GSP® NEONATAL CREATINE KINASE –MM KIT

PerkinElmer's new assay measures muscle specific creatine kinase in dried blood spots, helping to detect newborns affected by Duchenne Muscular Dystrophy.

WHAT IS DMD?

Duchenne muscular dystrophy (DMD) is the most prevalent form of muscular dystrophies affecting ~1:3000-1:6000 boys worldwide [1]. DMD is caused by mutations in the gene coding for dystrophin protein, which provides structural stability to muscle cells. Although DMD is an X-linked recessive disease and females are typically asymptomatic carriers, some female carriers may manifest symptoms varying from mild muscle weakness to a more severe phenotype. DMD causes progressive muscle wasting and loss of ambulation leading to premature death. Although no cure exists for DMD, corticosteroid treatment improves muscle strength, and with supportive medical care is associated with delayed loss of ambulation and markedly improved survival.

WHY IS EARLY DETECTION SO IMPORTANT?

Early screening prevents unnecessary diagnostic odysseys and allows appropriate early interventions and clinical surveillance. Recent therapeutic developments for DMD, along with new evidence of the importance of early intervention with therapy, such as corticosteroid treatment, have underscored the need for newborn screening of DMD.

INTRODUCING THE NEW METHOD FOR NEWBORN SCREENING OF DMD

Creatine kinase (CK) is an enzyme present in muscle cells that leaks into the bloodstream upon muscle damage. There are several different isoforms of CK (CK-MM, CK-MB, and CK-BB). The CK-MM form is found predominantly in the skeletal muscle cells and is consequently the isoform most specific to skeletal muscle damage. CK-MM is typically highly elevated in DMD patients and total serum creatine kinase is the standard clinical chemistry test used to diagnose DMD. Elevated CK-MM levels in blood of DMD infants enable newborn screening using CK-MM as the marker. However, CK-MM may not be elevated in all DMD infants immediately after birth as it is a secondary marker of disease progression.

PerkinElmer's GSP® Neonatal Creatine Kinase –MM kit is an immunoassay for measuring CK-MM in dried blood spot samples of newborn babies [2]. As the assay measures the muscle specific isoform, it enables the screening process to find the babies affected by DMD. The kit is available on PerkinElmer's GSP® instrument, a fully automated, high throughput biochemical analyzer.

[1] Mendell JR, Shilling C, Leslie ND, Flanigan KM, et. al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol 2012;71:304-313

[2] Moat SJ, Korpimäki T, Furu P, Hakala H, Polari H, Meriö L, Mäkinen P, Weeks I. Characterization of a blood spot creatine kinase skeletal muscle isoform immunoassay for high-throughput newborn screening of Duchenne muscular dystrophy. Clin Chem 2017;63:908-14.

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For the Better

VALIDATED, SENSITIVE ASSAY METHOD

The kit is intended for the quantitative in vitro determination of the creatine kinase isoform MM (CK-MM) concentration in blood specimens dried on filter paper as an aid in screening newborns for DMD using the GSP® instrument. The kit is not intended for use as a diagnostic test for DMD, for screening of other forms of muscular dystrophies, or for screening of female DMD carriers.

The assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique (Figure 1). Calibrators and controls are dried blood spots on barcoded filter paper cassettes. Sample disks are punched into the assay wells, where the assay buffer elutes the analyte (CK-MM) from the paper matrix. The analyte reacts simultaneously with immobilized mouse monoclonal antibodies and europium chelate labeled mouse monoclonal antibodies, which recognize two separate antigenic sites on the molecular surface of CK-MM. The excess unbound label is then washed away from the wells.

DELFIA® Inducer dissociates europium ions from the labeled antibodies into solution where they form highly fluorescent chelates with the components of DELFIA® Inducer. The fluorescence is proportional to the concentration of CK-MM in the blood.

ASSAY PROTOCOL PERFORMED BY THE GSP® INSTRUMENT

- Dispensing CK-MM Assay Buffer
- Dispensing Anti-CK-MM Eu-tracer
- Interval Shaking/Incubation
- Dispensing CK-MM Assay Buffer
- Shaking/Incubation
- Measurement (Elution control)
- Disk removal
- Wash
- Dispensing DELFIA Inducer
- Shaking/Incubation
- Measurement
- Liquid removal
- Runtime 4 hrs 50 min/plate, 26 plates / 15 hrs

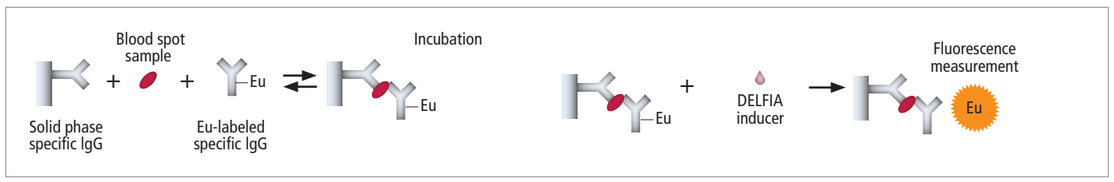

Figure 1. Assay principle of GSP CK-MM kit

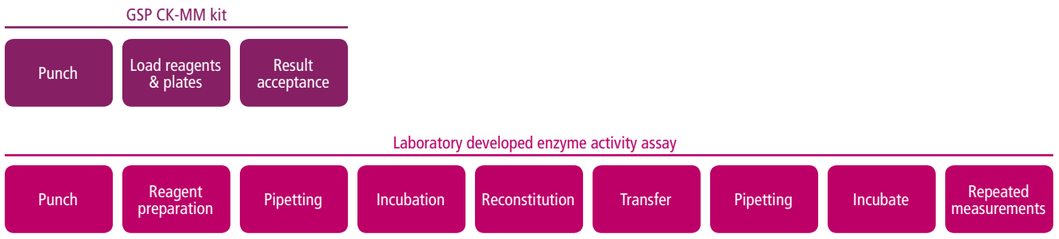

Figure 2. Workflow comparison of GSP® CK-MM kit and laboratory developed enzyme assay

KEY BENEFITS OF THE GSP® CK-MM KIT

- Fully automated – no reagent preparation (Fig. 2)
- Specific for CK-MM muscle isoenzyme
- Minimal cross-reactivity to other isoforms
- High sensitivity and specificity – good separation between affected and unaffected cases
- Low variation
- Long 14 days on-board stability
- Measures mass concentration, not enzyme activity
- Robust and reliable DELFIA® performance

RELIABLE SCREENING EFFICIENCY

SAMPLE STABILITY

The influence of storage time, temperature and humidity on CK-MM concentration was studied using DBS samples at four CK-MM concentration levels ranging from ~60 to 1500 ng/ml.Sample stability is sufficient for routine newborn screening. For short term storage, DBS samples are recommended to be stored at room temperature (+19 to +23 °C) or lower in dry conditions. For long term storage, DBS samples should be stored frozen (e.g. at -30 to -16 °C) in dry conditions (Figure 3.).

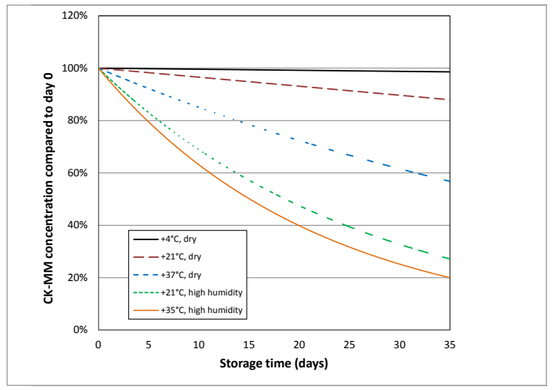

Figure 3. The change of CK-MM concentration during storage at different temperatures and humidity

ANALYTICAL PERFORMANCE [2]

The analytical limits were determined in accordance with CLSI document EP17-A2 and linearity in accordance with EP06-A. The cross-reactivity with CK-MB and CK-BB isoforms and the hook effect were also determined. Results are shown in Table 1. The precision was determined in accordance with CLSI document EP05-A3 using three kit lots and three GSP instruments. Total variation is shown in Table 2.

Table 1. Analytical performance characteristics

LoB, LoD and LoQ	0.7 ng/ml, 2.2 ng/ml and 6.8 ng/ml
Assay cross reactivity	CK-MM 100%, CK-MB <5%, CK-BB 0%
Linearity	6.8-8780 ng/mL
Measurement range	6.8 – 8000 ng/ml
Hook effect	>54 700 ng/ml

Table 2. Precision data using one calibration curve valid for 24hrs

Sample	n	Mean CK-MM conc. (ng/mL)	Total variation
			CV%
1	230	22.7	17.6
2	230	71.3	9.3
3	230	119	12.4
4	230	251	11.8
5	230	457	10.7
6	230	1155	9.4
7	230	5589	8.3

SCREENING PERFORMANCE

The GSP CK-MM kit was tested in a routine screening laboratory (external study) and in an internal study to generate newborn population distribution data and to assess the performance of the test. The samples used were archived newborn screening DBS samples obtained from the Danish Newborn Screening Biobank (external study) and from the California Biobank (internal study). In Denmark study, 2099 presumed negative were used in the cut-off phase and 16 confirmed positive and 1408 presumed negative in the screening performance phase. 700 presumed negative and 19 confirmed positive were used in the internal study. The descriptive statistics of the studies are shown in Table 3 and the frequency distribution data of the external study is shown in Figure 4. The CK-MM concentration is affected by the age of the newborn (Table 3. and Figure 5.). Therefore it is recommended that laboratories generate their own screening cut-offs based on the age of the newborn. Screening performance was calculated using 99.5th and 99th percentiles in external and internal studies, respectively (Table 4. and 5.)

Table 3.Descriptive statistics of the studies

Study	Sample type	n	Age of newborn at sampling	Range (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Percentile values	
							95%	99%
External	Presumed negative	2099	2-28 days	6.8 - 1740	124	96.6	291	513
External	Confirmed positive	16	2-11 days	919* - 6230	N/A	N/A	N/A	N/A
Internal	Presumed negative	700	25-48 hours	35.2 - 2390	328	251	867	1190
Internal	Confirmed positive	19	12-61 hours	2750-21600	N/A	N/A	N/A	N/A

* False negative sample result not included within the range.

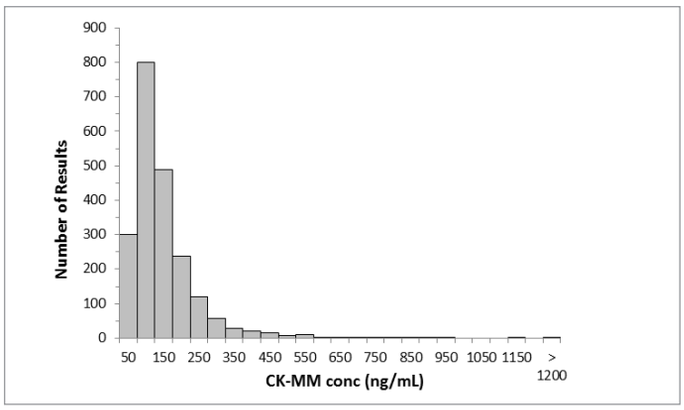

Figure 4. Frequency distribution of CK-MM concentrations (n=2099) in an external study (Denmark).

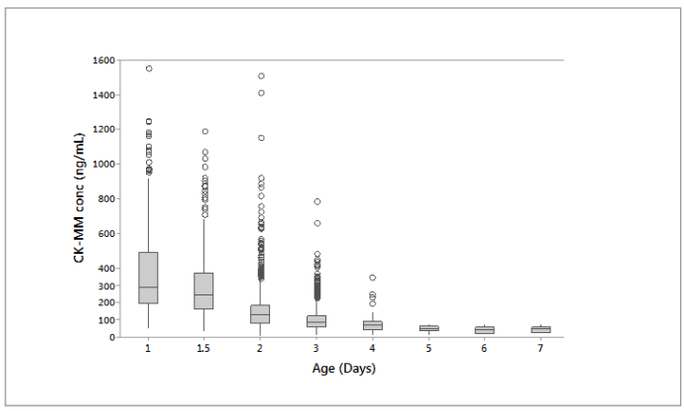

Figure 5. The effect of age of newborn at sampling on CK-MM concentration (n=2773) analysed from the combined data (external and internal study)

* The biospecimens and/or data used in this study were obtained from the California Biobank Program (SIS request number 684). The California Department of Public Health is not responsible for the results or conclusions drawn by the authors of this publication.

Table 4. Screening performance using the 99.5th percentile in external (Denmark) study

Sample		Clinical status		Total
		DMD positive	Presumptive negative	
Screening result (cut-off 675 ng/mL)	Screening positive	15	4	19
	Screening negative	1*	1404	1405
	Total	16	1408	1424

*CK-MM concentration was not elevated in one DMD positive sample taken from an extremely preterm newborn with a very low birth weight.

Table 5. Screening performance using the 99th percentile in internal study (California samples)

Sample		Clinical status		Total
		DMD positive	Presumptive negative	
Screening result (cut-off 1190 ng/mL)	Screening positive	19	8	27
	Screening negative	0	692	692
	Total	19	700	719

KIT INCLUDES ALL COMPONENTS FOR EFFECTIVE NEWBORN SCREENING

Kit contains reagents for running 12 plates

- 3 bottles of CK-MM Assay Buffer – ready for use
- 3 vials of Anti-CK-MM Eu-tracer – ready for use
- 6 levels of CK-MM calibrators, 7 series
- 3 levels of CK-MM controls, 10 series
- 12 barcoded, Anti-CK-MM coated plates
- Kit insert
- QC certificate
- 6 pcs of extra barcodes

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