QuickGel® Chamber Lipoprotein Electrophoresis Procedure

Cat. No. 3544T

The QuickGel Lipoprotein electrophoresis method is intended for the separation and quantitation of plasma lipoproteins by agarose gel electrophoresis using the QuickGel Chamber.

SUMMARY

Since Fredrickson and Lees proposed a system for phenotyping hyperlipoproteinemia in 1965[1], the concept of coronary artery disease detection and prevention utilizing lipoprotein electrophoresis has become relatively common.

Epidemiologic studies have related dietary intake of fats, especially cholesterol and blood levels of the lipids to the incidence of atherosclerosis, the major manifestations of which are cardiovascular disease and stroke. Ischemic heart disease has also been related to hypercholesterolemia.[2, 3]

The need for accurate determination of lipoprotein phenotypes resulted from the recognition that hyperlipoproteinemia is symptomatic of a group of disorders dissimilar in clinical features, prognosis and responsiveness to treatment. Since treatments of the disorders vary with the different phenotypes, it is absolutely necessary that the correct phenotype be established before therapy is begun.[4] In the classification system proposed by Fredrickson and Lees, only types II, III and IV have a proven relationship to atherosclerosis.

Plasma lipids do not circulate freely in the plasma, but are transported bound to protein and can thus be classified as lipoproteins. These various fractions are made of different combinations of protein, cholesterol, glycerides, cholesterol esters, phosphatides and free fatty acids.[5]

Several techniques have been employed to separate the plasma lipoproteins, including ultracentrifugation, thin layer chromatography, immunological techniques, and electrophoresis. Electrophoresis and ultracentrifugation are two of the most widely used methods and each has given rise to its own terminology. Table 1 shows the correlation of these classifications and the relative lipid and protein composition of each fraction.

Table 1: Classification and Composition of Lipoprotein Fractions

Classification according to		Composition - % in each Fraction			
Electrophoretic Mobility	Ultra-Centrifuge	Protein	Glyceride	Cholesterol	Phospho-lipids
Chylomicrons		2%	98%		
Beta	LDL*	21%	12%	45%	22%
Pre-Beta	VLDL*	10%	55%	13%	22%
Alpha	HDL*	50%	6%	18%	26%

**Non standard abbreviations: LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein).*

Various exceptions to the above classifications inevitably exist. One of these is the "sinking pre-beta," which is pre-beta migrating material that "sinks" in the ultracentrifuge, along with the LDL (beta migrating) fraction.[6] This is the Lp(a) lipoprotein reported by Dahlen.[7] It is considered a variant found in 20% of the population.[15] If a fourth band appears between pre-beta and alpha, it is Lp(a) and should be quantitated with pre-beta.

Another exception is the "floating beta," which is beta migrating material "floating" in the ultracentrifuge with the VLDL. The abnormal lipoprotein appears in Type III hyperlipoproteinemias.

Various types of support media have been used for the electrophoretic separation of lipoproteins. When Fredrickson originally devised the classification system, he used paper electrophoresis.[1, 8] More recently agarose gel, starch block, and polyacrylamide gel have been used.[5, 7]

PRINCIPLE

The specimen is applied to an agarose gel, the lipoprotein fractions are separated by electrophoresis and stained with Fat Red 7B. The stained bands may be visually inspected for qualitative results or may be quantitated in a scanning densitometer using a 525 nm filter or in a QuickScan Touch/2000.

REAGENTS

1. QuickGel Lipoprotein Gel

Ingredients: Each gel contains agarose in a sodium barbital buffer with EDTA, guanidine hydrochloride, 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. Lipoprotein Stain

Ingredients: When reconstituted as directed, the stain contains 0.1% (w/v) Fat Red 7B in 95% methanol.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

Preparation of Stock Stain Solution: Dissolve the stain (entire contents of vial) in 1 L methanol. Stir overnight, allow stock solution to stand for 1 day and filter before use.

Storage and Stability: The stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The diluted stain is stable for two months stored at 15 to 30°C. The filtered stain should be stored in a screw top container at 15 to 30°C.

Signs of Deterioration: The dissolved stain should be a homogeneous mixture free of precipitate.

INSTRUMENTS

A QuickGel Chamber must be used to electrophorese the gel. The gel can be scanned on a densitometer such as the QuickScan Touch/2000 (Cat. No. 1690/1660). Refer to the appropriate Operator's Manual for detailed operating instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Serum or plasma from samples collected in EDTA may be used. Do not use plasma collected in heparin. Fresh serum is the specimen of choice.

Patient Preparation: For the most accurate phenotyping of lipoprotein patterns, the following precautions should be observed before sampling.[9]

1. Discontinue all drugs, if possible, for 3 to 4 weeks.
2. The patient should be maintaining a standard weight and on a diet considered normal for at least one week.
3. Wait 4 to 8 weeks after a myocardial infarction or similar traumatic episode.
4. The patient should be fasting for a 12 to 14 hour period. Chylomicrons normally appear in the blood 2 to 10 hours after a meal; therefore, a 12 to 14 hour fast is necessary to define hyperlipoproteinemia.

Interfering Substances: Heparin therapy causes activation of lipoprotein lipase, which increases the relative migration rates of the fractions, especially the beta lipoprotein.[10]

Serum Storage: For best results, fresh serum should be used. Storage at 2 to 8°C for no more than 5 days yields satisfactory results. Prolonged storage increases the migration rate of the pre-beta fraction. Do not freeze.[11]

PROCEDURE

Materials Provided: The following materials needed for the procedure are contained in the QuickGel Lipoprotein Kit (Cat. No. 3544T).

- QuickGel Lipoprotein Gels (10)
- Lipoprotein Stain (1 vial)
- QuickGel Blotter C (10)
- QuickGel Blotter X (20)
- QuickGel Templates (10)
- QuickGel Blotter A (10)

Materials provided but not contained in the kit:

Item	Cat. No.
QuickGel Chamber	1284
QuickScan 2000	1660
QuickScan Touch	1690
Lipotrol	5069
REP Prep	3100
QuickGel Gel Block Remover	1262
REP Gel Staining Dish (10)	1362

Materials Needed but not Supplied:

5 mL serological pipette
Power Supply capable of providing at least 220 Volts.
Methanol
Destaining Solution: Mix 75 mL methanol with 25 mL deionized water.

STEP BY STEP METHOD

I. Chamber Preparation

1. The QuickGel Chamber must be plugged into a power supply.
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. 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 and dispose of template as biohazardous waste.
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.

III. Electrophoresis and Drying

1. 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. Electrophorese the gel, then turn off the power supply and the QuickGel Chamber.
3. Open the lid and remove the Blotters X's.
4. Using the Gel Block Remover, remove the two gel blocks from the gel. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture.
5. Replace the Electrophoresis Lid with the Drying Lid. Close the lid and turn on the QuickGel chamber.
6. Dry the gel for 15 minutes or until dry. Turn off the QuickGel chamber and remove the gel.

IV. Visualization of the Lipoprotein Bands

1. Preparation of Staining Solutions
   - **a. Stock Stain Solution:** Mix the Lipoprotein Stain in 1 L methanol. Allow to stir overnight and stand for one day. Filter before use. Store at 15 to 30°C.
   - **b. Working Stain Solution:** Approximately 5 minutes before use, prepare a working solution of Lipoprotein Stain by adding 5 mL deionized water to 25 mL of Stock Lipoprotein stain solution. For best results, add the water to the stain in a dropwise manner while swirling the solution.
   - **c. Destaining Solution:** Mix 75 mL methanol with 25 mL deionized water. Mix thoroughly.
2. Recommended Staining Procedure
   - a. Place the gel in the REP Gel Staining Dish, agarose side up. Carefully pour 30 mL of the Working Stain Solution directly on the agarose. Wait 4 minutes. Pour off the stain.
   - b. Place the gel in Destain Solution for 10 to 20 seconds. Remove excess stain from the sample area using a gloved finger to gently and evenly wipe the gel surface. Pour off the Destain.
   - c. Destain the gel again for 10 to 20 seconds. Pour off the Destain Solution.
   - d. A brief final water wash may be necessary if trace amounts of background stain remain on the gel. Excessive destaining may cause light or faded bands and/or non-quantitation of control.
3. Return the gel to the QuickGel chamber. Close the lid and turn on the QuickGel chamber. Dry the gel for 5 minutes or until dry.
4. Turn off the QuickGel chamber and remove the gel.

Evaluation and Quantitation

1. Qualitative evaluation: The QuickGel Lipoprotein Gel may be visually inspected for the presence of bands.
2. Quantitative evaluation: Scan the Lipoprotein Gel in the QuickScan Touch/2000 using slit 5.

Stability of End Product: Gels to be scanned for the quantitative determination of the bands must be scanned as soon as possible. Gels to be visually inspected for qualitative evaluation only may be kept an indefinite period of time after being processed.

Calibration: A calibration curve is not necessary because relative concentration of the bands is the only parameter determined.

Quality Control:

Lipotrol (Cat. No. 5069) can be used to verify all phases of the procedure and should be used on each gel run. The control should be used as a marker for location of the lipid bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with each control for assay values. Additional QC controls may be required for federal, state or local regulations.

REFERENCE VALUES

Reference range studies were established using 37 male and female adults with a total cholesterol of ≤ 200 mg/dL.

Lipoprotein Fraction	% of Total Lipoprotein
Alpha	12.6 - 46.6
Pre-Beta	0 - 57.1
Beta	21.7 - 67.7
Chylomicrons	< 1.0

Any quantitation of Lp(a) must be added to pre-beta for an accurate total pre-beta.

These values are intended as guidelines. Each laboratory should establish its own normal range study because of population differences in various regions.

RESULTS

The Alpha-lipoprotein (HDL) band is the fastest moving fraction and is located closest to the anode. The Beta-lipoprotein (LDL) band is usually the most prominent fraction and is near the origin, migrating cathodic to the point of application. The pre-Beta lipoprotein (VLDL) band migrates between Alpha and Beta lipoprotein. The mobility of the pre-Beta fraction varies with the degree of resolution obtained, the type of pre-Beta present, and the percent of Beta present. Sometimes pre-Beta will be seen as a smear just ahead of the Beta fraction. Other times it may be split into two or more fractions or may be lacking altogether. The integrity of the pre-Beta fraction decreases with sample age. Chylomicrons, when present, stay at the point of application.

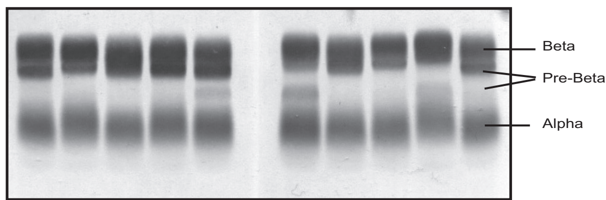

Figure 1: A QuickGel Lipoprotein gel illustrating the band positions.

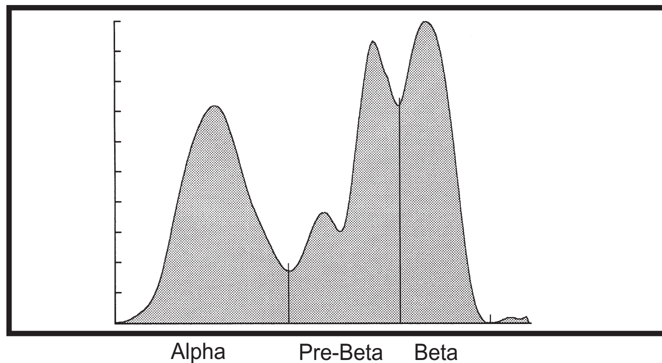

Figure 2: A scan of a QuickGel Lipoprotein pattern with an Lp(a) band.

Calculations of the Unknown: Figure 2 shows a typical lipoprotein scan produced by a QuickScan 2000. The relative percent of each band is computed and printed automatically by the densitometer. The calculated percent of the Lp(a) band must be added to the percent of the pre-Beta band for a total pre-Beta value.

Calculating the mg/dL of total lipids from the relative percent values obtained is not recommended. (See LIMITATIONS)

INTERPRETATION OF RESULTS

Lipoprotein Phenotyping Using the SPIFE Lipoprotein Electrophoresis Method:

Normal Pattern - A normal fasting serum can be defined as a clear serum with negligible chylomicrons and normal cholesterol and triglyceride levels. On electrophoresis, the Beta lipoprotein appears as the major fraction with the pre-Beta lipoprotein faint or absent and the Alpha band definite but less intense than the Beta. If a fourth band appears between pre-Beta and Alpha, it is Lp(a) and should be quantitated with the pre-Beta band.

Abnormal Patterns - A patient must have an elevated cholesterol or triglyceride to have hyperlipoproteinemia. The elevation must be determined to be primary or secondary to metabolic disorders such as hypothyroidism, obstructive jaundice, nephrotic syndrome, dysproteinemias, or poorly controlled insulinopenic diabetes mellitus. Primary lipidemia arises from genetically determined factors or environmental factors of unknown mechanism such as diet, alcohol intake, and drugs, especially estrogen or steroid hormones.[14] Also considered primary are those lipoproteinemias associated with ketosis-resistant diabetes, pancreatitis, and obesity. Diabetes mellitus and pancreatitis can be confusing, for it is often difficult to tell whether the hyperlipoproteinemia or the disease is the causative factor.

LIMITATIONS

Limiting Factors: Fat Red 7B, as well as the Sudan black stains, has a much greater affinity for triglycerides and cholesterol esters than it has for free cholesterol and phospholipids. Bands seen after staining with these dyes do not reflect a true quantitation of the total plasma lipids.[12] For this reason it is not recommended that relative percentages of lipoprotein bands be used to calculate the total lipid content in each fraction from a total plasma lipid value. Since most laboratories routinely offer total cholesterol and triglyceride levels, this information is unnecessary.

Interfering Factors: Specimens collected in heparin should not be used since heparin alters the migration patterns of the lipoprotein fractions.

Further Testing Required: Since the lipid composition of each lipoprotein fraction is variable, it is essential to determine total cholesterol and triglyceride levels before attempting to classify a pattern.[8, 9] When it becomes necessary to diagnose or rule out a Type III hyperlipoproteinemia, a more definitive quantitation of the lipoproteins such as ultracentrifugation[4] or electrophoresis on polyacrylamide gel[13] is essential.

PRIMARY LIPOPROTEINEMIAS

The Fredrickson Classification

Type I: Hyperchylomicronemia

Criteria: Chylomicrons present, pre-Beta normal or only slightly elevated. Alpha and Beta decreased, often markedly so. Standing plasma with marked creamy layer.

Confirmation: A measurement of post-heparin lipolytic activity (PHLA) and the demonstration of severe intolerance to exogenous fat. The condition is rare and always familial. There has been no correlation to vascular disease. It is thought to be due to a genetic deficiency of lipoprotein lipase.[8]

Type II: Hyperbetalipoproteinemia

Criteria: Increased total cholesterol due to an increased Beta-lipoprotein cholesterol. Alpha cholesterol usually normal or low.

Type IIa: normal pre-Beta, normal triglycerides, plasma clear.

Type IIb: increased pre-Beta and triglycerides, plasma clear to slightly turbid with no creamy layer.

This is one of the most common familial forms of hyperproteinemias.

Secondary causes: Myxedema, myelomas, macroglobulinemias, nephrosis, liver disease, excesses in dietary cholesterol and saturated fats.

Type III: "Broad Beta" - Abnormal Lipoprotein

Criteria: Presence of triglyceride burdened lipoprotein of abnormal composition and density. Cholesterol and triglyceride elevated. The abnormal material has broad beta electrophoretic mobility but separates with VLDL in the ultracentrifuge. Plasma turbid to cloudy. The abnormal lipoprotein is also known as "floating Beta". The condition is rare.

Confirmation: Polyacrylamide gel electrophoresis[13] or ultracentrifuge studies to demonstrate the abnormal lipoprotein.

Type IV: Carbohydrate Induced Endogenous Hypertriglyceridemia

Criteria: Increased pre-Beta, increased triglycerides, normal or slightly increased total cholesterol. Alpha and Beta lipoprotein usually normal. (An increased pre-Beta with normal triglyceride level is seen with the normal variant "sinking pre-Beta." Such samples do not belong to Type IV.)

Secondary causes: Nephrotic syndrome, diabetes mellitus, pancreatitis, glycogen storage disease, and other acute metabolism changes where mobilization of free fatty acids is increased. Endogenous triglycerides are very sensitive to alcohol intake, emotional stress, diet and changes in weight. Little effect is seen with exogenous triglyceride intake. Ninety percent of people with familial Type IV have an abnormal glucose tolerance. Probably the most common type of hyperlipoproteinemia reflecting an imbalance in synthesis and clearance of endogenous triglycerides.

Type V: Mixed Triglyceridemia (carbohydrate and fat included)

Criteria: Increased exogenous and endogenous triglycerides, cholesterol increased, chylomicrons present, pre-Beta increased, Beta normal to slightly increased.

Secondary causes: Nephrosis, myxedema, diabetic acidosis, alcoholism, pancreatitis, glycogen storage disease and other acute metabolic processes.[4]

Note: Only Types II, III, and IV have been correlated to vascular disease.

THE ALPHA LIPOPROTEINS IN DISEASE

Marked increases in the Alpha lipoproteins are seen in obstructive liver disease and cirrhosis. Marked decreases are seen in parenchymal liver disease. Tangier's disease is a rare genetic disorder characterized by the total absence of normal Alpha lipoproteins. Heterozygotes exhibit decreased levels of Alpha lipoproteins.[8] It should be noted that hyperestrogenemia (pregnancy or oral contraceptive use) may cause moderate elevations in the Alpha lipoproteins.[12]

DECREASES IN THE BETA LIPOPROTEINS

Abetalipoproteinemia is a primary inherited defect characterized by severe deficiency of all lipoproteins of density less than 1.063 (all but the Alpha lipoproteins). It is accompanied by numerous clinical symptoms and life expectancy is limited. A few cases of familial hypobetalipoproteinemia have been reported. There is some evidence that the mutation is different from that producing abetalipoproteinemia.

LIPOPROTEIN-X

Lipoprotein-X is an abnormal lipoprotein often seen in patients with liver disease. It consists of unesterified (free) cholesterol, phospholipids and protein. It migrates slower than LDL. Because of its particular lipid contents, it stains poorly or not at all with the usual lipid stains and so is not usually detected by standard lipoprotein electrophoretic methods.

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run: A patient sample was run 10 times on one gel. N = 10

Lipoprotein Fraction	Mean %	SD	CV
Alpha	39.2	1.1	2.8%
Pre-Beta	17.6	0.8	4.3%
Beta	43.2	1.5	3.4%

Between Run: A control and a patient sample were run alternately on eight gels. N = 40

Control

Lipoprotein Fraction	Mean %	SD	CV
Alpha	39.3	2.7	6.8%
Pre-Beta	20.5	1.1	5.4%
Beta	40.2	2.6	6.6%

Patient

Lipoprotein Fraction	Mean %	SD	CV
Alpha	39.7	2.4	6.0%
Pre-Beta	17.5	0.7	4.0%
Beta	42.8	2.3	5.3%

CORRELATION

Correlation studies were performed on 27 normal and abnormal specimens. The QuickGel Lipoprotein method on QuickGel chamber was compared to QuickGel Lipoprotein run on SPIFE resulting in the following linear regression:

N = 27
Y = 0.992X + 0.258
R = 0.974
X = QuickGel Lipoprotein on SPIFE
Y = QuickGel Lipoprotein on QuickGel chamber

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