EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
GSP Neonatal Creatine Kinase-MM kit

DECISION SUMMARY

A. DEN Number:

DEN180056

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the GSP Neonatal Creatine Kinase-MM kit

C. Measurand:

Creatine Kinase MM-isoform

D. Type of Test:

Quantitative, fluoroimmunometric assay

E. Applicant:

PerkinElmer, Inc.

F. Proprietary and Established Names:

GSP Neonatal Creatine Kinase-MM kit

G. Regulatory Information:

Regulation	Name	Product Code	Panel
21 CFR 862.1506	Muscular dystrophy newborn screening test	QJE	Chemistry (75)

H. Indications for Use:
1. <u>Indications for Use:</u>
   The GSP Neonatal Creatine Kinase-MM kit, is intended for the quantitative in vitro determination of creatine kinase MM-isoform (CK-MM) concentration in blood specimens dried on filter paper as an aid in screening newborns for Duchenne Muscular Dystrophy (DMD) using the GSP instrument.

2. Special conditions for use statement(s)

- For prescription use only
- This kit is not intended for use as a diagnostic test for DMD or for screening of other forms of muscular dystrophies
- Due to the complexity of the DMD carrier phenotype, there is a possibility that asymptomatic female carriers may have a false positive screening result and that symptomatic/manifesting female carriers may have a false negative screening result.
- Test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, and clinical evaluation as appropriate.
- Storage of samples in an environment with elevated temperatures (37℃) and humidity (80%) increases the risk of false negative screening results.
- CK-MM may not be elevated in all DMD infants immediately after birth as it is a marker of skeletal muscle damage and therefore an indirect marker of DMD. In cases such as extremely preterm birth or very low birth weight, the muscle damage sustained by the newborn due to DMD may be limited, and thus false negative DMD screening results may be obtained. While no newborns with DMD were missed in the clinical validity study, in another study a known DMD positive, extremely preterm (<28 weeks) newborn with very low birth weight (<1500 g) resulted in a false negative result.
- The cut-off values described in the labeling have only been validated with samples obtained less than 72 hours after birth. Since CK-MM enzyme levels have been shown to decrease with age, separate cut-offs should be determined for use with samples taken more than 72 hours after birth.

3. Special instrument requirements:

For use on the GSP instrument only.

I. Device Description:

The GSP Neonatal Creatine Kinase-MM assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique and utilizes standard PerkinElmer DELFIA chemistry with the GSP instrument. The kit contains:

- The CK-MM Calibrators (containing 0, 30, 120, 500, 2000 and 8000 ng/mL of creatine kinase) consisting of 7 cassettes each containing 1 set of dried blood spots.
- The CK-MM Controls (containing 130, 500 and 2000 ng/mL of creatine kinase) consisting of 5 cassettes each containing 2 set of dried blood spots.
- Anti-CK-MM-Eu Tracer
- CK-MM Assay Buffer
- Anti-CK-MM Microtitration strips
- Extra barcodes for the plates

J. Standard/Guidance Documents Referenced:

Clinical and Laboratory Standards Institute (CLSI) EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Third Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

K. Test Principle:

The GSP Neonatal Creatine Kinase -MM assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. Calibrators, controls, and test samples are dried blood spot specimens. Sample disks are punched into the assay wells, where the assay buffer elutes the analyte (MM isozyme of creatine kinase, 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 antibody into solution where they form highly fluorescent chelates with the components of the DELFIA Inducer. The fluorescence in each well is then measured. The fluorescence of each sample is proportional to the concentration of CK-MM in the sample.

L. Performance Characteristics:
1. <u>Analytical performance:</u>

   a. *Reproducibility/Precision:*

   The precision studies were performed following the recommendations in the CLSI EP05-A3 guideline. Results were calculated with a full calibration curve in duplicate for each plate.

   Samples (DBS) included in precision studies:
   C1 to C3: Controls, sheep blood spiked with purified human CK-MM
   (b) (4)

Study 1: This study was conducted over 20 days, with 2 plates per day and 2 replicates per plate for a total of 80 determinations. This study used 1 lot of reagents and 1 instrument. Within-lab precision was calculated with the repeatability (within-plate), between-plate and between day measurements.

Sample	Mean CK-MM (ng/mL)	N	Repeatability (Within-plate)		(b) (4)		(b) (4)		Within-laboratory	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
(b) (4)										
(b) (4)										

Study 2: A five-day precision study was performed to determine lot-to-lot precision using a single instrument. Five replicates of each sample were run once daily for three kit lots over five days on (n = 75 total across 3 lots). Within-lab precision was calculated with the repeatability (within-plate) and between day measurements. The reproducibility with multiple kit lots includes the within-laboratory and between lot measurements. The results for the five-day precision study are shown in the table below:

Sample	Mean CK-MM (ng/mL)	n	Within laboratory		Between-lot		Reproducibility with multiple kit lots	
			SD	CV%	SD	CV%	SD	CV%
(b) (4)								

Study 3: This study was conducted to look at precision across multiple instruments. The study included (b) (4)
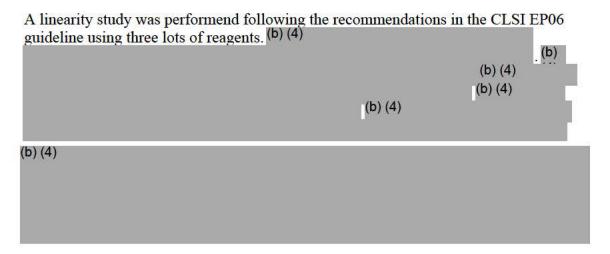
Within-lab precision was calculated with the repeatability (within-plate), and (b) (4) measurements. The reproducibility with multiple instruments includes the within-laboratory and between instrument measurements. During this study, a single measurement from the (b) (4) sample was extremely high compared to all other replicates of this sample. The results for the (b) (4) precision study are shown in the table below, with values for (b) (4) calculated both with and (b) (4) :

Sample	Mean CK-MM (ng/mL)	n	Within-laboratory		Between-instrument		Reproducibility with multiple instruments	
			SD	CV%	SD	CV%	SD	CV%
(b) (4)								

Reproducibility (site-to-site):
The sponsor provided information documenting that the precision of the device is not different across three clinical sites.

b. *Linearity/assay reportable range:*

A linearity study was performend following the recommendations in the CLSI EP06 guideline using three lots of reagents. (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Based on the results of the linearity study, the sponsor claims that the reportable range of the device is 29.2-8000 ng/mL.

Hook Effect

No significant hook effect was observed up to concentrations >50,000 ng/mL.

c. *Traceability, Stability, Expected values:*

In the absence of an international reference preparation or a reference method for CK-MM concentration, the calibration is anchored to an in-house CK-MM reference preparation. The traceability scheme was reviewed and found acceptable.

The sponsor included the following statement in the labeling:

As a result of possible variability and systematic bias among lots, at the 99.5th percentile the false negative screen rate of future lots could range from 0% to 0.48% (based on the upper 95% confidence interval) and the false positive screen rate of future lots could range from 0.4 to 0.7%. At the 97.5th percentile the false negative screen rate of future lots could range from 0% to 0.05% (based on the upper 95% confidence interval) and the false positive screen rate of future lots could range from 2.0 to 3.7%.

Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the test system was determined. The analysis of the LoB, LoD, and LoQ were performed following the recommendations in the CLSI EP17-A2 guideline.

To determine the LoB, five blank samples were prepared from a (b) (4) human red blood cells (RBCs) (b) (4) . Each sample was prepared from a different lot of RBCs. Hematocrit (b) (4) (b) (4) The samples were assayed in (b) (4) The LoB was determined for each lot using the non-parametric approach described in CLSI EP17-A2. The LoB for the worse performing lot is reported in the package insert.

To determine LoD, (b) (4) Each sample was prepared from a different lot of RBCs. Hematocrit (b) (4) The samples were assayed (b) (4) LoD was determined for each lot using the precision profile approach described in CLSI EP17-A2. The LoD for the worse performing lot is reported in the package insert.

LoQ was estimated by first fitting a linear model with SD as the response variable and then determining the point where the fitted variation model reaches the LoQ study acceptance limit ((b) (4) % CV).

The LoB, LoD, and LoQ are summarized in the table below:

LoB	LoD	LoQ
0.7 ng/mL	2.2 ng/mL	6.8 ng/mL

d. *Analytical specificity:*

Analytical specificity studies were performed following the recommendations in the CLSI EP07-A2 guideline. Potential interferents tested included unconjugated bilirubin, conjugated bilirubin, triglycerides (intralipid), free hemoglobin, albumin, acetaminophen, calcifediol, chlorhexidine, glucose, and galactose. Three blood pools with different CK-MM concentration levels (approximately 200 ng/mL, 500 ng/mL and 1500 ng/mL) were prepared by spiking human whole blood with purified human CK-MM. The hematocrit values of the blood pools used in the sample preparation were (b) (4)   %. The test pools were prepared by (b) (4)
Control pools were prepared by (b) (4)   Test and control pools were (b) (4)   Samples were assayed in replicates of (b) (4) For the hematocrit interference studies, pools were prepared by (b) (4)
% for the control pool, and hematocrit targets of (b) (4) for the test pools. The limit for significant interference was defined as (b) (4) The following substances at the listed concentrations did not interfere with the performance of the test:

Potential Interferent	Highest Concentration of interferent tested that did not show significant interference (Endogenous + added concentration of tested substance in whole blood)
Unconjugated bilirubin	20 mg/dL (20 mg/dL)
Conjugated bilirubin	33 mg/dL (33 mg/dL)
Triglycerides	1500 mg/dL (1590mg/dL)
Albumin	30 g/L (47 g/L)
Acetaminophen	5.5 mg/dL
Ascorbic acid	1.5 mg/dL
Ampicillin	18 mg/L
Calcifediol	250 nmol/L (283 nmol/L)
Gamma Globulin	30 g/L (36 g/L)
Folate	3 mg/L
EDTA	9 mg/mL
Glucose	500 mg/dL
Galactose	7.5 mg/dL
Hemoglobin	3.75 g/dL (20 g/dL)

The following is included in the package insert:

Chlorhexidine digluconate was found to result in significant interference to the device at a concentration of 0.04%. This interference resulted in an increased CK-MM level, which could result in false positive screen results from the test. This information is included in the test labeling, with instructions that when chlorhexidine digluconate is used for cleaning of the skin prior to specimen collection, the skin is allowed to thoroughly air dry before puncture to avoid contamination of the sample with the disinfectant.

The effect of hematocrit was tested by adjusting the amount of red blood cells with plasma on three whole blood DBS samples with different CK-MM concentrations (159, 514, and 1870 ng/mL) and testing the blood samples with the GSP Neonatal Creatine Kinase–MM kit for hematocrit interference according to CLSI document EP07-A2. The labeling includes a notation that the device is subject to interference in samples with low hematocrit (35-45%) with the lowest CK-MM concentration tested (159 ng/mL). The other tested CK-MM concentration results were equivalent within the tested range of hematocrit (35–65%).

Cross-reactivity

To assess cross reactivity of CK-BB, three blood pools with different CK-MM concentration levels (b) (4) were prepared by spiking human whole blood with purified human CK-MM. An additional blood pool with CK-MM was prepared from a suspension of red blood cells (b) (4) The hematocrit values of the blood pools used in the sample preparation (b) (4) The test pools were prepared by (b) (4)

Control pools were prepared by (b) (4)
(b) (4)   . Cross-reaction percentages were calculated by determining the (b) (4)
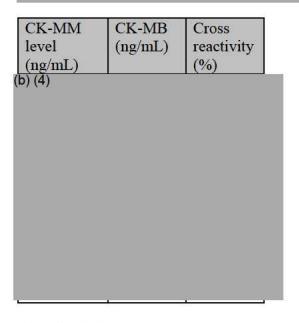
          Samples were assayed in replicates of (b) (4) The limit for significant cross-reactivity was defined as (b) (4)

CK-BB level (ng/mL)	CK-MM (ng/mL)	Cross reactivity (%)
(b) (4)		

In a separate study, clinically relevant levels of CK-MB were assessed for cross-reactivity. Three blood pools with different CK-MM concentration level (approximately 200 ng/mL, 500 ng/mL and 1500 ng/mL) were prepared by (b) (4) The hematocrit values of the

blood pools used in the sample preparation (b) (4)   (b) (4)

. The results are summarized below.

CK-MM level (ng/mL)	CK-MB (ng/mL)	Cross reactivity (%)
(b) (4)		

e. *Assay Cut-off:*

Not applicable.

f. *Specimen Stability*

The sponsor provided information to support the following information regarding DBS sample stability in their labeling:

- CK-MM is stable for up to 200 days at +4°C in dry conditions
- CK-MM may have moderate loss of concentration (up to 27%) after 6 days at +4°C in ambient conditions
- CK-MM is stable for up to 25 days at -20°C in ambient conditions
- CK-MM is stable for up to 20 days at +21°C in dry conditions
- CK-MM may have moderate loss of concentration (up to 30%) after 2 days at +21°C in ambient conditions
- CK-MM is unstable in humid (RH 80%) conditions at +21°C and +35°C (<80% recovery at 2 days)
- CK-MM is unstable in hot conditions at +37°C (<80% recovery at 3 days).

g. *Shipping Stability*

Based on the stability of the analyte, the sponsor includes the following recommendations for shipping DBS samples for CK-MM determination:

- Since humid conditions can lead to <80% recovery in 2 days at +21°C and above, it is recommended to pack the samples in low permeability containers together with desiccant pouches, if humid conditions are expected during transport.
- Since hot conditions (+37°C) can lead to <80% recovery in 3 days, it is recommended to avoid multiday continuous exposure to temperatures significantly above +21°C during transport.

2. Comparison studies:

   a. *Method comparison:*
      Not applicable.

   b. *Matrix Comparison*

      Not applicable.

3. Clinical studies:

   a. *Clinical Sensitivity*

      Not applicable.

   b. *Clinical Specificity*

      Not applicable.

   c. *Other clinical supportive data (when a. and b. are not applicable)*

      The screening performance of the creatine kinase-MM kit was determined in a prospective clinical study of routine newborn screening samples and retrospectively confirmed positive DMD samples from newborns. Three thousand forty-one routine newborn samples and 30 clinically confirmed DMD positive newborn samples were tested. Most routine samples (97.3%) tested were from newborns ≤72 hours old. Routine samples were stored for (b) (4) days prior to testing and confirmed DMD positive samples ranged from 1 to 12 years of storage.

      Screening algorithm:
      Samples were initially tested in singlicate. If the CK-MM concentration was greater than the cut-off, new dried blood spot punches were re-tested in duplicate to confirm the high concentration results. For the specimens that were re-tested, final screening categorization was based on the mean value of the replicate retest results.

      A cut-off value was applied to all specimens to classify samples into screen positives and screen negatives to estimate the false positive and false negative screening rates of the test. The outcome of all routine samples with CK-MM concentrations above

1250 ng/mL when initially tested (prior to duplicate re-test) was evaluated using next generation sequencing of the DMD gene. To estimate the false negative rate of the test, the outcome of samples with CK-MM concentrations between 984 and 1210 ng/mL when initially tested were also evaluated using next generation sequencing of the DMD gene. During the review of this submission, FDA consulted with experts in the field of DMD diagnosis who confirmed that sequencing of the DMD gene is a widely accepted method of diagnosing DMD. Clinical diagnosis of the 30 DMD confirmed retrospective positive samples was known. All confirmed DMD positive samples were screen positive.

Using next generation sequencing, four routine samples that were screen positive were determined to be DMD positive based on the genetic variant detected. Genetic variants of unknown significance were detected in three routine samples that were screen negative.

Routine samples only:

Cut-off	Screened samples	Above initial cutoff	Below initial cutoff	Retest rate	Screen Positive (above cut-off after repeat testing)	Screen Negative (below cut-off after repeat testing)	False positive rate
1250	3041	86	2955	(b) (4)	73*	2968**	2.26%
2040	3041	21	3020		20*	3021**	0.53%

*Includes samples identified as positive by NGS
**Includes samples identified by NGS as VOUS

Routine samples and DMD confirmed positives:

Cut-off	Screened samples	Above initial cutoff	Below initial cutoff	Retest rate	Screen Positive (above cut-off after repeat testing)	Screen Negative (above cut-off after repeat testing)	False positive rate
1250	3071	(b) (4)					
2040	3071						

*Includes both samples identified as positive by NGS and the confirmed positive.
**Includes samples identified by NGS as VOUS

Screening Performance:

This information corresponds to the screening performance from the clinical study. Refer to the section above for additional information on the possible false negative

screen rates and false positive screen rates for future lots of this device.

Cut-off: 1250 ng/mL (corresponding to the 97.5% percentile value from the cut-off study):

Screening Results		Outcome, DMD				
		DMD positive (pathogenic variant or clinical diagnosis)	Indeterminate (variant of unknown significance)	Presumed DMD negative (benign or no variant)	Not determined	Total
Creatine Kinase MM kit	Positive	34*	0	69	0	103
	Negative	0	3**	97	2868	2968
	Total	34	3**	166	2868	3071

*Includes 30 retrospective confirmed positive samples and 4 samples identified as positive during the clinical study.

Cut-off: 2040 ng/mL (corresponding to the 99.5% percentile value from the cut-off study):

Screening Results		Outcome, DMD				
		DMD positive (pathogenic variant or clinical diagnosis)	Indeterminate (variant of unknown significance)	Presumed DMD negative (benign or no variant)	Not determined	Total
Creatine Kinase MM kit	Positive	34*	0	16	0	50
	Negative	0	3**	150	2868	3021
	Total	34	3**	166	2868	3071

*Includes 30 retrospective confirmed positive samples and 4 samples identified as positive during the clinical study.

Regarding the use of genetic testing to determine the positive status of 4 specimens in the clinical trial the sponsor states: "For DMD molecular assay 1-5% of patients with a clinical diagnosis of DMD may get a negative molecular result."

For a full list of limitations, see section H. 2. "Special conditions for use statement(s)".

4. Expected Values

In a different study, creatine kinase-MM values by percentile from the testing of 2019 routine newborn screening specimens completed with the GSP Neonatal Creatine Kinase-MM kit at a state U.S. laboratory:

GSP Neonatal Creatine Kinase MM (ng/mL)					Upper percentiles		
N	Min	Max	Mean	Median	97.5%	99%	99.5%
2019	6.8	11600	405	320	1250	1610	2040

The labeling states that each laboratory should establish its own reference range and cut-off values. The cut-off values described in the labeling have only been validated with samples obtained less than 72 hours after birth. Since CK-MM enzyme levels have been shown to decrease with age, separate cut-offs should be determined for use with samples taken more than 72 hours after birth.

c. Instrument Name:

GSP Instrument

d. System Description:

1. Modes of Operation:

Same as referenced in K090846.

2. Software:

Same as referenced in K090846.

3. Specimen Identification:

Same as referenced in K090846.

4. Specimen Sampling and Handling:

Same as referenced in K090846.

5. Calibration:

The six levels of calibrators are provided in the kit. A calibration curve must be run in duplicate for each kit lot and DELFIA Inducer lot. Thereafter, the calibration curve is valid for up to 24 hours, or until a new calibration curve is run.

6. Quality Control:

Controls at three different levels are provided in the kit. Control samples should always be used to assure the day-to-day validity of results. Controls at three different levels are included in the kit. These controls should be run in duplicate on each plate. Each laboratory should establish its own mean and acceptable range. The established mean

should be within ± 2 SD of the values stated on the quality control certificate. It is recommended that the laboratories establish their own controls at different levels in addition to the controls included in the kit. Sample results should only be reported if control results for the assay meet the laboratory's established criteria for acceptability.

M. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable.

N. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

O. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

Identified Risks to Health and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Risk of False Negative Results	Certain design verification and validation activities Certain labeling information
Risk of False Positive Results	Certain design verification and validation activities Certain labeling information

P. Benefit/Risk Analysis

Summary of the Assessment of Benefit

There is currently no available FDA cleared/approved device for DMD screening and under current conditions of care, the clinical diagnosis of DMD remains persistently delayed, which not only exacerbates underlying health disparities but also keeps DMD clinical care centers from delivering high quality clinical care that could meaningfully improve outcomes (Kwon, 2016). Newborns may benefit from the use of the device because earlier diagnosis can potentially lead to improvement in clinical course if steroids are initiated at symptom onset and interdisciplinary care and emerging therapies are initiated before symptom onset. Additionally, earlier diagnosis may decrease the emotional and financial cost during the years it may take to gain a final DMD diagnosis. There may be a further potential benefit in terms of family planning for the families of newborns screened and diagnosed earlier.

The test correctly screened for DMD in 30 confirmed positive newborn specimens included in the clinical study and an additional 4 specimens from the 3041 leftover NBS samples used

in the clinical study. Given the low prevalence of DMD (~ 1 in every 3000 to 6000 boys worldwide), the detection of DMD in 4 out of 3041 leftover samples is supportive of device performance and the benefit of the test.

Summary of the Assessment of Risk

Associated device risks include false negative and false positive test results. False negative test results may extend the time to diagnosis if initial clinical evaluation excludes the initial workup for DMD due to the negative screening result. False positive test results could lead a newborn to have unnecessary additional confirmatory testing and to add emotional burden and cost to the family of the newborn.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks associated with the device. However, the probable clinical benefits outweigh the probable risks for the assay, considering the mitigation of the risks provided for in the special controls. Design verification and validation, including a clinical validation study, the results of which will be included in the labeling, along with limitations and performance information, will help ensure that the device functions as intended and mitigate the risk of false positive and negative test results. Overall, the potential benefits outweigh the temporary risk of additional testing and potential delay in diagnosis which can occur with erroneous testing results for the proposed indications for use, in light of the special controls and in combination with the general controls.

Q. Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

       Product Code: QJE
       Device Type: Muscular dystrophy newborn screening test
       Class: II (special controls)
       Regulation: 21 CFR 862.1506