

Evaluation of a Creatine Kinase-MM Assay for Use in Newborn Screening for Duchenne Muscular Dystrophy

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Objective

The NYS Newborn Screening program, in collaboration with Parent Project Muscular Dystrophy (PPMD) and PerkinElmer (PKI, Waltham, Mass), investigated the use of the GSP® Neonatal Creatine Kinase-MM kit as a first-tier screen to determine the feasibility of performing newborn screening for Duchenne Muscular Dystrophy.

Introduction

Creatine kinase (CK) activity is increased in asymptomatic individuals with Duchenne Muscular Dystrophy leading to the use of CK enzyme activity as a marker for Duchenne. However, existing tests for CK activity are nonspecific in that they measure total enzyme activity whereas it is the CK-MM isoform that is significantly increased in the serum of patients with muscular dystrophies. PerkinElmer (PKI, Waltham, Mass) has developed a standardized and automated high-throughput immunoassay, which measures the concentration of CK-MM in dried blood spots (DBS). We investigated the use of the GSP® Neonatal Creatine Kinase-MM kit as a first-tier screen to determine its suitability in detecting Duchenne in newborns.

Methods

The GSP Neonatal CK-MM assay is a solid phase, 2-site immunofluorometric assay for the quantitative measurement of CK-MM in dried blood spots using the GSP instrument. We investigated the performance of the assay by determining inter-assay and intra-assay reproducibility and stability. In addition, after obtaining consent and IRB-approval we tested 15 NBS specimens from known Duchenne patients. As part of assay validation and cut-off determination, de-identified specimens from 8,621 different babies were screened.

Results

Validation studies indicated that intra-assay and inter-assay reproducibility were <6% showing acceptable reproducibility (Table 1). CK-MM stability after one week of storage at 4°C was not significantly affected. All 15 specimens from known Duchenne patients had elevated CK-MM values (Table 2). The CK-MM concentration and frequency distribution were determined for 8,621 newborns (Figures 1 and 2). Data from screening specimens from these newborns indicated that mean CK-MM values are significantly higher at earlier collection times and decrease with increasing age of collection (Figure 3a). The mean CK-MM values were slightly lower for female newborns than male newborns (Figure 3b). Of the 8,621 newborns screened, three with significant CK-MM elevation were detected (2 females with CK-MM values of 5690 and 5430 ng/ml; 1 male with CK-MM >7040 ng/ml)(Table 3).

Table 1. Intra-assay and Inter-assay Reproducibility of the GSP Neonatal CK-MM Kit

Standard	Intra-assay Reproducibility (%CV)	Inter-assay Reproducibility (%CV)
Low (104 ng/ml)	5.8%	3.1%
Medium (476 ng/ml)	4.9%	4.7%
High (1780 ng/ml)	4.9%	5.2%

For intra-assay reproducibility 60 points were tested, in duplicate, for each standard. For inter-assay reproducibility 12 points were tested, 10 times, for each standard.

Table 2. CK-MM Values of Confirmed Duchenne Patients Tested Retrospectively

Birth weight (g)	Age at collection (days)	Sex	Birth Year	CK-MM (ng/ml)	CK-MM (ng/ml)
3370	2	M	2008	>7040	6560
3620	3	M	2009	>7040	>7040
3485	2	M	2009	2050	1830
4230	2	M	2010	>7040	>7040
3460	2	M	2012	>7040	>7040
3210	1	M	2013	>7040	>7040
3145	1	M	2013	>7040	6850
3360	1	M	2014	6530	>7040
3367	2	M	2014	>7040	>7040
4238	1	M	2015	6430	4870
3620	2	M	2009	4968	5696
3602	2	M	2009	3643	4289
3599	3	M	2013	2039	2165
3311	2	M	2014	>7040	>7040
3600	2	M	2015	>7040	>7040

Cut-off determination for the assay must take into account the lowest CK-MM values obtained for confirmed Duchenne patients.

Figure 1. CK-MM Values of 8,621 Blinded Specimens

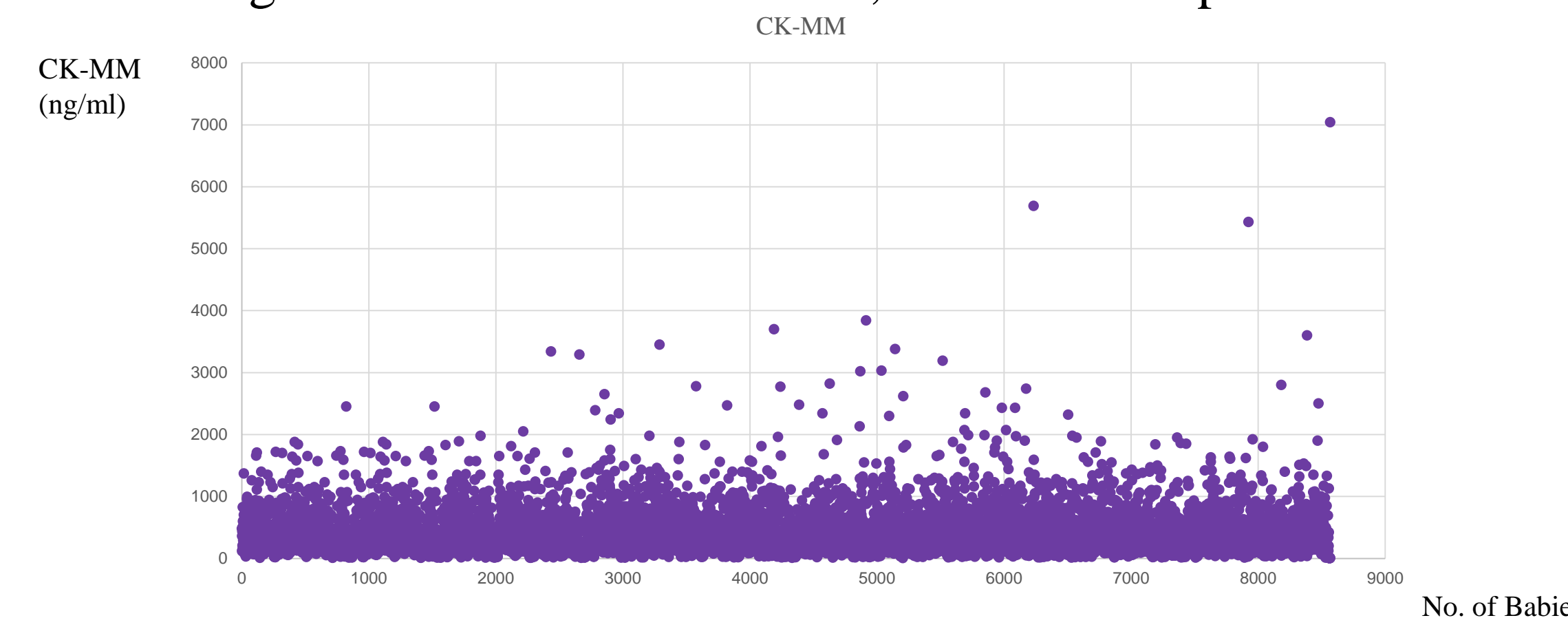


Figure 2. CK-MM Frequency Distribution for 8,621 Blinded Specimens

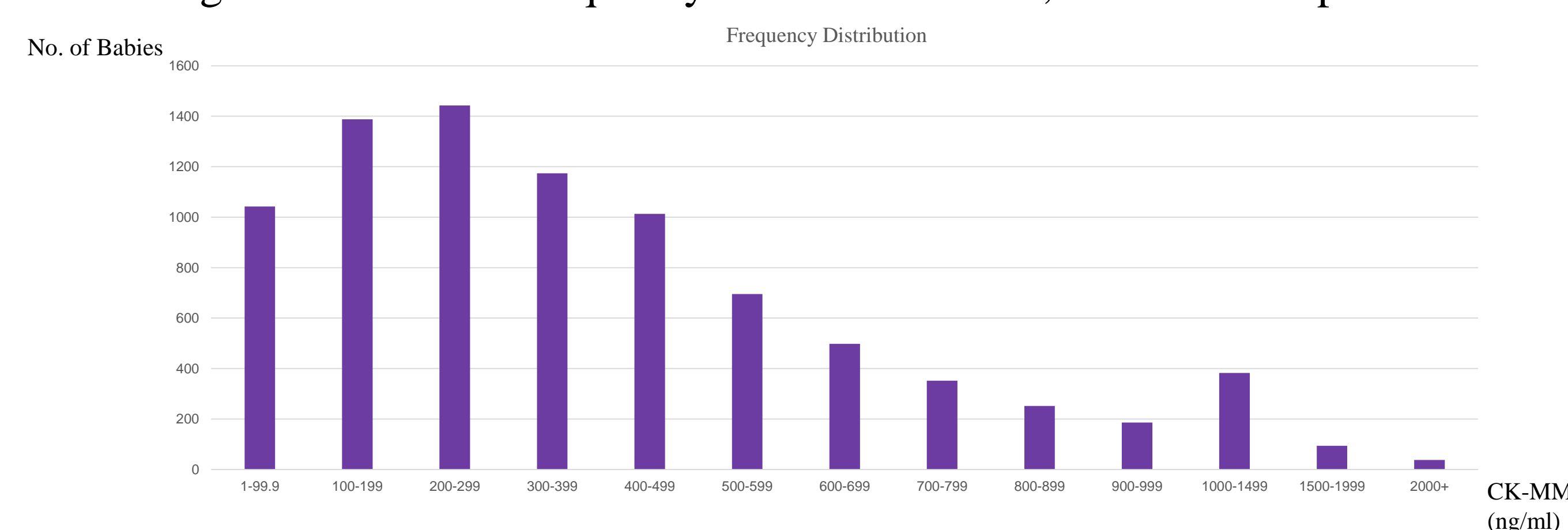


Figure 3. Effect of Time of Collection and Sex on CK-MM Values

a) Time of collection



b) Sex

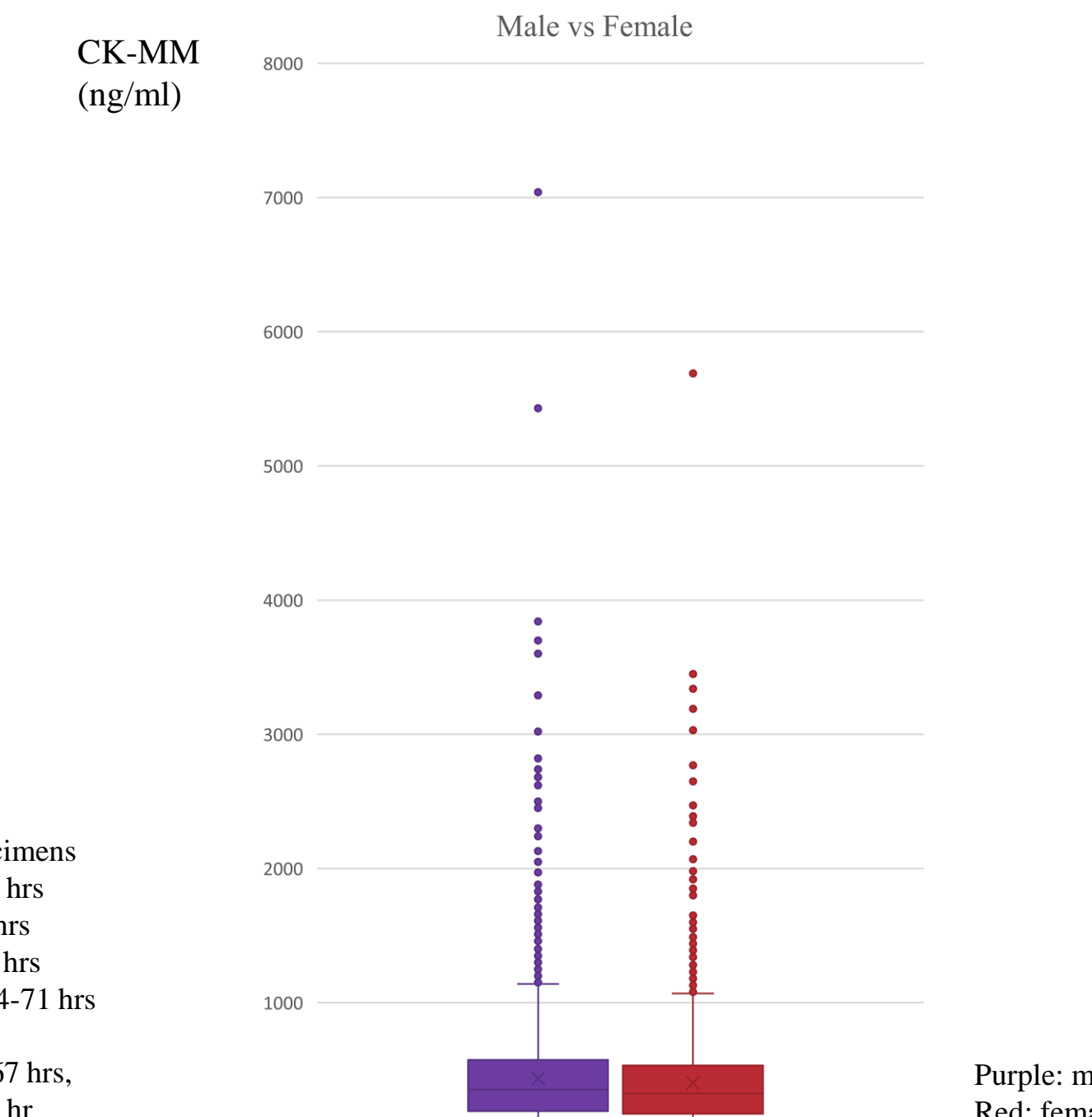


Table 3. Blinded specimens with elevated CK-MM values

Baby	Age at Collection (hrs)	Sex	CK-MM (ng/ml)
1	30	F	5690
2	72	F	5430
3	31	M	>7040

Conclusion

The GSP Neonatal CK-MM assay showed acceptable inter-assay and intra-assay reproducibility. The assay cut-offs selected were based on time of specimen collection because time of collection and CK-MM levels were inversely correlated. The CK-MM concentrations observed in the Duchenne affected patients showed satisfactory separation from unaffected newborns. We identified three newborns (one male and two female) with significant CK-MM elevation who would be candidates for second-tier testing. These validation studies indicate that the GSP CK-MM assay is a suitable candidate for a first-tier newborn screen for Duchenne.

Acknowledgements

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