamber.

Evaluation of Fractions

For quantitation of the lipoprotein cholesterol fractions scan the gel, agarose side up, in the QuickScan Touch Plus.

Stability of End Product: For best results, scan the QuickGel 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[26], and wide variations among different geographical locations and races have been reported[6]. Therefore, it is essential that each laboratory establish its own expected range for its particular population.

A total of 54 patients with normal total cholesterol (total cholesterol ≤ 200 mg/dL) were tested using the QuickGel Cholesterol system. These patients have not been differentiated by age, race or sex. These values should only serve as guidelines.

	Range ($\bar{x}$ ± 2 SD)
HDL (%)	11.8 - 45.0
Lp(a)-C%	0.0 - 12.2
VLDL (%)	0.0 - 20.3
LDL (%)	47.6 - 77.5

Each laboratory should establish its own range for age, sex and race.

RESULTS

The QuickGel 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.[27] 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.

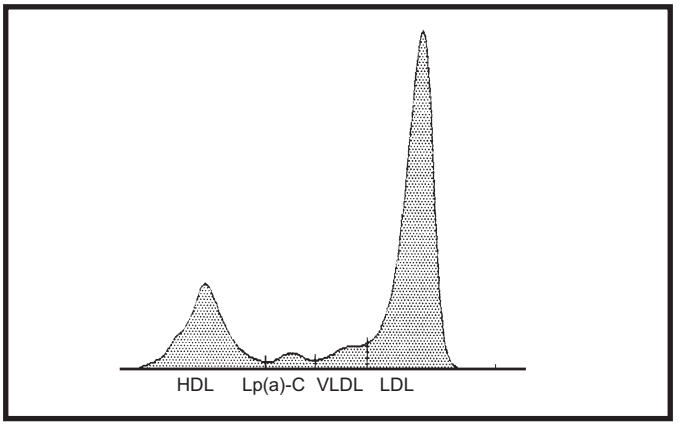

Figure 1: A scan of a QuickGel Cholesterol pattern.

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[10]. 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[10]

Total Cholesterol	
Desirable Blood Cholesterol	< 200 mg/dL
Borderline-High Blood Cholesterol	200-239 mg/dL
High Blood Cholesterol	≥ 240 mg/dL
HDL-Cholesterol	
Low HDL Cholesterol	< 40 mg/dL
Protective HDL-Cholesterol	≥ 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
Dietary Therapy

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

LDL-Cholesterol
Drug Treatment

	Initiation Level	LDL Goal
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

Precision studies were done using a control and a normal patient specimen on both the SPIFE and the QuickGel Chamber methods.

SPIFE

Within Run - A single patient sample and a control were run in replicate on one gel. N = 5

Control

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	22.8	2.3	11.3	63.6
SD	0.8	0.1	0.5	0.6
CV	3.5%	3.7%	4.5%	0.9%

Patient	HDL %	Lp(a)-C		LDL %
Mean	27.2	7.7		65.0
SD	0.6	0.2		0.4
CV	2.2%	3.1%		0.7%

Between Run - A patient sample and a control were run in replicate on 9 gels. N = 45

Control

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	22.9	2.4	11.6	63.2
SD	0.8	0.2	1.0	1.4
CV	3.6%	7.3%	8.7%	2.3%

Patient	HDL %	Lp(a)-C		LDL %
Mean	27.3	7.5		65.2
SD	0.8	0.4		0.7
CV	2.9%	5.7%		1.1%

QuickGel Chamber

Within Run - A single patient sample and a control were run in replicate on one gel. N = 5

Control

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	23.9	2.4	10.5	63.3
SD	0.7	0.2	0.3	0.7
CV	3.1%	6.4%	3.1%	1.1%

Patient	HDL %	Lp(a)-C		LDL %
Mean	29.5	6.9		63.6
SD	0.8	0.1		0.8
CV	2.7%	0.8%		1.3%

Between Run - A patient sample and a control were run in replicate each on 9 gels. N = 45

Control

	HDL %	Lp(a)-C	VLDL %	LDL %
Mean	23.4	2.4	10.5	63.7
SD	0.9	0.2	0.3	1.0
CV	3.8%	9.4%	3.2%	1.6%

Patient	HDL %	Lp(a)-C		LDL %
Mean	29.4	6.8		63.8
SD	1.5	0.5		1.5
CV	5.3%	6.8%		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 81 patient samples, were run using SPIFE Vis Cholesterol as the reference method. The following is the correlation data produced.

N = 81

SPIFE	QuickGel Chamber
R = 0.998	R = 0.998
Y = 1.024X - 0.753	Y = 1.010X - 0.322
X = SPIFE Vis Cholesterol	X = SPIFE Vis Cholesterol
Y = QuickGel Cholesterol on SPIFE	Y = QuickGel Cholesterol on QuickGel Chamber

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