ELISA Quantitation of Creatine Kinase Isoform MM

A-108

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BACKGROUND

Creatine Kinase (CK) is a dimeric enzyme that exists as three isoforms: BB, MM and MB. These isoforms are found in brain tissue, skeletal muscle and cardiac muscle, respectively. Levels of CK-MM are elevated in the serum of patients with muscular dystrophy. Elevated levels of CK-MM can be used as a biomarker for muscular dystrophy screening.

METHODS

A two-site sandwich immunoassay was developed with two commercially available anti-human CK-MM monoclonal antibodies (MAb). The capture MAb was passively coated to a 96-well microtiter plate. The open sites on the plate were blocked with a nonreactive protein solution. A second MAb was biotinylated and paired with streptavidin-horseradish peroxidase (HRP) conjugate for detection. Human CK-MM antigen was used as the substrate for determining linear range, limit of detection (LOD), limit of quantification (LOQ) and precision. Dilution linearity and spike recovery assessments were performed with human serum specimens (n=3) and sample diluent (n=3) prepared with human CK-MM antigen. Human CK-MB and CK-BB antigens were used to assess cross-reactivity at concentrations ranging from 0.20 ng/mL to 800 ng/mL. Dilutions of human CK-MM in sample diluent (n=5) were used to assess precision (intra-assay and inter-assay) and were assayed in four replicates on five different days. Recovery samples were diluted 100-fold and spiked with human CK-MM antigen.

RESULTS

Precision			
Sample Type	**Average [C] (ng/mL)**	**Average Intra-run %CV**	**Inter-Run %CV**
Diluent	50.8	10.5	14.0
Diluent	10.4	5.7	10.1
Diluent	2.8	4.5	12.8
Diluent	0.9	5.3	16.3
Diluent	0.2	9.9	17.2

Dilution Linearity			
Sample Type	**Dilution**	**Average % Recovery**	**% Recovery Range**
Serum (n=3)	20-fold	104.8	100.1-109.9
	40-fold	104.4	102.6-105.7
	80-fold	102.5	100.1-106.2
	160-fold	101.6	99.5-102.7
Diluent (n=3)	20-fold	94.9	92.8-97.5
	40-fold	97.9	94.5-102.3
	80-fold	98.0	96.8-99.4
	160-fold	101.7	99.9-104.2

Spike Recovery			
Sample Type	**Spike [C] (ng/mL)**	**Average % Recovery**	**% Recovery Range**
Serum (n=3)	0.8	67.5	65.5-70.3
	8.1	89.7	85.6-95.0
	16.1	95.6	94.6-96.8
Diluent (n=3)	0.8	73.8	72.2-75.6
	8.1	95.6	88.2-110.0
	16.1	92.4	90.1-94.6

The standard curve was generated via the 5-parameter logistic model within the plate reader software. This assay exhibited a linear range (R^2=0.9854) from 0.08 ng/mL to 80 ng/mL. The LOD was 0.02 ng/mL. The LOQ was 0.14 ng/mL. Intra-assay variation (repeatability) ranged from 4.5% to 10.5% with a mean of 7.2% (n=25). Inter-assay variation (reproducibility) ranged from 10.1% to 17.2% with a mean of 14.1% (n=25). Percent recovery for dilution linearity ranged from 92.8% to 109.9% with a mean of 103.3% (n=6). Spike recovery ranged from 65.5% to 110.0% with a mean of 85.8% (n=6). Cross-reactivity with human CK-MB averaged 8.7%. Cross-reactivity with human CK-BB was not observed.

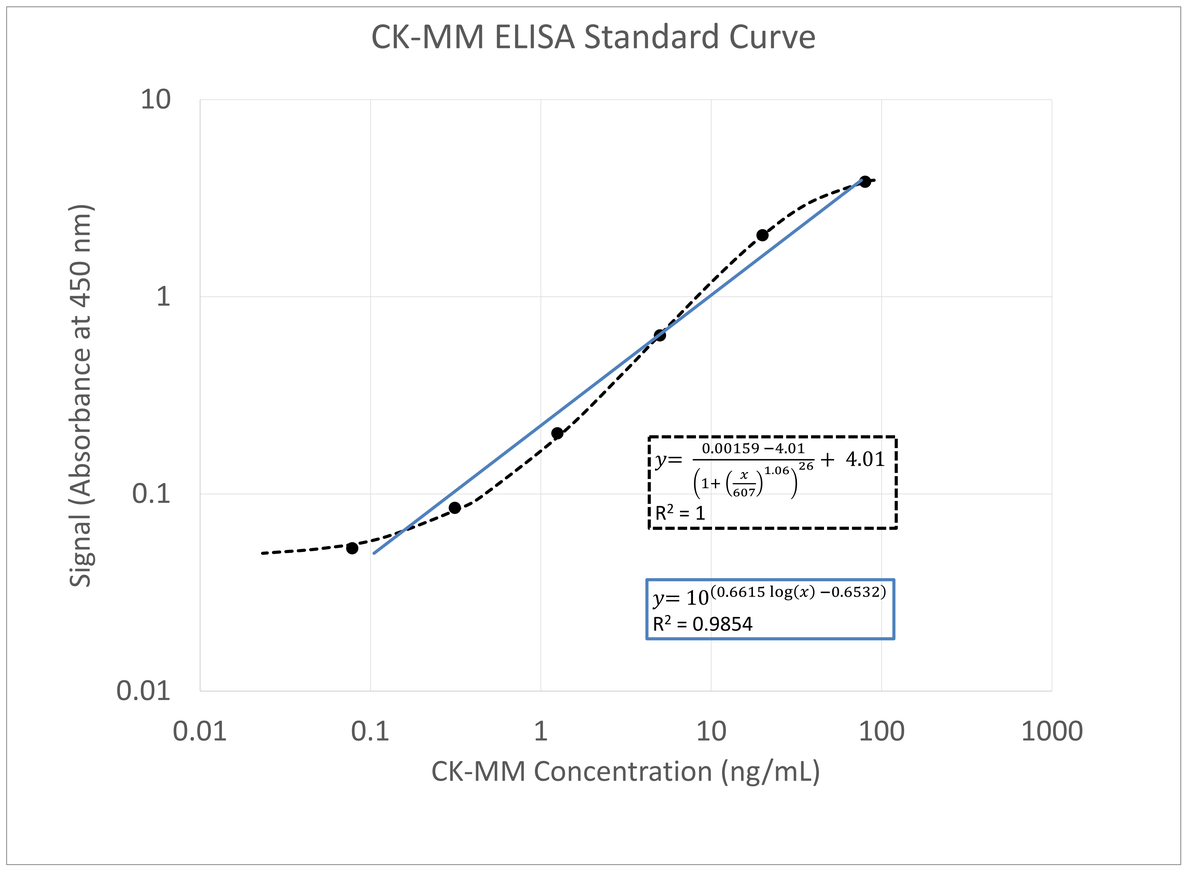

Figure 1: CK-MM Standard Curve with 5-parameter logistic fit (black dashed line) and log-log transform linear fit (blue solid line).

CONCLUSIONS

This study demonstrates that human CK-MM concentrations can be determined with a highly sensitive non-competitive ELISA without reliance upon measurement of CK enzymatic activity. It should be possible to use this method for muscular dystrophy screening in newborns, monitoring disease progression or monitoring response to therapy.