# SPIFE® 3000 CK Vis Isoenzyme Procedure For Plastic Blades

**Cat. No. 3332, 3333**

The SPIFE CK Vis Isoenzyme method is intended for the qualitative and quantitative analysis of the creatine phosphokinase isoenzymes in serum by agarose electrophoresis using the SPIFE 3000 system.

## 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 18-24 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 LD1 and LD2).[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 25-40% 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 Vis Isoenzyme Reagent.

The SPIFE CK Vis Isoenzyme Reagent (substrate) utilizes the following reactions:

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

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

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

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

**1. SPIFE CK Vis Isoenzyme 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 two vials of CK Vis Isoenzyme Reagent each with 1.5 mL of CK Isoenzyme 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 Vis Isoenzyme 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 Vis Chromogen**

**Ingredients:** 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 dissolved Reagent, invert several times 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 Vis 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 must be used to apply samples, electrophorese, apply reagent, incubate, wash 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°C to 37°C water bath for thawing. Repeated freezing and thawing destroys CK activity and should be avoided.

## PROCEDURE

**Materials Provided:** The following materials are provided in the SPIFE CK Vis Isoenzyme Kit. Individual items are not available.

| Sample Test Size | Cat. No. |
|---|---|
| 40 Sample | 3332 |
| 20 Sample | 3333 |

SPIFE CK Vis Isoenzyme Gels (10)
CK Vis Isoenzyme Reagent (20 x 1.5 mL)
CK Vis Isoenzyme Diluent (2 x 15 mL)
CK Vis Chromogen (2 x 1.5 mL)
CK Vis Activator (2 x 0.2 mL)
REP Blotter C (10)
Citric Acid Destain (1 pkg)
Blade Applicator Kit-20 Sample

**Materials provided by Helena but not contained in the kit:**

| | Cat. No. |
|---|---|
| SPIFE 3000 | 1088 |
| QuickScan Touch | 1690 |
| QuickScan 2000 | 1660 |
| Disposable Sample Cups (Deep Well) | 3360 |
| SPIFE Dispo Cup Tray | 3370 |
| CK/LD Control | 5134 |
| REP Prep | 3100 |
| Gel Block Remover | 1115 |
| SPIFE Reagent Spreaders | 3706 |
| Applicator Blade Weights | 3387 |

## STEP-BY-STEP METHOD

**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.

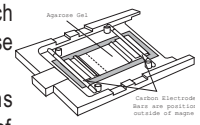
The new software version 1.20 has an automatic wash cycle prompted by initiation of a test which does not use the stainer unit for staining when the previous test did use the stainer for staining. To avoid delays after electrophoresis, this wash cycle should be initiated at least seven (7) minutes prior 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 incubation.

**I. 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 21-40 samples, remove two Applicator Blades from the packaging. If testing fewer samples, remove one Applicator Blade from the packaging.
3. Place the two Applicator Blades into the vertical slots in the Applicator Assembly identified as 8 and 14. If using one Applicator Blade, place it into either of the two locations noted above.
   **NOTE: The Applicator Blade will only fit into the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.**
4. Place an Applicator Blade Weight on top of each Applicator Blade. When placing the weight on the blades, position the weight with the thick side to the right
5. Slide the appropriate number of Disposable Cup Strips into the middle or bottom rows of the Cup Tray. If testing less than 21 samples, place the cups into the row that corresponds with applicator placement.
6. Pipette 75-80 µL of pretreated patient serum or control into each cup. Cover the tray until ready to use.

**II. Gel Preparation**

1. Remove the gel from the protective packaging and discard overlay. Using a REP Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. 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 no bubbles remain under the gel.
4. Clean and wipe the electrodes and the Reagent Spreaders with lint-free tissue.
5. Place a carbon electrode on the outer ledge of each gel block on the outside of the magnetic posts. Close the chamber lid.




6. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **CK** option appears on the displays.

**III. Preparation of Isoenzyme Reagent**

1. Reconstitute each of two vials of the CK Vis Isoenzyme Reagent with 1.5 mL of CK Vis Isoenzyme Diluent. Mix well by inversion. Do not add the Chromogen until ready to use as it can cause excess background on the gel.

**IV. Electrophoresis Parameters**

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

**Electrophoresis Unit**

| | | | | |
|---|---|---|---|---|
| 1) No Prompt<br>Load Sample 1 | 00:02 | 21°C | SPD6 | |
| 2) No Prompt<br>Load Sample 2 | 00:02 | 21°C | SPD6 | |
| 3) No Prompt<br>Load Sample 3 | 00:02 | 21°C | SPD6 | |
| 4) No Prompt<br>Load Sample 4 | 00:30 | 21°C | SPD6 | |
| 5) No Prompt<br>Apply Sample 1 | 1:00 | 21°C | SPD6 | LOC1 |

| | | | | | |
|---|---|---|---|---|---|
| 6) No Prompt | | | | | |
| Load Sample 5 | 00:30 | 21°C | SPD6 | | |
| 7) No Prompt | | | | | |
| Apply Sample 2 | 1:00 | 21°C | SPD6 | LOC1 | |
| 8) No Prompt | | | | | |
| Electrophoresis 1 | 4:30 | 13°C | 750 Volt | 65mA | |
| 9) Remove gel blocks (Continue) | | | | | |
| Apply Reagent 1 | | 37°C | 8 cycles | | |
| 10) No Prompt | | | | | |
| Incubate 1 | 20:00 | 45°C | | | |
| 11) No Prompt | | | | | |
| END OF TEST | | | | | |

**Stainer Unit**

| | | | |
|---|---|---|---|
| 1) No Prompt | | | |
| Destain 1 | 2:30 | REC=ON | VALVE=2 |
| 2) No Prompt | | | |
| Destain 2 | 2:30 | REC=ON | VALVE=2 |
| 3) No Prompt | | | |
| Wash 1 | 2:30 | REC=ON | VALVE=7 |
| 4) No Prompt | | | |
| Wash 2 | 2:30 | REC=ON | VALVE=7 |
| 5) No Prompt | | | |
| Dry 1 | 25:00 | 63°C | |
| 6) No Prompt | | | |
| END OF TEST | | | |

**V. Electrophoresis**

1. Open the chamber lid. Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Place a reconstituted vial of reagent (without chromogen) into each outer hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.
3. With **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.
4. Open the chamber lid, remove the electrodes and dispose of blades and cups as biohazardous waste.
5. With the gel still in the chamber, use a Gel Block Remover or straight edge to completely remove and discard the two gel blocks.
6. Use a lint-free tissue to wipe around the edges of the gel including the gel block area.
7. Place a Reagent Spreader rod (glass rod) across each end of the gel inside the magnetic posts.
8. Remove the reagent vials and add 150 µL of Chromogen to each vial of prepared Reagent. Invert several times to mix and replace immediately into each outer hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.

**VI. Incubation**

1. Press the **TEST SELECT/CONTINUE** button to apply reagent and start the incubation timer.
2. At the end of the incubation, the instrument will beep. Remove the gel from the chamber.
3. Attach the gel to the holder by placing the round hole in the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.
4. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
5. With **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 instrument will destain and dry the gel.
6. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer and scan the bands.

**VII. Evaluation of the CK Isoenzyme Bands**

1. **Qualitative evaluation:** The SPIFE CK Vis Isoenzyme Gel may be visually inspected for the presence of the bands.
2. **Quantitative evaluation:** Scan the SPIFE CK Vis Isoenzyme Gel in the Quick Scan Touch/2000 using the Acid Violet filter and a slit size of 5.

**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 | 96.7-100 |
| % MB | 0- 3.3 |
| % 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]

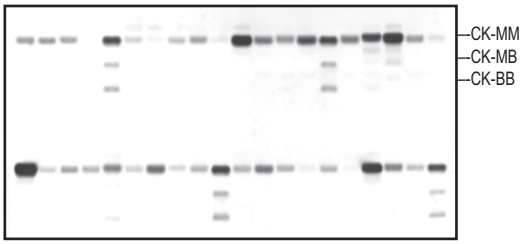



**Figure 1:** A representation of a SPIFE CK Vis Isoenzyme Gel showing the relative position of the CK Isoenzyme bands.

**Calculation of the Unknown**

The QuickScan Touch/2000 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.

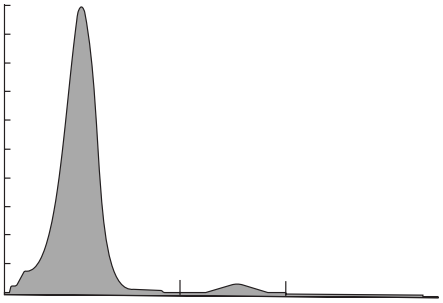


**Figure 2:** SPIFE CK Vis electrophoresis scan.

## LIMITATIONS

The 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 2.5 U/L.

**NOTE:** The 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 0-5%.[23,36] Note that although 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 U/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

## PRECISION

**Within Run** studies were run using one patient sample and one control run in replicate on one gel. n = 20

| Control | Fraction | Mean | SD | CV% |
|---|---|---|---|---|
| | % MM | 68.4 | 1.0 | 1.5 |
| | % MB | 12.8 | 0.5 | 3.6 |
| | % BB | 18.8 | 0.6 | 3.4 |
| Patient | Fraction | Mean | SD | CV% |
| | % MM | 83.1 | 0.7 | 0.8 |
| | % MB | 16.9 | 0.7 | 3.9 |

**Between Run** studies were done using one patient sample and one control run in replicate on four gels. n = 84

| Control | Fraction | Mean | SD | CV% |
|---|---|---|---|---|
| | % MM | 67.6 | 1.5 | 2.2 |
| | % MB | 13.4 | 0.8 | 6.3 |
| | % BB | 19.0 | 0.9 | 4.5 |
| Patient | Fraction | Mean | SD | CV% |
| | % MM | 82.1 | 1.0 | 1.2 |
| | % MB | 17.9 | 1.0 | 5.6 |

## CORRELATION STUDIES

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

| | | |
|---|---|---|
| n | = 100 | Y = 1.006X - 0.552 |
| Slope | = 1.006 | X = Reference method |
| Intercept | = -0.552 | Y = SPIFE CK Vis |
| R | = 0.9998 | |

## LINEARITY

The system has been validated to be linear to 700 U/L total CK.

## SENSITIVITY

Results from validation studies show that the system is sensitive to 2.5

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