C-KB

Often seen in the serum of patients with prostatic carcinoma and occasionally in the serum of patients with other carcinomas and malignant tumors.1

3. Rarely seen in the serum of patients with brain injury due to damage to the blood–brain barrier.1


ATYPICAL CK BANDS

A number of atypical bands of CK have been reported. Atypical bands migrating between CK-MM and CK-MB have been attributed to CK-MM complexed to lipoprotein2 as well as without others positive identification.3,4 Mitochondrial CK migrates cathodically to CK-MM5, and a band designated “macro CK,” isolated from a cancer patient, also migrated cathodically to CK-MB.

PERFORMANCE CHARACTERISTICS

PRECISION

The following studies were run using one patient sample and one control sample run in duplicate on one gel. N = 20

Control

Fraction Mean SD CV% % MM 63.6 0.5 0.8 % BB 22.6 1.2 5.4

Between Run studies were done using one patient sample and one control run in repli
cates on nine gels N = 160

Control

Fraction Mean SD CV% % MM 64.0 1.8 2.8 % BB 33.4 0.7 2.1 % BR 22.6 1.2 5.4

CORRELATION STUDIES

34 patient specimens were listed on the SPIFE CK Vis Method and SPIFE Touch CK Vis Method.

n = 34

Y = 0.0126X + 0.164

R² = 0.998

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 U/L.

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

In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damages. Helena’s liability shall be limited to repairing or replacing goods or to refunding to the Buyer the purchase price attributable to the goods as to which such claim is made. These alternatives shall be buyer’s exclusive remedies.

Ingredients:

- Adenosine 5'-diphosphate (ADP)
- G-6-PD
- Acetyl CoA
- Bovine Serum Albumin (BSA)
- Diadenosine pentaphosphate
- ADP + creatine phosphate  ATP + creatine
- D-G-6-P + NAD  NADH + 6 phosphogluconate + H
- ADP + creatine phosphate  ATP + creatine
- ATP + glycose  D-Glucose-6-phosphate + ATP
- D-G-6-P + NAD  D-Glucose-6-phosphate + NADH + H
- PMS + NAD

Additional enzyme studies in accurately diagnosing MI.

REFERENCES

1. SPIFE CK Vis Isoenzyme Gel

2. SPIFE CK Vis Isoenzyme Kit

3. SPIFE CK Vis Isoenzyme Control

4. SPIFE CK Vis Isoenzyme Reagent

5. SPIFE CK Vis Isoenzyme Procedure

6. SPIFE CK Vis Isoenzyme Gel

7. SPIFE CK Vis Isoenzyme Kit

8. SPIFE CK Vis Isoenzyme Control

9. SPIFE CK Vis Isoenzyme Reagent

10. SPIFE CK Vis Isoenzyme Procedure

The diluent contains MES, sucrose, Triton X and sodium azide added as a preservative.

For additional enzyme studies in accurately diagnosing MI. The most important con
densation in the interpretation of CK localization and pattern detection is the characteristic change of pattern of multiple examinations (the relatively fast appearance and disappearance of CK-MB and the flip of LD1 and LD2).1,14 Persistent elevation in CK-MB is not indicative of myocardial infarction. CK-MB may be helpful in diagnosing a small infarct in which total CK never exceeds the upper limit of normality.

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 the CK-MB is present in 100% of the patients with MI as well as in some cases of severe coronary insufficiency.

Definitive testing laboratory in the diagnosis of MI is accomplished by performing studies of CK isoenzymes in conjunction with lactate dehydrogenase (LD) isoenzymes.10 The specificity and sensitivity achieved with these two tests eliminated the necessity for additional enzyme studies in accurately diagnosing MI.10,11 The most important con

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**PROCEDURE**

**STAINER UNIT**

The QuickScan Touch/2000 densitometer will automatically calculate and print the relative and absolute value for each band. Refer to the Operator’s Manual provided with the instrument.

**Step-by-Step Method**

**NOTE:** The lab technician should be trained in the use of the densitometer before using it for the first time.

**STEP 1: Preparatory Steps**

1. Reconstitute each of two vials of the CK Vis Isoenzyme Reagent with 1.5 mL of SPIFE Reagent Spreaders.

2. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.

3. Attach the gel to the holder by placing the round hole in the gel mylar over the left cathodic band, and CK-MB migrates intermediate to CK-MM and CK-BB.

4. The QuickScan Touch/2000 densitometer will automatically calculate and print the relative and absolute value for each band. Refer to the Operator’s Manual provided with the instrument.

**INTERPRETATION OF RESULTS**

**CK-MM**

- Often the only isoenzyme of CK found in normal serum.
- Small amounts of CK-MB activity have been interpreted as an alert to possible myocardial ischemia.

**CK-MB**

- More sensitive and specific than CK-MM for detection of myocardial infarction.

**MB**

- More specific than CK-MB for detection of myocardial necrosis.

**MB**

- More specific than CK-MB for detection of myocardial necrosis.

**REFERENCES**

Range

<table>
<thead>
<tr>
<th>% MM</th>
<th>CK-MB</th>
<th>CK-BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63-100</td>
<td>3-33</td>
</tr>
</tbody>
</table>

**NOTE:** The CK-MB increases after starting to decline. The total CK may or may not show an increase after starting to decline.

**VALUES following open heart surgery**

- CK-MB and CK-LD 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. The LD is flipped secondary to hemolysis from extra corporeal circulation. The CK-MB activity, 5% of total CK activity and a myoglobinuria (C) brain injury.

**REFERENCES**

Range: 50-200 IU/L

<table>
<thead>
<tr>
<th>% MM</th>
<th>CK-MB</th>
<th>CK-BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63-100</td>
<td>3-33</td>
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The following materials are provided in the SPIFE CK Vis Isoenzyme kit. Individual items are not available.

Sample Test Qty Cat. No.
40 Sample 3332
20 Sample 3333

SPIFE CK Vis Isoenzyme Gels (10) 20 Sample 3333
CK Vis Isoenzyme Diluent (2 x 1.5 mL) 20 Sample 3333
CK Vis Chromogen (2 x 1.5 mL) 20 Sample 3333
CK-Vis Activator (2 x 0.2 mL) 20 Sample 3333
REP Brotter C (1) 20 Sample 3333
Citrin Add:Den-1 (1 pkg) 20 Sample 3333
Blade Applicator Kit - 20 Sample 3333

Materials provided by Helena but not contained in the kit:

Item Cat. No.
SPIFE Touch 1158
Quick Scan Touch 1690
Quick Scan 2000 1690

Disposable Sample Cups (Deep Well) 3360
SPIFE 20, 40, 60 Deep Cup Tray 3370
CKD Control 5134
REP Prep 3100
Gel Block Remover 1115
SPIFE Reagent Spreader 3706
Applicator Blade Weights 3387

STEP-BY-STEP METHOD

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 SPIFE Touch CK test. To verify the status of the staining chamber, use the arrows under the STAINER UNIT to select the appropriate test, place the empty Gel Holder into the staining chamber and place the chamber lid. If washing of the staining chamber is necessary, the prompt “wash is needed” will appear. Use Prompt RETRY to begin the staining wash. The cleaning process will complete automatically in about seven minutes. To avoid delays after incubation, this wash cycle should be initiated at least 10 minutes prior to the end of the run.

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. Load 21-40 samples, removing two disposable Applicator Blades from the packaging. If loading 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 slots noted above.

NOTE: The Applicator Blade will only fit into the slots one way; do not try to force the blades into the slots.

4. Place the Applicator Blade weights on top of each Applicator Blade. When placing the weight on the blades, position the weight with the flat 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. Qualification Protocol

1. Remove the gel from the protective packaging and discard overlay.

2. Place a REP Brotter C, with the longer end parallel with the gel blocks, into the SPIFE Touch. The REP Brotter is removeable. Place the REP Brotter and the blodter.

3. Disassemble the ISOENZYME 2 mL of REP Prep onto the left side of the electrophoresis chamber.

4. Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently tilt the entire surface of the gel using slight finger pressure on the borders and remove the blodter.

5. Position the correct ISOENZYME 2 mL of REP Prep into the slots of the electrophoresis chamber.

6. Place the left edge of the gel over the Prep aligning the round hole on the left pin of the chamber. Gently tilt the entire surface of the gel using slight finger pressure on the borders and remove the blodter.

7. Dispose of the ISOENZYME 2 mL of REP Prep and wash the electrodes and the Reagent Spreader with lint-free tissue. Close the chamber lid.

8. Preheating of the Reagent Spreader

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 Chemstrip until ready to use as it can cause excess background on the gel.

IV. Electrophoresis/Visualization

Use the instructions provided in the Operator’s Manual, set up the parameters as follows for the SPIFE Touch.

<table>
<thead>
<tr>
<th>Step</th>
<th>Prompt</th>
<th>Time</th>
<th>Temperature</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>None</td>
<td>0:00</td>
<td>Temperature: 21°C</td>
<td>Speed: 6</td>
</tr>
<tr>
<td>2.</td>
<td>None</td>
<td>0:00</td>
<td>Temperature: 21°C</td>
<td>Speed: 6</td>
</tr>
<tr>
<td>3.</td>
<td>None</td>
<td>0:00</td>
<td>Temperature: 21°C</td>
<td>Speed: 6</td>
</tr>
<tr>
<td>4.</td>
<td>None</td>
<td>0:00</td>
<td>Temperature: 21°C</td>
<td>Speed: 6</td>
</tr>
</tbody>
</table>

Applicator Blade Location

1. Prompts: None
2. Prompt: None
3. Prompt: None
4. Prompt: None
5. Prompt: None
6. Prompt: None

Electrophoresis

<table>
<thead>
<tr>
<th>Step</th>
<th>Prompt</th>
<th>Time</th>
<th>Temperature</th>
<th>Voltage</th>
<th>Voltage</th>
<th>Voltage</th>
<th>Voltage</th>
<th>Voltage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>None</td>
<td>0:30</td>
<td>Temperature: 13°C</td>
<td>75 Volts</td>
<td>270 Volts</td>
<td>65 mA</td>
<td>45 mA</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Prompt: Remove Gel Blocks</td>
<td>0:30</td>
<td>Temperature: 37°C</td>
<td>Cycles: 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Prompt: None</td>
<td>0:00</td>
<td>Temperature: 45°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 calidad is not variance 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. 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 quantification. 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

Range: 667-700

% MM: 0-33

% BB: 667-700

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

RESULTS

CK-MB 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.

Calculation of the Uncertainty

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.

Calibration

The QuickScan Touch 2000 will be calibrated using a standard curve generated from a known concentration of CK-MM isoenzyme activity, a known concentration of CK-MB isoenzyme activity, and a known concentration of CK-BB isoenzyme activity.

INTERPRETATION OF RESULTS

CK-MM

1. Often the only isoenzyme of CK found in normal serum.

2. Elevated in: (a) Skeletal muscle injury (b) Myocardial injury (c) Brain injury.

CK-MB

1. May be present in serum from normal subjects in the amount of 0-5%.

2. May be present in serum from normal subjects in the amount of 0-5%.

3. Positive indication of myocardial infarct when the following criteria are met:

Figure 2: A representation of a SPIFE CK Vis Isoenzyme Gel showing the relative position of the CK isoenzyme bands.

Figure 2: SPIFE CK Vis electrosorption 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 Details (Required for Proper Location of the Isoenzyme Bands) and Additional controls may be required for federal, state or local regulations.

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The SPIFE Touch CK Vis Isoenzyme System

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

\[
\text{D-G-6-P + NAD} \rightarrow \text{NADH + 6 phosphogluconate + H}_2\text{O}
\]

The isoenzymes of CK are separated according to their electrophoretic mobility on agarose gels loaded with the SPIFE CK Vis Isoenzyme Reagents. To prevent the formation of toxic vapors, sodium azide should not be mixed with alkaline phosphatase. The Activator should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

Storage and Stability: The CK Reagents should be stored at 2 to 8°C until the expiration date indicated on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

1. CK Isocratic Label

Ingredients: The activator contains NIT (Nitrosoimidazole) in 3% Tween.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

2. CK Activator

Ingredients: The activator contains 144 ml of BME (Bovine Methyl Alcohol) in 3% Tween.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

3. CK Isocron Label

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

4. CK Chromogen

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

5. G-6-PD

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

6. Glucose 6-phosphate dehydrogenase

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

7. TBA

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

8. glucose 6-phosphate

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.

9. D-glucose

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INJECT.

Preparation for Use: The CK is ready for use on packaging.

Storage and Stability: The CK should be stored at 2 to 8°C and is stable until the expiration date on the vial.

Signs of Deterioration: Discard if the IVT deteriorates or changes in color.