Intended Use
For the quantitative determination of creatine kinase activity in serum.
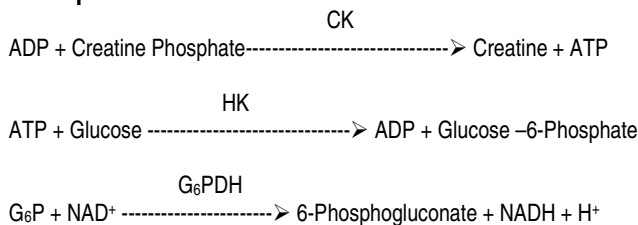
Clinical Significance
Creatine kinase (CK) is primarily found in skeletal muscle, cardiac muscle and brain tissue. Damage to any of these tissues may result in increased levels of CK activity in serum. Cardiac muscle damage following myocardial infarction usually results in an increase to 7-12 times the upper limit of normal within 18 to 30 hours of infarction. Elevated CK activity is also noted in hypothyroidism, various types of muscular dystrophy, viral myositis and similar types of skeletal muscle diseases.[1] The determination of serum CK activity is used to aid in the diagnosis of myocardial infarction and various types of muscle disease.

Method History
The first procedure for determining CK, based on the rate of ATP formation, was presented by Oliver in 1955.[2] A modified method was described by Nielson and Ludvigsen[3] in 1963 with Rosalki[4] adding a sulfhydryl compound and AMP in 1967 to assure maximum CK activity and inhibit adenylate kinase activity. Optimized conditions for measuring CK were published by Szasz[5] in 1976 as well as by the Scandinavian Committee on Enzymes.[6] The above procedure was modified again in 1979[7] to include EDTA. The present reagent is modification of the above revision.

Principle

$$ADP + Creatine\ Phosphate \xrightarrow{\quad CK \quad} Creatine + ATP$$

$$ATP + Glucose \xrightarrow{\quad HK \quad} ADP + Glucose\ –6\text{-}Phosphate$$

$$G_6P + NAD^+ \xrightarrow{\quad G_6PDH \quad} 6\text{-}Phosphogluconate + NADH + H^+$$

CK catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed, to produce ADP and glucose-6-phosphate (G_6P). The G_6P is oxidizes to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK activity.

Reagents
After combining R1 and R2 the reagent contains: Creatine Phosphate 30mM, ADP 2mM, AMP 5mM, NAD 2mM, NAC 20mM, Hexokinase (microbial) 2500 U/L, G_6PDH (microbial) 2000 U/L, D-Glucose 20mM, Magnesium Ions 10mM, EDTA 2.0mM, Diadenosine Pentaphosphate 10uM, Buffer 100mM, pH 6.7±0.1, non-reactive stabilizers and fillers with sodium azide (0.05%) as preservative.

Reagent Preparation
Reagents are supplied as ready to use liquids. To prepare working reagent mix 5 parts of R1 with 1 part R2.

Reagent Storage
1. Store R1 and R2 at 2-8°C. If stored as directed the reagents are stable until the expiration date.

2. Working reagent is stable for twenty-four hours at room temperature (18-25°C) or 14 days at 2-8°C.

Reagent Deterioration
Do not use if:
1. Bacterial contamination is evident (turbidity).
2. The reconstituted reagent has an absorbance greater than 0.700 at 340 nm against water.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagents may be irritating to skin. Flush skin with water if contacted.
3. This reagent contains sodium azide (0.05%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Handling
1. Serum is the specimen of choice. Rosalki has reported that plasma (Heparin or EDTA) may be used.[4]
2. Red cells have very little CK, therefore slight hemolysis is acceptable.[8,4]
3. CK in serum is stable for forty-eight hours at room temperature (18-25°C) and seven days refrigerated (2-8°C). The sample may be frozen for up to one month when protected against evaporation.[4]
4. Strenuous exercise or physical activity can produce elevated levels of CK in serum.[1]

Interferences
1. Certain drugs and medications may affect the activity of CK, see Young, et al.[9]
2. Elevated levels of bilirubin (20mg/dl) and hemoglobin (500mg/dl) have been found to have negligible effect on this assay.

Materials Provided
CK R1 and R2 Reagent.

Materials Required but not Provided
1. Test tubes/rack
2. Pipetting devices
3. Spectrophotometer with the ability to read at 340nm
4. Timer
5. Heating Block (37°C)

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Prepare working reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm at 37°C for five minutes.
3. Zero spectrophotometer with water at 340nm.
4. Transfer 0.025ml (25ul) of sample to reagent, mix and incubate at 37°C for two minutes.
5. After two minutes, read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes.
6. Calculate the average absorbance difference per minute ΔAbs./min.
7. The ΔAbs./min multiplied by the factor 6592 (see Calculations) will yield results in IU/L.

Procedure Notes

1. Samples with values above 1500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. If the spectrophotometer being used is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

Limitations

1. The present procedure measures total CK, regardless of its source.
2. Samples with values above 1500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calibration[10,11]

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the described test conditions. Refer to the "Calculations" section for information on the calculation of the enzyme activity of the sample.

Calculations

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined conditions.

$$U/L = \frac{\Delta Abs./min \times 1.025 \times 1000}{1 \times 6.22 \times .025} = \Delta Abs./min \times 6592$$

Where: ΔAbs./min = Average absorbance change per minute
1.25 = Total reaction volume
1000 = Conversion of U/ml to U/L
1 = Light path in cm
6.22 = Millimolar absoptivity of NADH
.025 = Sample volume in ml

Example: If your average absorbance change per minute is .01, then .01 x 6592 = 66 IU/L

Note: If any of the test parameters are changed, a new factor has to be determined using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply U/L by 16.67

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal creatine kinase values. These conditions should be run at least with every working shift in which creatine kinase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values[12]

The following values are based on measurements performed at 37°C.
Males: Up to 160 U/L
Females: Up to 130 U/L
Newborns: 2 to 3 times adult values
It is strongly recommended that each laboratory establish its own reference range.

Performance

1. Linearity: 1500IU/L

2. Correlation: Studies done between this procedure and a similar procedure yielded a correlation coefficient of 0.999 with a regression equation of
3. y=1.07 x - 5.6. (n=103, range=25-2047 IU/L)
4. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
152	1.3	0.9	155	1.6	1.0
483	1.7	0.4	486	2.2	0.5

5. Sensitivity: Under the reaction conditions described, 1 U/L of creatine kinase gives a ΔAbs./min of 0.00015.

References

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