The product should be discarded if it shows L.mesenteroides.

Refer to the diluent if it shows signs of bacterial contamination, as surgical procedures, intramuscular injections and myocardial infarct induce increased CK activity in the serum. The source of elevated CK activity may be isolated primarily from skeletal muscle), CK-MB (myocardium) and CK-BB (primarily from the brain). CK-BB is one of the important procedures used in the detection of myocardial damage. 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.

Definitive laboratory testing in the diagnosis of MI is accomplished by performing studies of CK isoenzymes in conjunction with lactate dehydrogenase (LD) activity. This specificity and sensitivity achieved with these tests has diminished the necessity for additional enzymes in studies accurately diagnosing MI.

The most important consideration in the interpretation of CK and LD isoenzyme analysis is the detection of the characteristic change of pattern of multiple examinations (the relatively fast appearance and disappearance of CK-MB and the fall of LD1 and LD2). Persistent elevation in CK-MB is not indicative of myocardial infarction but may be helpful in diagnosing MI in which total CK never exceeds the upper limit of normal. CK produced by myocardium is only 25-40% CK-MB, the remainder being CK-MM (isolated primarily from skeletal muscle), CK-MB (myocardium) and CK-BB (primarily from the brain). The isoenzymes of CK have been assessed by various methods.

The specificity of the isoenzymes of CK are separated according to their electrophoretic mobility on agarose gel electrophoresis. The specificity and sensitivity achieved with these tests has diminished the necessity for additional enzymes in studies accurately diagnosing MI.

The specificity of the isoenzymes of CK are separated according to their electrophoretic mobility on agarose gel electrophoresis. The specificity and sensitivity achieved with these tests has diminished the necessity for additional enzymes in studies accurately diagnosing MI.
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 unit for staining. To avoid delays after electrophoresis, this wash cycle should be initiated at least 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” prompt will appear, followed by “Place out, Holder in” prompts. Press “Continue” to begin the stainer wash. The cleaning process will complete automatically in about 5 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 aligned as 1 and 4. 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 Stips 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 overwrap. Using a REP Blocker C, gently blot the entire gel using slight fingertip pressure on the surface.

2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.

3. Place the left blade 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, lifting the obround hole off the right pin. Use lint-free tissue to wipe around the edges of the plastic gel block, especially next to electrode ports, to remove excess REP Prep. Make sure no bubbles remain under the gel.

4. Clean and wipe the electrodes and the Reactor Spreader with lint-free tissue.

5. Place a carbon electrode on the outer edge 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 parameters provided in the appropriate Operator’s Manual, set up the electrophoresis parameters as follows for the SPIFE 3000:

Electrophoresis Unit

1) No Prompt

Load Sample 1 00:02 21°C SPD6

2) No Prompt

Load Sample 2 00:02 21°C SPD6

3) No Prompt

Load Sample 3 00:02 21°C SPD6

4) No Prompt

Load Sample 4 00:02 21°C SPD6

5) No Prompt

Apply Sample 1 00:00 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 4:30 13°C 750 Volt 65mA

9) Remove gel blocks (Continue) Apply Reactant 1 37°C 8 cycles

10) No Prompt

Apply Sample 3 20:00 45°C SPD6

I. End of Test

Stainer Unit

1) No Prompt

Wash 1 2:30 REC=ON VALVE=2

2) No Prompt

Wash 2 2:30 REC=ON VALVE=2

3) No Prompt

Wash 3 2:30 REC=ON VALVE=2

4) No Prompt

Dry 1 25:00 63°C

5) No Prompt

Dry 2 25:00 63°C

6) No Prompt

END OF TEST

V. Electrophoresis

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.

1. Place a solidified 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.

2. When the channel tray is cleaned, 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, electrophoresis and begin the wash cycle.

3. Open the chamber lid, remove the electrodes and dispose of blades and reagent vials. Place the waste into designated waste containers in the laboratory.

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 including the gel block area.

6. Place a Reactant Spreader rod (glass rod) across each end of the gel inside the magnetic post.

7. Remove the reagent vials and add 150 µL of Chromogen to each vial of prepared Reagent. Insert 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 on the right pin on the holder.

4. Place the Gel Holder with the attached gel facing backwards into the Stainer unit. If cleaning is required, the “Wash” prompt will appear, followed by “Place out, Holder in” prompts. Press “Continue” to begin the stainer wash. The cleaning process will complete automatically in about 5 minutes. The unit is then ready to process the gel after incubation.

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.

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/CKL 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 or an indicator of the isoenzyme bands and may also be quantitative to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay variables. 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:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>% MM</td>
<td>967.100</td>
</tr>
<tr>
<td>% MB</td>
<td>0.3-3.0</td>
</tr>
<tr>
<td>% BB</td>
<td>0.1</td>
</tr>
</tbody>
</table>

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

RESULTS

CK-MM is the fastest moving, most anodic band, and 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 (LDH) and isoenzyme studies performed in conjunction with the CK isoenzymes provide a much more definitive test in the diagnosis of myocardial infarct.1,2,3

INTERPRETATION OF RESULTS

U1

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

2. Elevated in: (a) Skeletal muscle injury (b) Myocardial injury (c) Brain injury1,2,3
To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When dispensing 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**

1. A SPIFE 3000 Analyzer
2. QuickScan Touch/2000

**REQUIRED MATERIALS**

- Sodium Azide Warning
- Destain at 15 to 30°C. It is stable until the expiration date on the package.

**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

**Sample Preparation**

1. Add a 1:10 dilution to 150 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 lower samples, remove one Applicator Blade from the packaging.
3. Place the two Applicator Blades into the vertical slots in the Applicator Assembly and select A & B if using one Applicator Blade, place it into either of the two locations noted above.
4. NOTE: The Applicator Blade will only fit into the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.
5. 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.
6. Place a carbon electrode on the outer ledge of each Cup Tray. Gently lay the gel down on the REP Prep, making sure the electrodes are inserted into the magnetic posts.
7. Clean and wipe the electrodes and the Reagent Spreaders with lint-free tissue.
8. Place an Applicator Blade on top of each Applicator Blade. When placing the weight on the blades, position the weight with the thick side to the right.
9. Place a Reagent Spreader rod (glass rod) across each end of the gel inside the electrophoresis chamber.
10. Open the chamber lid, remove the electrodes and dispose of blades and electrodes. Replace the gel block area.
11. Load Sample 1 00:02 21°C SPD6

**Calibration**

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

**Staining and Drying**

- 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.

**Limitations**

The CK Vis Isoenzyme Reagent is linear to 700 U/L total CK. The CK Vis method is not designed to identify tumor markers.

**Interfering Factors**

- Refer to SPECIMEN COLLECTION AND HANDLING.

Further Testing Required: Lactate dehydrogenase (LDH) levels, CK-II levels, and CK-MB levels may be required as a marker of myocardial infarction. The CK Isoenzyme bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay validity. 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:

- % MM
- % MB
- % BB

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.

**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.

**Further Testing**

- Lactate dehydrogenase (LDH) levels, CK-II levels, and CK-MB levels may be required as a marker of myocardial infarction. The CK Isoenzyme bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay validity. Additional controls may be required for federal, state or local regulations.

**INTERPRETATION OF RESULTS**

**GENERAL**

- Only the CK isoenzyme of CK found in normal serum.

**2,3**

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

**4,5**

- Elevated in: (d) Myocardial injury (e) Periventricular leukomalacia (f) Cardiac surgery

**LIMITATIONS**

The CK Vis Isoenzyme Reagent is linear to 700 U/L total CK. The CK Vis method is not designed to identify tumor markers. 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.
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