QuickGel® CK Vis Isoenzyme Procedure

Cat. No. 3334, 3534T

INTENDED USE

The QuickGel® CK Vis Isoenzyme Procedure is intended for the qualitative and quantitative analysis of the creatine phosphokinase isoenzymes in serum by agarose electrophoresis using the SPIFE 3000 system or QuickGel Chamber.
Cat. No. 3334 - SPIFE 3000
Cat. No. 3534T - QuickGel Chamber
For In Vitro Diagnostic use.
Rx Only

SUMMARY

Creatine phosphokinase (CK) (EC 2.7.3.2) is an energy transfer enzyme which catalyzes the reversible reaction

$$\text{ADP + creatine phosphate} \xrightleftharpoons{\text{CK}} \text{ATP + creatine}$$

CK exists primarily in skeletal muscle, cardiac muscle and the brain, with small amounts in several other tissues.[1] A number of diverse clinical episodes such as surgical procedures, intramuscular injections and myocardial infarct induce increased CK activity in the serum.[2, 3] The source of elevated CK activity may be narrowed by isoenzyme assessment. There are two molecular CK subunits, designated M and B, the combinations of which produce three isoenzymes: CK-MM (isolated primarily from skeletal muscle), CK-MB (myocardium) and CK-BB (primarily from the brain).[3]
CK isoenzyme analysis is one of the important procedures used in the detection of myocardial damage.[4] After an acute myocardial infarction (MI), CK-MB appears in the serum in approximately 4 to 6 hours, reaches peak activity at 18 to 24 hours, and may disappear completely within 72 hours. Within the first 48 hours after MI, CK-MB is present in 100% of the patients with MI as well as in some cases of severe coronary insufficiency.[1, 3, 7]
Definitive laboratory testing in the diagnosis of MI is accomplished by performing studies of CK isoenzymes in conjunction with lactate dehydrogenase (LD) isoenzymes.[3, 5-8] The specificity and sensitivity achieved with these two tests has eliminated the necessity for additional enzyme studies in accurately diagnosing MI.[6] The most important consideration in the interpretation of CK and LD isoenzyme patterns is the detection of the characteristic change of pattern of multiple examinations (the relatively fast appearance and disappearance of CK-MB and the flip of LD_1 and LD_2).[1, 3, 35] Persistent elevation in CK-MB is not indicative of myocardial infarct. CK-MB may be helpful in diagnosing a small infarct in which total CK never exceeds the upper limit of normal.[9]
CK produced by myocardium is only 25 to 40% CK-MB, the remainder being CK-MM.[1, 4] Therefore, an elevation in CK due to myocardial infarction produces not only a rise in CK-MB but in CK-MM as well.[3] The isoenzymes of CK have been assessed by various methods.[10-19] Electrophoresis offers the distinct advantage of complete separation of the isoenzymes without risk of carryover.[3]

PRINCIPLE

The isoenzymes of CK are separated according to their electrophoretic mobility on agarose gel. After separation the gels are incubated with the SPIFE CK Isoenzyme Reagent which utilizes the following reactions:

$$\text{Creatine phosphate + ADP} \xrightleftharpoons{\text{CK}} \text{Creatine + ATP}$$

$$\text{ATP + D-glucose} \xrightleftharpoons{\text{Hexokinase}} \text{D-Glucose-6-phosphate + ADP}$$

$$\text{D-G-6-P + NAD} \xrightleftharpoons{\text{G-6-PD}} \text{NADH + 6 phosphogluconate + } H^+$$

$$\text{NADH + } H^+ \xrightleftharpoons{\text{PMS}} \text{NAD + Formazan}$$

REAGENTS

1. QuickGel CK Gel

Ingredients: Each gel contains agarose in a AMP/MOPSO buffer. Sodium azide has been added as a preservative.
WARNING: FOR IN-VITRO DIAGNOSTIC USE. Refer to Sodium Azide Warning.
Preparation for Use: The gels are ready for use as packaged.
Storage and Stability: The gels should be stored 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. CK Vis Isoenzyme Reagent

Ingredients:

Adenosine 5'-diphosphate (ADP)	12 mM
Creatine phosphate	90 mM
Adenosine 5'-monophosphate (AMP)	15 mM
Magnesium Acetate	60 mM
Diadenosine pentaphosphate	0.1 mM
Nicotinamide adenine dinucleotide (NAD)	10 mM
D-glucose	60 mM
Glucose-6-phosphate dehydrogenase (L.mesenteroides)	7,500 IU/L
Hexokinase (Yeast)	9,000 IU/L
PMS	0.15 mM
Bovine Serum Albumin (BSA)	4.5 g/L

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
Preparation for Use: Reconstitute one vial of CK Reagent with 1.5 mL of CK Diluent.
Storage and Stability: The dry reagent should be stored at 2 to 8°C and is stable until the expiration date on the vial. Reconstituted reagent is stable for 1 hour at 15 to 30°C.
Signs of Deterioration: If the unreconstituted reagent is not a uniformly white or slightly off white dry powder, it should not be used.

3. CK Diluent

Ingredients: The diluent contains MES, sucrose, Triton X and sodium azide added as a preservative.
WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. Refer to Sodium Azide Warning.
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. CK Chromogen

Ingredients: The chromogen contains 0.023 g Tetranitro Blue Tetrazolium (TNBT) per mL Dimethyl-formamide.
WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST – IRRITANT.
Preparation for Use: Add 150 µL of Chromogen to each vial of reconstituted Reagent, invert and use immediately.
Storage and Stability: The Chromogen should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.
Signs of Deterioration: The product should be discarded if it shows noticeable signs of turbidity.

5. CK Activator

Ingredients: The Activator contains 114 mM BME (Beta Mercapto Ethanol) in Tris base.
WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST.
Preparation for Use: The product is ready for use as packaged.
Storage and Stability: The Activator should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.
Signs of Deterioration: The product should be discarded if it shows noticeable signs of turbidity.

6. Citric Acid Destain

Ingredients: After dissolution, the destain contains 0.3% (w/v) citric acid.
WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST -IRRITANT.
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.

Sodium Azide Warning

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, 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.

INSTRUMENTS

A SPIFE 3000 Analyzer or the QuickGel Chamber must be used to electrophorese and dry the gel. Refer to the Operator's Manual for detailed operating instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Serum is the specimen of choice.

Collection of Specimen: Proper timing of specimen collections is critical to accurate interpretation of CK isoenzyme analysis. A blood specimen should be obtained immediately upon admission of the patient to the hospital and at 8 to 12 hour intervals thereafter for a minimum of 36 hours.

Interfering Substances:

1. Mature red blood cells contain no CK; however, some of the side reactions may occur in the coupled enzyme assay resulting in lower estimated CK activity. Non-hemolyzed samples are, therefore, preferred.[20]
2. CK is inactivated by heat.[20]
3. Repeated freezing and thawing destroys activity (see Serum Storage).
4. For the effects of various drugs on CK activity, refer to Young, et al.[21]

Serum Storage:

1. The blood specimen should be refrigerated (2 to 8°C) immediately after collection. Serum should be separated from the red blood cells as soon as possible.
2. Serum specimens may be stored at 2 to 8°C for up to 48 hours.[22]
3. Specimens may be stored frozen (-20°C) for up to two weeks.[22] Frozen specimens should be thawed at room temperatures and should never be placed in a 30 to 37°C water bath for thawing. Repeated freezing and thawing destroys CK activity and should be avoided.

PROCEDURE FOR SPIFE

Materials Provided: The following materials are provided in the QuickGel CK Vis Isoenzyme Kit (Cat. No. 3334). Individual items are not available.

QuickGel CK Gels (10)
CK Vis Reagent (10 x 1.5 mL)
CK Diluent (1 x 15 mL)
CK Chromogen (1 x 1.5 mL)
CK Activator (1 x 0.2 mL)
QuickGel Blotter C (10)
Citric Acid Destain (1 pkg)
Applicator Blade Assembly-20 Sample

Materials provided but not contained in the kit:

	Cat. No.
SPIFE 3000	1088
Disposable Sample Cups	3369
QuickScan Touch Plus	1640
CK/LD Control	5134
REP Prep	3100
Gel Block Remover	1115
SPIFE 3000/REP 3 Reagent Spreaders	3706
Applicator Blade Weights	3387
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrode	1111
SPIFE QuickGel Holder	3358
SPIFE QuickGel Chamber Alignment Guide	86541003
Chamber Cover	8JP34012
SPIFE Applicator Blades	3450

STEP-BY-STEP METHOD

I. Stainer Preparation

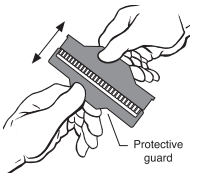
NOTE: If a SPIFE procedure requiring a stain has been run prior to running the CK gels, the stainer unit must be cleaned/washed before washing the CK gel.

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 to process the gel after electrophoresis.

II. Chamber Preparation

1. The SPIFE Quick Gel Chamber Alignment Guide must be used to mark the location for gel placement if the chamber floor hasn't 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 and 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.

III. Sample Preparation



1. Add 1 µL Activator to 100 µL patient sample or control. Mix and allow to sit at room temperature for 10 minutes.
2. If testing fewer than 10 samples, remove one Applicator Blade Assembly from the packaging. If testing 11 to 20 samples, remove two disposable Applicator Blade Assemblies from the packaging. Remove the protective guards from the blades by gently bending the protective piece back and forth until it breaks free.
3. Place the Applicator Blade into the vertical slot 6 in the Applicator Assembly. If using two Applicator Blades, place them into the vertical slots numbered 6 and 12. Additional blades must be ordered (Cat. No. 3450) if testing 11 to 20 samples per gel. **NOTE: The blade assembly will only fit into the slots one way; do not try to force the blade assembly into the slots.**
4. Place an Applicator Weight on top of the Applicator Blade.
5. Slide the Disposable Sample Cups into the appropriately numbered top row of the Cup Tray. If testing more than 10 samples, place the cups into both rows.
6. Pipette 17 µL of pretreated patient serum or control into cups numbered 1 to 5 and 6 to 10. If testing more than 10 samples, pipette samples into cups 11 to 15 and 16 to 20. Cover the tray until ready to use.

IV. 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 electrophoresis chamber.
4. Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
5. Clean the QuickGel Electrodes and Reagent Spreaders with deionized water before and after each use. Wipe with a lint-free tissue.
6. Place a QuickGel Electrode on the outside ledge of each gel block inside the magnetic posts. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the SPIFE 3000 until **QG-CK** option appears on the display.

V. Preparation of Isoenzyme Reagent

1. Reconstitute the CK Reagent with 1.5 mL CK Diluent. Mix well by inversion during the electrophoresis process. Do not add the Chromogen until ready to use as it can cause excess background on the gel.
2. Place a reconstituted vial of reagent (without Chromogen) into the center hole of the reagent bar on SPIFE 3000, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.

VI. Electrophoresis/Visualization

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

NOTE: A "Dry 1" time of 15 minutes is recommended. However, due to variations in environmental conditions, the following ranges are acceptable.
***Dry 1 = 12 to 20 minutes.**

Electrophoresis Unit

1)	No Prompt Load Sample 1	00:30	21°C	SPD6	
2)	No Prompt Apply Sample 1	1:00	21°C	SPD6	LOC1
3)	No Prompt Load Sample 2	00:30	21°C	SPD6	
4)	No Prompt Apply Sample 2	1:00	21°C	SPD6	LOC1
5)	No Prompt Load Sample 3	00:30	21°C	SPD6	
6)	No Prompt Apply Sample 3	1:00	21°C	SPD6	LOC1
7)	No Prompt Electrophoresis 1	10:00	13°C	225 V	30 mA
8)	Remove gel blocks Apply Reagent 1		37°C	8 cycles	
9)	To continue (continue) Incubate 1	18:00	45°C		
10)	No Prompt END OF TEST				

Stainer Unit

1)	No Prompt Destain 1	2:30	REC=REV	VALVE=2
2)	No Prompt Destain 2	2:30	REC=REV	VALVE=2
3)	No Prompt Wash 1	2:30	REC=REV	VALVE=7
4)	No Prompt Wash 2	2:30	REC=REV	VALVE=7
5)	No Prompt Dry 1	*15:00	63°C	
6)	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. With **QG-CK** 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, electrophorese, and beep when finished.
3. Open the chamber lid, remove the electrodes and dispose of blades as biohazardous waste.
4. With the gel still in the chamber, use a Gel Block Remover or straight edge to completely remove and discard the two gel blocks.
5. Use a lint-free tissue to wipe around the edges of the gel.
6. Place a Reagent Spreader Rod (glass rod) across each end of the gel inside the magnetic posts.
7. Remove the reagent vial and add 150 µL of Chromogen to it. Invert to mix, and replace immediately into the center hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.
8. Press the **TEST SELECT/CONTINUE** button to spread the reagent.
9. After the reagent is spread, the instrument will beep. Open the chamber lid and insert a Chamber Cover in the grooves of the chamber. Close the chamber lid.

VII. Incubation

1. Press the **TEST SELECT/CONTINUE** button to start the incubation timer.
2. At the end of the incubation, the instrument will beep. Remove the gel from the chamber.
3. 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.
4. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
5. With the appropriate test name displayed, 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 destain and dry the gel.
6. When the gel has completed the process, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot.

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 CK Vis Kit (Cat. No. 3534T). Individual items are not available.

QuickGel CK Gels (10)
CK Vis Isoenzyme Reagent
CK Diluent
CK Chromogen
CK Activator
QuickGel Blotter C (10)
QuickGel Blotter D (10)
Citric Acid Destain (1 pkg)
QuickGel Templates (10)
QuickGel Blotter A (10)

Materials provided but not contained in the kit:

Item	Cat. No.
QuickScan Touch Plus	1640
Staining Dish	4061
QuickGel Chamber	1284
Incubation Chamber	4062
REP Prep	3100
QuickGel Gel Block Remover	1262
CK/LD Isoenzyme Control	5134
QuickGel Accessory Kit	3426

Materials needed but not provided:

Incubator capable of maintaining 45°C
5 mL serological pipette
Power Supply capable of providing at least 400 Volts.

STEP BY STEP METHOD

I. Chamber Preparation

1. The QuickGel Chamber must be plugged into a power supply. Set a timer for *4:15 minutes and the power at 400 Volts. *An electrophoresis time of 4:00 to 4:30 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 QuickGel Blotter D 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 45°C.
4. Allow the chamber and blotter to equilibrate to 45°C while performing the electrophoresis steps.
5. If more than one gel will be put in the incubator, it is recommended that each incubation chamber be placed between preheated Development Weights.
6. Reconstitute the CK Reagent with 1.5 mL CK Diluent. Mix well by inversion. **Do NOT add the Chromogen until ready to use as it can cause excess background on the gel.**

III. Sample Template Application

1. Add 1 µL Activator to 100 µL patient sample or control. Mix and allow to sit at room temperature for 10 minutes.
2. 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.
3. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
4. 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.
5. Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
6. Remove one QuickGel Template from the package if only 1 to 10 samples are tested. Remove two templates if 11 to 20 samples

are tested. Hold the template so that the small hole in the corner is toward the front right side of the chamber.

7. 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.
8. 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.**
9. Apply 2 µL of the appropriate sample to the template slits. After the last sample application, wait 3 minutes to allow time for the proper absorption.
10. Use the QuickGel Blotter A to gently blot the excess sample from the template. Carefully remove the blotter and template and dispose of them as biohazardous waste.
11. Close the lid, press the power switch to turn on the chamber and start the power supply.

IV. Electrophoresis and Incubation

1. Electrophorese the gel for *4:15 minutes at 400 Volts.
2. Turn off the Power Supply and the QuickGel Chamber.
3. Open the lid. 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.
4. 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.
5. Add 150 µL of Chromogen to the vial of prepared reagent. Invert to mix and pour the contents of the vial of reagent along the cathode edge of the gel.
6. 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.
7. Place the gel into the preheated (45°C) Incubation Chamber.
8. Place the Incubation Chamber in a laboratory incubator at 45°C for 18 minutes.
9. Two containers are needed; one for the Destain solution and one for the 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 water into the other container.
10. 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 get rid of excess destain.
11. Place the gel in the container of water for 5 minutes. Use the same technique as in Step 10. Tap the gel to remove the excess water.
12. 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, turn the chamber on and dry the gel for 20 minutes or until dry. Remove the gel when drying is completed, and turn off the chamber.

Evaluation of the CK Isoenzyme Bands

1. Qualitative evaluation: The QuickGel CK Vis Isoenzyme Gel may be visually inspected for the presence of the bands.
2. Quantitative evaluation: Scan the QuickGel CK Vis Isoenzyme Gel in the QuickScan Touch Plus using the Acid Violet filter.

Stability of End Product

The CK gels should be scanned for quantitative results within two hours after drying.

Calibration

A calibration curve is not necessary because relative intensity of the bands is the only parameter determined.

Quality Control

The CK/LD Isoenzyme Control (Cat. No. 5134) 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 proper location of the isoenzyme bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay values. Additional controls may be required for federal, state, or local regulations.

REFERENCE VALUES

Reference range studies including 50 normal men and women were performed by Helena Laboratories. The following results were obtained:

	Range
% MM	97 - 100
% MB	0 - 3
% BB	0

These values should only serve as guidelines. Each laboratory should establish its own expected value range with this procedure.

RESULTS

CK-BB is the fastest moving, most anodic band, CK-MM is the slowest moving, most cathodic band, and CK-MB migrates intermediate to CK-MM and CK-BB.[1, 2, 3]

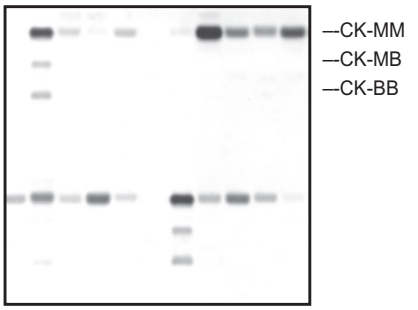


Figure 1: A representation of a QuickGel CK Vis Isoenzyme Gel showing the relative position of the CK Isoenzyme bands.

Calculation of the Unknown

The QuickScan Touch Plus densitometer will automatically calculate and print the relative percent and the absolute value for each band. Refer to the Operator's Manual provided with the instrument.

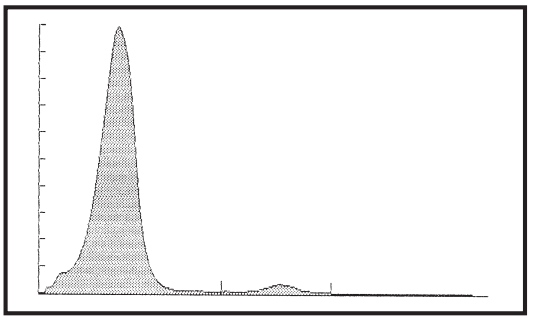


Figure 2: QuickGel CK Vis electrophoresis scan.

LIMITATIONS

The QuickGel CK Vis Isoenzyme Reagent is linear to 700 U/L total CK as determined with a UV kinetic method at 37°C. Results for sensitivity studies show that the CK Reagent is sensitive to 3 U/L.

NOTE: The QuickGel CK method is not designed to identify tumor markers.

Interfering Factors: Refer to SPECIMEN COLLECTION AND HANDLING.

Further Testing Required: Lactate dehydrogenase (LD) isoenzyme studies performed in conjunction with the CK isoenzymes provide a much more definitive test in the diagnosis of myocardial infarct.[2, 3]

INTERPRETATION OF RESULTS

CK-MM

1. Often the only isoenzyme of CK found in normal serum.[1]
2. Elevated in: (a) Skeletal muscle injury (b) Myocardial injury (c) Brain injury.[1, 3]

CK-MB

1. May be present in serum from normal subjects in the amount of 0 to 5%.[23] Note that small amounts of CK-MB activity have been interpreted as an alert to possible myocardial infarct and should be followed by serial CK and LD isoenzyme studies.
2. Positive indication of myocardial infarct when the following criteria are met:
   a. Proper clinical setting.[2]
   b. CK-MB activity > 5% of total CK activity and a minimum of 10 IU/L.[1, 14, 24]

c. CK-MB shows characteristic change in pattern (relatively rapid appearance and disappearance).[1, 3, 35]

3. Positive identification of second myocardial infarct: After the first MI the CK-MB increases after starting to decline. The total CK may or may not show an increase after starting to decline.
4. Values following open heart surgery[3]
   CK and LD isoenzymes are less specific following open heart surgery than in most diagnostic situations. The CK-MB will be elevated due to myocardial damage resulting from the operative procedure as well as trauma to the heart from manipulation and cannulation. The LD is flipped secondary to hemolysis from extra corporeal circulation. Infarct patients have higher levels of CK-MB activity, but the wide range of isoenzyme activity seen in non-MI patients overlaps that noted in patients with MI. This makes complete discrimination impossible. Despite this difficulty, accuracy in diagnosing MI can be increased by doing serial determinations of CK-MB in the post-operative period and analyzing its activity trend. Perioperative infarct patients will usually have a progressive rise in CK-MB levels, while non-MI patients exhibit a more precipitous post-operative decrease in that fraction.[2, 25]
5. Elevation in diseases other than myocardial infarct:[1, 3]

Severe coronary insufficiency	Dermatomyositis
Duchenne's muscular dystrophy	Myoglobinuria
Rocky Mountain Spotted Fever	Polymyositis
Rhabdomyolysis	Reye's Syndrome

CK-BB

1. Often seen in the serum of patients with prostatic carcinoma and occasionally in the serum of patients with other carcinomas and malignant tumors.[1]
2. Rarely seen in the serum of patients with brain injury due to damage to the blood-brain barrier.[1, 26]
3. Occasionally seen in the serum of patients with severe shock syndrome (probably due to lung or small bowel involvement).
4. Occasionally seen in the serum of patients with chronic renal failure, gastric cancer, women in labor, Reye's syndrome, oat cell carcinoma, and malignant hyperpyrexia.[1]

ATYPICAL CK BANDS

A number of atypical bands of CK have been reported. Atypical bands migrating between CK-MB and CK-MM have been attributed to CK-BB complexed to IgG[27, 28] and CK-MM complexed to lipoprotein,[29] as well as others without positive identification.[30-32] Mitochondrial CK migrates cathodically to CK-MM[33], and a band designated "macro" CK, isolated from a cancer patient, also migrated cathodic to CK-MM.[34]

PERFORMANCE CHARACTERISTICS

SPIFE

PRECISION

Within Run studies were done using one patient sample and one control run in replicate on one gel.

Control	Fraction	Mean	SD	CV%
N = 12				
	% MM	72.3	0.8	1.1
	% MB	12.3	0.5	3.9
	% BB	15.4	0.6	3.6
Patient	Fraction	Mean	SD	CV%
N = 8				
	% MM	85.5	0.5	0.6
	% MB	12.3	0.6	4.7
	% BB	2.2	0.2	9.2

Between Run studies were done using one patient sample and one control run in replicate on four gels.

Control	Fraction	Mean	SD	CV%
N = 48				
	% MM	70.7	1.5	2.1
	% MB	12.5	0.5	4.3
	% BB	16.7	1.2	6.9
Patient	Fraction	Mean	SD	CV%
N = 32				
	% MM	85.4	0.6	0.7
	% MB	12.3	0.6	4.8
	% BB	2.2	0.3	12.3

CORRELATION STUDIES

100 patient specimens were tested on the QuickGel CK Vis method and another commercially available product

N	= 100	Y = 1.023X - 1.922
Slope	= 1.023	X = SPIFE CK Vis
Intercept	= -1.922	Y = QuickGel CK Vis on SPIFE
R	= 0.9992	

QUICKGEL CHAMBER

PRECISION

Within Run studies were done using one control run in replicate on one gel.

Control	Fraction	Mean	SD	CV%
N = 20				
	% MM	69.8	0.8	1.1
	% MB	10.5	0.5	4.4
	% BB	19.7	0.4	2.3

Between Run studies were done using one control run in replicate on eight gels.

Control	Fraction	Mean	SD	CV%
N = 160				
	% MM	69.2	1.6	2.3
	% MB	11.1	0.8	7.6
	% BB	19.6	1.1	5.7

CORRELATION STUDIES

38 normal and abnormal patient specimens were tested on the QuickGel CK Vis method using the SPIFE and the QuickGel Chamber

N	= 38	Y = 1.0045X - 0.1478
Slope	= 1.0045	X = QuickGel CK Vis on SPIFE
Intercept	= -0.1478	Y = QuickGel CK Vis on QuickGel Chamber
R	= 0.9998	

LINEARITY

The systems have been validated to be linear to 700 U/L total CK.

SENSITIVITY

Results from validation studies show that the systems are sensitive to 3 U/L.

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