QuickGel® Touch Cholesterol Procedure

Cat. No. 3334

INTENDED USE

The QuickGel Touch Cholesterol procedure is for use in the quantitative determination of cholesterol and cholesterol esters in serum lipoproteins using the SPIFE Touch. 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[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 work-up 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.

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

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

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

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 with the QuickScan Touch/2000.

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 Touch 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 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

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)
Blade Applicator Kit - 20 Samples (10)

Materials provided by Helena but not contained in the kit:

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
Cholesterol Profile Control	3218
REP Prep	3100
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 Reagent Spreaders	3706
SPIFE QuickGel Disposable Stainless Steel Electrodes	3357
SPIFE Reagent Spreaders	3386
Applicator Blade Weights	3387

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 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 QuickGel Touch Cholesterol test. To verify the status of the stainer chamber, use the arrows under the **STAINER UNIT** to select the appropriate test, place the empty Gel Holder into the stainer chamber and press **START**. If washing of the staining chamber is necessary, the prompt "Vat must be washed. Remove gel and install gel holder." will appear. Press **RETRY** to begin the stainer wash. The cleaning process will complete automatically in about 7 minutes. To avoid delays after incubation, this wash cycle should be initiated at least 7 minutes prior to the end of the run.

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 Applicator Blade will only fit into the slots one way; do not try to force the Applicator Blade into the slots.**
3. Place an Applicator Weight on top of the Applicator Blade. When placing the weight on the blade, position the weight with the thick side to the right.
4. Slide a strip of Disposable Cups into top row numbered 1 to 10 of the appropriate Cup Tray.
5. 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. Place a QuickGel Blotter C on the gel with the longer edge parallel with gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and 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. Improper contact between the electrodes and the gel block can result in skewed patterns. Close the chamber lid.
9. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP**.

VI. Sample Application/Electrophoresis

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

	Separator Unit
Load Sample 1	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 2	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 3	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 4	Prompt: None Time: 0:30 Temperature: 20°C Speed: 6
Apply Sample	Prompt: None Time: 1:00 Temperature: 20°C Speed: 6 Location: 1
Electrophoresis	Prompt: To Continue Time: 25:00 Temperature: 16°C Voltage: 220 V mA: 60 mA
Apply Reagent	Prompt: Remove Blotter Temperature: 30°C Cycles: 8
Incubate	Prompt: None Time: 15:00 Temperature: 30°C
End	
	Stainer Unit
Wash 1	Prompt: None Time: 5:00 Recirculation: Rev Valve: 2 Fill,Drain
Wash 2	Prompt: None Time: 5:00 Recirculation: Rev Valve: 7 Fill,Drain
Dry	Prompt: None Time: 15:00 Temperature: 70°C
End	

1. Place the Cup Tray with samples on the SPIFE Touch. 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. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples and beep.
4. Remove the two QuickGel Blotter X's 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 Touch will electrophorese and beep when completed.

6. Open the chamber lid, remove electrodes and dispose of Blotter X's. Dispose of blades as biohazardous waste. Using a 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 Reagent Spreader (glass rod) inside the magnetic posts at each end of the chamber.
9. Close the chamber lid and press the **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. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. 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.

EVALUATION OF FRACTIONS

For quantitation of the lipoprotein cholesterol fractions scan the gel, agarose side up, in the Quick Scan Touch/2000 on the acid violet setting. A slit size of 4 is recommended. Auto Edit is used with this test.

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.

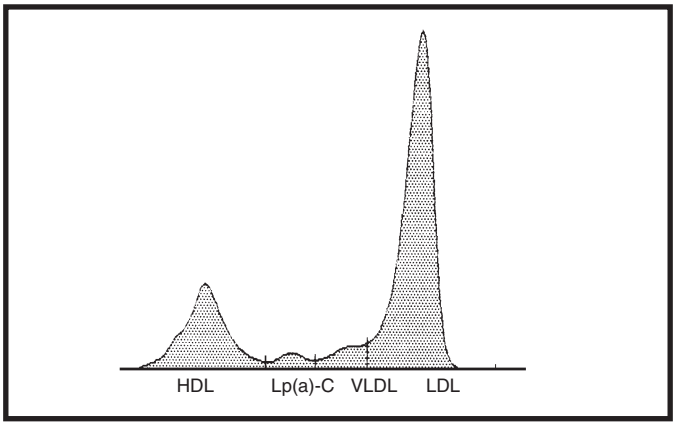

Figure 1: A scan of a QuickGel Cholesterol pattern.

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 the SPIFE Touch.

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	19.4	5.8	17.2	57.6
SD	0.9	0.4	0.4	1.2
CV	4.6%	6.9%	2.3%	2.1%

Patient	HDL %		VLDL %	LDL %
Mean	33.4		16.5	50.1
SD	1.2		1.1	0.9
CV	3.6%		6.7%	1.8%

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	20.3	5.9	17.4	56.4
SD	1.1	0.4	1.1	1.4
CV	5.4%	6.8%	6.3%	2.5%

Patient	HDL %		VLDL %	LDL %
Mean	34.4		16.0	49.6
SD	1.2		1.8	2.5
CV	3.5%		11.3	5.0%

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 36 patient samples, were run using QuickGel Cholesterol on SPIFE 3000 as the reference method. The following is the correlation data produced.

N = 36

R = 0.9988

Y = 1.0193 - 0.476

X = QuickGel Cholesterol on SPIFE 3000

Y = QuickGel Cholesterol on SPIFE Touch

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QuickGel Cholesterol System

Cat. No. 3443

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)
Blade Applicator Kit - 20 Sample (10)

Other Supplies and Equipment

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

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
Cholesterol Profile Control	3218
REP Prep	3100
Gel Block Remover	1115
SPIFE Disposable Cups (Deep Well)	3360
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrodes	1111
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