510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K190335

B Applicant

PerkinElmer Inc.

C Proprietary and Established Names

GSP Neonatal Total Galactose kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JIA	Class I, reserved	21 CFR 862.1310 - Galactose Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modification to a previously cleared device to extend the assay incubation time

B Measurand:

Galactose and galactose-1-phosphate

C Type of Test:

Quantitative, fluorescent galactose oxidase method

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

III Intended Use/Indications for Use:

A Intended Use(s):

The GSP Neonatal Total Galactose kit is intended for the quantitative determination of total galactose (galactose and galactose-1-phosphate) concentrations in blood specimens dried on filter paper as an aid in screening newborns for galactosemia using the GSP® instrument.

B Indication(s) for Use:

See intended use above.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For In-Vitro Diagnostic Use Only

Screening specimens that result in values at or above cut-off should be considered as presumptive positives for galactosemia, retested and/or confirmed by a diagnostic test procedure.

Blood samples that have been obtained from infants before 24 hours or who have not ingested sufficient breast milk or formula containing lactose may give low values, resulting in a false negative result.

If a sample collected before 24 hours, or from an infant who has not been on a lactose-containing diet, is tested and returns a negative result, a follow-up sample collected at or after 24 hours and after lactose-containing diet should be tested.

The GSP Neonatal Total Galactose kit may result in:

- False negatives by not detecting galactosemia in samples collected at <24 hours
- False negatives by not detecting galactosemia in infants who have not had a lactose containing diet prior to sampling
- False negatives by not detecting Duarte variant galactosemia

D Special Instrument Requirements:

For use on the GSP instrument only.

GSP instrument software version 1.4 rev 6 is required to support the updated GSP Neonatal Total Galactose assay.

IV Device/System Characteristics:

A Device Description:

The GSP Neonatal Total Galactose test kit contains the following components:

Calibrators have been prepared from human red blood cells enriched with galactose, and with ProClin 300 as preservative. The hematocrit value is 50 - 55 % to correspond to a hematocrit of a

newborn. The calibrators have been calibrated against primary calibrators gravimetrically prepared using a U.S. Pharmacopeia Reference Standard Preparation for galactose.

Controls have been prepared from human blood enriched with galactose and galactose-1-phosphate, and with ProClin 300 as preservative. Prior to dispensing the blood onto the filter paper, the hematocrit value of blood used in the controls preparation is adjusted to 50 - 55 % to correspond to a hematocrit of a newborn. The low control is approximately 4.0 mg/dL and the high control approximately 12 mg/dL.

All human source materials used in the preparation of kit components were tested and found to be non-reactive for the presence of HBsAg, anti-HIV 1 and 2, and HCV by FDA approved methods.

B Principle of Operation:

The Neonatal Total Galactose assay measures total galactose, i.e. both galactose and galactose-1-phosphate, using a fluorescent galactose oxidase method. The fluorescence is measured using an excitation wavelength of 505 nm and an emission wavelength of 580 nm. The following illustration summarizes the reactions that occur during the assay procedure:

$$\text{Gal-1-P} + H_2O \xrightarrow{\text{AP}} \text{Gal} + \text{P}$$

$$\text{Gal} + O_2 \xrightarrow{\text{GAO}} \text{GHD} + H_2O_2$$

$$\text{ADHP} + H_2O_2 \xrightarrow{\text{HRP}} \text{Resorufin}^* + H_2O$$

Gal-1-P = Galactose-1-phosphate
AP = Alkaline phosphatase
Gal = Galactose
P = Phosphate
GAO = Galactose oxidase
GHD = D-galacto-hexadialdose
ADHP = 10-Acetyl-3,7-dihydroxyphenoxazine
HRP = Horseradish peroxidase
* = Fluorescent

V Substantial Equivalence Information:

A Predicate Device Name(s):
Gsp Neonatal Total Galactose kit

B Predicate 510(k) Number(s):
K133652

C Comparison with Predicate(s):

Device & Predicate Device(s):	K190335	K133652
Device Trade Name	GSP Neonatal Total Galactose kit	GSP Neonatal Total Galactose kit
General Device Characteristic Similarities		
Intended Use/Indications For Use	This kit is intended for the quantitative determination of total galactose (galactose and galactose-1-phosphate) concentrations in blood specimens dried on filter paper as an aid in screening newborns for galactosemia using the GSP® instrument.	Same
Test Methodology	Enzymatic Assay	Same
Detection Method	The fluorescence is measured using an excitation wavelength of 505 nm and an emission wavelength of 580 nm.	Same
Instrument Platform	GSP Instrument, automated	Same
Sample Type	Dried blood spot	Same
Calibrators	A - 0.5 mg/dL B - 2.5 mg/dL C - 5.0 mg/dL D - 10 mg/dL E - 20 mg/dL F - 50 mg/dL	Same
Sample Type	Dried blood spot	Same
General Device Characteristic Differences		
Assay reaction incubation time	58 minutes	25 minutes
Reportable Range	1.2-50 mg/dL	1.15-50 mg/dL
Lower limits of measurement	LoB = 0.3 mg/dL LoD = 0.7 mg/dL LoQ = 1.2 mg/dL	LoB = 0.34 mg/dL LoD = 0.97 mg/dL LoQ = 1.15 mg/dL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3: Evaluation of precision of quantitative measurement procedures; Approved Guideline - Third Edition

CLSI EP06-A: Evaluation of 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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The GSP Neonatal Galactose kit precision was determined based on the recommendations of the CLSI guideline EP5-A3.

The precision performance was determined in three separate studies.

- In study 1 was performed over 20 days. Two plates were run each day and each plate contained two replicates of each sample. Each plate contained a full calibration curve in duplicate. The repeatability (within-plate variation), and within-laboratory precision (between-day, between-plate, and within-plate) were determined for the product. Study 1 also produced information on the between-plate, and between-day variation.
- Study 2 was run on 5 different days over a 9 day period. Three plates were run each day, with five replicates per plate, and each plate was analyzed with a different kit lot by the same operator. Each plate contained a full calibration curve in duplicate. Between-lot variation of the product was determined.
- Study 3 was run over 5 days. Three plates were run each day, each with a different GSP instrument by the same operator, with five replicates per plate. Each plate contained a full calibration curve in duplicate. Between-instrument variation of the product was determined.

The eight dried blood spot samples used as precision samples (PS1–PS8) were prepared by spiking normal adult human blood with equimolar amounts (50:50) of galactose-1-phosphate and galactose.

The hematocrit values of the blood samples used in sample preparation were adjusted to 40-55 % to correspond to the hematocrit of neonates.

Study 1:

Sample	Mean total galactose concentration (mg/dL)	n	Repeatability (Within-plate variation)		Between-plate variation		Between-day variation		Total variation (Within-lab)	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
PS1	4.5	80	0.4	8.8	0.3	5.7	0.0	0.8	0.5	10.5
PS2	8.2	80	0.9	10.8	0.6	7	0.2	2.2	1.1	13.1
PS3	10.5	80	0.8	7.6	0.4	3.9	0.5	4.8	1.0	9.9
PS4	14.0	80	1.2	8.2	0.7	5.3	0.5	3.6	1.5	10.4
PS5	16.5	80	1.7	10.1	0.0	0.0	1.2	7.5	2.1	12.6
PS6	34.5	80	3.2	9.2	0.3	0.9	1.8	5.2	3.7	10.6
PS7	51.5	80	4.8	9.3	3.4	6.5	0.4	0.8	5.9	11.4
PS8	52.2	80	5.3	10.1	5.1	9.7	0.4	0.7	7.4	14.1

Study 2:

Sample	Mean total galactose concentration (mg/dL)	n	Total variation (Within-lab)		Between-lot variation		Total variation with multiple kit lots	
			SD	CV%	SD	CV%	SD	CV%
PS1	4.5	75	0.5	11.1	0.1	3.3	0.5	11.6
PS2	8.1	75	1.0	12.1	0.3	4.1	1.0	12.8
PS3	10.5	75	1.0	9.8	0.3	3.1	1.1	10.3
PS4	14.6	75	1.5	10.5	0.5	3.3	1.6	11.0
PS5	16.0	75	1.7	10.7	0.4	2.2	1.8	11.0
PS6	33.4	75	3.0	9.1	1.3	3.9	3.3	9.9
PS7	48.2	75	4.9	10.1	0.4	0.8	4.9	10.1
PS8	49.0	75	4.7	9.5	0.7	1.3	4.7	9.6

Study 3:

Sample	Mean total galactose concentration (mg/dL)	n	Total variation (Within-lab)		Between-instrument variation		Total variation with multiple instruments	
			SD	CV%	SD	CV%	SD	CV%
PS1	4.3	75	0.4	8.7	0.1	2.0	0.4	9.0
PS2	8.3	75	1.0	11.7	0.0	0.0	1.0	11.7
PS3	10.3	75	1.1	10.8	0.0	0.0	1.1	10.8
PS4	13.9	75	1.4	10.4	0.0	0.0	1.4	10.4
PS5	15.8	75	1.5	9.7	0.0	0.0	1.5	9.7
PS6	33.4	75	2.9	8.6	0.0	0.0	2.9	8.6
PS7	49.9	75	4.9	9.9	0.0	0.0	4.9	9.9
PS8	50.0	75	6.7	13.3	0.0	0.0	6.7	13.3

2. Linearity:

The linearity was assessed in this study following the principles described in the CLSI Guideline EP06-A. The study used one kit lot and one GSP instrument. A full calibration curve in duplicate was included in the plate, and each linearity study sample was measured in 4 replicates.

Linearity study samples were prepared to cover approximately a 20–30 % wider range than the anticipated measuring range. The linearity study sample series with samples at 18 different concentration levels were prepared by proportionally mixing two human whole blood samples with high and low total galactose concentrations. The low total galactose concentration sample was normal human whole blood. The high total galactose concentration sample was prepared by spiking normal human whole blood with equimolar amounts (50:50) of galactose and galactose-1-phosphate. Expected concentration of the high and low total galactose samples were based on measured values with the GSP Neonatal Total Galactose kit. Expected values for dilution samples between these two endpoints were based on the volumetric ratio and measured value of each of these two components.

The hematocrit values of the blood samples used in sample preparation were adjusted to 50 % to correspond to the hematocrit of neonates. The blood sample series was dispensed on filter paper and dried overnight at room temperature.

A polynomial evaluation of linearity was used for the data analysis.
The significance of the second and third order polynomials were evaluated by performing a t-test. The regression analysis results showed that the third order polynomial had statistically significant non-linear terms (β_3). Thus, the third order model was compared with the linear model.

The results showed that the maximum deviation from linearity within the claimed measuring range between the measured total galactose and the best fit linear model was 6.0% at a measured value of 35.2 mg/dL.

The measured TGAL concentrations are compared to the expected concentrations. A weighted regression analysis was performed (measured vs. expected TGAL concentration), the fitted regression model is:

$$y = 1.044x + 1.211 \ (R^2 = 0.991)$$

where y = Measured TGAL concentration (mg/dL)

and x = Expected TGAL concentration (mg/dL).

3. Analytical Specificity/Interference:

The objective of the interference study was to define the effect that common substances in clinical samples might have on the GSP Neonatal Total Galactose kit. The interference study was performed in accordance with the principles described in the CLSI Guideline EP07-A2. The study used one kit lot and one GSP instrument.

Three blood pools with different total galactose concentration levels (approximately 5 mg/dL, 10 mg/dL and 15 mg/dL) covering the screening cut-off area were prepared by spiking normal human whole blood with equimolar amounts (50:50) of galactose and galactose-1-phosphate. The hematocrit values of the blood pools used in the sample preparation were adjusted to 40-55%. Samples were prepared as dried blood spots.

The control pools were prepared adding equal volume of solvent (without the interfering substances) to the base pool as was added to create the test pools. The test pools were prepared by spiking in the interfering substance at an appropriate concentration into the base pool. The added spiked substance concentration was in addition to any amount of substance already present in the sample prior to spiking. For some potential interferents, the total concentration in the sample is higher than the added concentration. A paired-difference test was performed for the potential interfering substances. A bias exceeding ±15% is considered a significant interference by the sponsor. The substances indicated in the table below were found not to interfere with the proposed device at the added concentration indicated.

Tested substance	**Added concentration with ≤15% interference**
Acetaminophen	1.38 mg/dL
Ampicillin	152 µmol/L
Ascorbate	6 mg/dL
Bilirubin (conjugated)	8.3 mg/dL
Bilirubin (unconjugated)	20 mg/dL
D-fructose	18 mg/dL
D-glucose	1000 mg/dL
D-mannose	100 mg/dL
Glutathione	3 mmol/L
HSA	30 mg/mL
Hemoglobin	223 g/L
Intralipid	125 mg/dL
NADH	100 µmol/L

The sponsor's packages insert states:

> Acetaminophen and conjugated bilirubin were found to interfere with the assay by decreasing the measured total galactose concentration. Acetaminophen concentrations above 1.38 mg/dL and conjugated bilirubin concentrations above 8.3 mg/dL may cause a false negative screening result for a sample with measured total galactose concentration close to the cutoff value.
>
> Intralipid (used to mimic high triglycerides concentration interference) was found not to interfere up to added concentrations of 125 mg/dL (total concentration of 174 mg/dL) at 5 mg/dL total galactose, up to 375 mg/dL (total concentration of 424 mg/dL) at 10 mg/dL total galactose, and up to 500 mg/dL (total concentration of 549 mg/dL) at 15 mg/dL total galactose. When present above these amounts of triglycerides may cause a false positive screening result for a sample with measured total galactose concentration close to the cutoff value.
>
> In addition, hemoglobin in combination with elevated bilirubin concentration of 15 mg/dL was found to interfere with the assay by increasing the measured total galactose concentration (see the table below). Therefore, a hemoglobin level at 237 g/L and above in combination with elevated bilirubin levels may cause a false positive screening result for a sample with measured total galactose concentrations close to the cut-off value.

Total Galactose conc. (mg/dL)	Hemoglobin concentration tested g/L at bilirubin level 15 mg/dL	Percent change in measured galactose (%)	Significant change
5	103	-11.7	No
	204	10.3	No
	223	7.1	No
	237	17.6	Yes
10	103	-5.9	No
	204	14.2	No
	223	12.1	No
	237	17.6	Yes
15	103	-12.3	No
	204	6.9	No
	223	12.0	No
	237	13.2	No

Hematocrit levels from 35% to 65% (Hemoglobin levels 12–22 g/dL, i.e. 120–220 g/L) were studied and found not to interfere at total galactose concentrations of 5, 10, and 15 mg/dL.

4. Assay Reportable Range:

The claimed measuring range for the GSP Neonatal Total Galactose kit is 1.2 – 50 mg/dL based on the Limit of Quantitation and Linearity studies.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

As established in k133652.

6. Detection Limit:

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

Limit of the Blank (LoB): The LoB was determined separately for each reagent lot and the highest result was considered to be the most conservative estimate. The five samples for the LoB study were dried blood spot specimens prepared from five pools of washed red blood cells in sodium chloride-sucrose solution and suspended in an artificial serum solution (0.9% NaCl and 120 g/L sucrose in water). The hematocrit value was adjusted to 40-55% and dried blood spots were prepared. The samples were assayed in replicates of 6 over 5 working days using two reagent lots.

The LoB was determined for each lot using the non-parametric classical approach described in the guideline. The LoB for total galactose is 0.30 mg/dL (17 µmol/L).

Limit of Detection (LoD): The LoD was determined separately for each reagent lot and the highest result was considered to be the most conservative estimate. The six samples for the LoD study were dried blood spot specimens prepared from five pools of washed red blood cells in sodium chloride-sucrose solution and suspended in an artificial serum solution (0.9% NaCl and 120 g/L sucrose in water). The samples were then spiked with galactose and galactose-1-phosphate (50:50 molar amounts). The hematocrit value was adjusted to 40-55% and dried blood spots were prepared. The samples were assayed in replicates of 6 over 5 working days using two reagent lots.

LoD was set at the concentration where the 95th percentile of measurement results exceeds LoB. The LoD was calculated to be 0.71 mg/dL

Limit of Quantitation (LoQ): The LoQ was determined separately for each reagent lot and the highest result was considered to be the most conservative estimate. The LoD samples were also used to determine LoQ.

The LoQ is defined as the lowest concentration with a total CV equal to or less than 20%. The LoQ for total galactose is 1.22 mg/dL (68 μmol/L).

The sponsor's claimed measuring range of the device is 1.2 – 50 mg/dL.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   See C3 below for screening performance.

2. Matrix Comparison:

   Not applicable. This assay only uses neonatal dried blood spots on filter paper.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   The screening performance of the candidate device was evaluated in a clinical study at a US newborn screening laboratory. In the study, 2,154 routine newborn screening samples were

tested. The study population was enriched with 7 archived confirmed positive galactosemia newborn dried blood spot specimens added in a blinded fashion.

The 7 confirmed positive specimens consisted of 6 cases of classic galactosemia and 1 case of Duarte galactosemia.

All the assayed specimens, i.e. the routine specimens and retrospective confirmed galactosemia positive specimens, were classified as screen positive or screen negative based on the measured galactose concentrations. The classification was performed using 99.5%, 99.0% and 95.0% percentile-based cutoffs (see values in the table below).

	N	Min	Max	Mean	Median	99.5%	99.0%	95%
GSP	2154	0.0	11.3	1.5	1.0	7.9	6.8	4.1
Predicate	2154	0.0	11.4	1.0	0.5	8.4	7.0	3.9

Screening results:

Summary of accuracy – 99.5th percentile (All specimens)				
		Predicate device (8.4 mg/dL)		
		Positive	Negative	Total
GSP (7.9 mg/dL)	Positive	13*	2	15
	Negative	4***	2142**	2146
	Total	17	2144	2161

*Includes 4 clinically confirmed classic galactosemia specimens.
**Includes one clinically confirmed classic galactosemia specimen that was collected 22 hours after birth, and one clinically confirmed Duarte galactosemia specimen where the infant had not been fed a diet containing lactose prior to sampling.
***Includes one clinically confirmed classic galactosemia specimen collected 16 hours after birth.

Summary of accuracy – 99th percentile (All specimens)				
		Predicate device (7.0 mg/dL)		
		Positive	Negative	Total
GSP (6.8 mg/dL)	Positive	20*	7	27
	Negative	7***	2127**	2134
	Total	27	2134	2161

*Includes 4 clinically confirmed classic galactosemia specimens.
**Includes one clinically confirmed classic galactosemia specimen that was collected 22 hours after birth, and one clinically confirmed Duarte galactosemia specimen where the infant had not been fed a diet containing lactose prior to sampling.
***Includes one clinically confirmed classic galactosemia specimen collected 16 hours after birth.

Summary of accuracy – 95th percentile (All specimens)				
		Predicate device (3.9 mg/dL)		
		Positive	Negative	Total
GSP (4.1 mg/dL)	Positive	100*	14	114
	Negative	14	2033**	2047
	Total	114	2047	2161

*Includes 5 clinically confirmed classic galactosemia specimens.
**Includes one clinically confirmed classic galactosemia specimen that was collected 22 hours after birth, and one clinically confirmed Duarte galactosemia specimen where the infant had not been fed a diet containing lactose prior to sampling.

Results for the neonatal samples from confirmed Galactosemia patients:

Specimen no.	Predicate device (mg/dL)	GSP Neonatal Galactose (mg/dL)
1	>50	>50
2	>50	>50
3	22.3	18.1
4	22.3	25.2
5	8.5	5.9
6	2.2	2.5
7*	0	0

*Clinically confirmed Duarte galactosemia specimen where the infant had not been fed a diet containing lactose prior to sampling.

D Clinical Cut-Off:

See expected values.

E Expected Values/Reference Range:

The labeling states that each laboratory should establish its own reference range and cut-off values.

Total galactose values by percentile from the testing of routine screening specimens completed with the GSP Neonatal Galactose kit at a state U.S. laboratory:

	N	Min	Max	Mean	Median	99.5%	99.0%	95%
GSP Neonatal Galactose (mg/dL)	2137	0.0	11.3	1.5	1.0	8.0	6.8	4.1

VIII Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.