

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k071649

B. Purpose for Submission:

New device

C. Measurand:

Galactose and galactose-1-phosphate

D. Type of Test:

Quantitative, fluorescent galactose oxidase method

E. Applicant:

Wallac Oy

F. Proprietary and Established Names:

Neonatal Total Galactose Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JIA	Class I	21 CFR 862.1310 - Galactose test system	Chemistry

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

See intended use above.

3. Special conditions for use statement(s):

For prescription use only, presumptive positive results are to be tested using a confirmatory diagnostic method.

4. Special instrument requirements:

The Neonatal Total Galactose kit requires a fluorometer capable of measuring fluorescence using excitation central wavelength of 340 nm (or 320 nm) and emission central wavelength of 405 nm on 96 well microtiter plates. Performance characteristics were determined using the PerkinElmer 1420 Victor D fluorometer.

I. Device Description:

Each Neonatal Total Galactose kit contains reagents for either 960 assays or 4800 assays. The kit contains the following components:

Galactose calibrators and controls- Prepared from washed human blood, with preservative, adjusted to a hematocrit of 55% and spotted onto Whatman 903 specimen collection paper.

The six calibrators contain galactose at the following concentrations:

A	0 mg/dL	D	9.0 mg/dL
B	1.5 mg/dL	E	18 mg/dL
C	4.0 mg/dL	F	40 mg/dL

The two Normal and Abnormal Controls contain galactose and galactose-1-phosphate at approximately 190 $\mu\text{mol/L}$ blood (3.5 mg/dL) and 530 $\mu\text{mol/L}$ blood (9.5 mg/dL) respectively.

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

Zinc Sulfate Reagent -1 bottle, 30 mL
Galactose Reconstitution Buffer- 1 bottle, 240 mL
Galactose Substrate Reagent -10 bottles, dried
Galactose Oxidase Reagent- 10 bottles, lyophilized
Stop Solution- 1 bottle, 240 mL

J. Substantial Equivalence Information:

1. Predicate device name(s):
Quantase Total Galactose Screening Assay
2. Predicate K number(s):
k990654
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	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	Same
Test methodology	Enzymatic assay	Same

Differences		
Item	Device	Predicate
Reportable range	1.3-56 mg/dL (approximate)	0.6-55 mg/dL
Detection method	Fluorescence- measured at 340 nm and 405 nm wavelengths	Colorimetric- absorbance read at 570 nm and 690 nm wavelengths

K. Standard/Guidance Document Referenced (if applicable):

- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A).

L. Test Principle:

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 excitation central wavelength of 340 nm (or 320 nm) and emission central wavelength of 405 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Two studies were performed to evaluate precision of the Neonatal Total Galactose test. Samples were prepared from adult human whole blood spiked with different concentrations of galactose. The samples were then dispensed onto filter paper and dried at room temperature overnight. Since the testing was performed using dried blood spots, the extraction procedure was captured

in the evaluation process.

In study 1, three levels of spiked dried blood samples were assayed in replicates of four in 18 runs over 6 days. The testing was conducted using three kit lots, two fluorometers, and three operators. The total number of replicates was n=144. The results are summarized in the table below.

Study 1

Sample	Galactose mg/dL	Within plate variation (CV%)	Total within lot variation (CV%)	Between lot variation (CV%)	Total variation (CV%)
1	7.1	6.6	12.1	2.1	12.3
2	13.8	6.5	10.6	2.4	10.8
3	20.2	5.3	8.6	0.4	8.6

In study 2, four levels of spiked dried blood samples were assayed in replicates of four in 20 runs over 20 days. The testing was conducted using one kit lot, one fluorometer, and one operator. The results are summarized in the table below.

Study 2

Sample	Galactose mg/dL	Within plate variation (CV%)	Total variation (CV%)
A	3.5	7.8	12.7
B	11	7.2	11.8
C	23	6.0	8.4
D	36	5.2	8.6

b. *Linearity/assay reportable range:*

The study was performed following the CLSI protocol EP6-A. Whole blood heparinized sample pools were spiked with analyte to create samples with high concentrations of galactose and galactose-1-phosphate. The high samples were mixed in varying proportions with the sample pools containing very low galactose to create a dilution series. Each test sample was spotted on filter paper in quadruplicate (n=4), dried overnight, and tested.

The data was analyzed using linear regression as well as second and third order non-linear fitted polynomial regression. The second order model fit the data better than the linear model. However, for all dilution points the relative differences between the two models were within the sponsor's defined acceptance criterion of +/- 25%. The results are summarized below.

Fitted regression models are:

Linear: $y = 1.6 + 52x$

Second order: $y = 1.3 + 58x - 7.4x^2$

Third order: $y = 1.2 + 60x - 16x^2 + 7.5x^3$

Where y = Total galactose concentration (mg/dL) and x = Dilution point.

Dilution point	Predicted 1 st order mg/dL	Predicted 2 nd order mg/dL	Absolute difference mg/dL	Relative difference (%)
1	53.5	51.4	-2.1	-4
0.9	48.3	47.0	-1.3	-3
0.85	45.7	44.8	-0.9	-2
0.8	43.1	42.6	-0.6	-1
0.6	32.7	33.1	0.4	1
0.35	19.7	20.5	0.8	4
0.2	12.0	12.5	0.5	5
0.12	7.79	8.08	0.29	4
0.08	5.72	5.84	0.12	2
0.05	4.16	4.14	0.12	0
0.03	3.12	3	-0.11	-4
0	1.56	1.29	-0.27	-17

The maximum observed percent difference (–17 %) between the models was seen with the undiluted low level sample which is near the limit of detection of the test. The relative differences (%) of all other dilutions points were within ± 5 %. Therefore, the Neonatal Total Galactose kit is linear in the range 1.3 mg/dL to approximately 56 mg/dL.

The values of the highest kit calibrator may vary slightly but does not exceed 56 mg/dL. The package insert states that samples with values above the highest calibrator are not accurate and should be reported as presumptive positives.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The kit calibrators are traceable to a validated HPLC-MS/MS method. Six dried blood spot calibrators and two dried blood spot controls, normal and abnormal, are provided with the kit. Real-time stability including shelf-life, transport, and in-use stability studies for the entire kit are performed, including the controls and calibrators. Additionally, accelerated stability studies are also performed separately for the kit calibrators and controls. The study protocols and statistically calculated acceptance limits were reviewed and found to be acceptable.

d. *Detection limit:*

The sponsor states that the limit of blank (LoB) study was performed in accordance with the recommendations of CLSI EP-17. The LoB was calculated from data using two kit lots over five testing days and a total of 100 measurements of the zero calibrator. Calibrators and controls were tested in duplicate for each assay. Since the assay does not report values less than zero, the LoB was estimated non-parametrically, as CLSI EP-17 advises, as the 95th percentile of the measurements. The limit of blank was observed to be 0.7 mg/mL.

To estimate the limit of detection (LoD), five samples with low levels of galactose were also assayed in the same study for a total of 100 measurements at each level. These samples were prepared from heparinized whole blood with added galactose and galactose-1-phosphate, spotted onto filter paper, and dried. Statistical analysis yielded a limit of detection of 1.3 mg/dL.

The package insert states that values below 1.3 mg/dL should be reported as “<1.3 mg/dL.”

e. *Analytical specificity:*

Interference from elevated lipid, bilirubin, and hemoglobin was evaluated at a galactose concentration of approximately 10 mg/dL. The sponsor used a two sample t-test at the 95% confidence interval to evaluate the significance of any difference seen between test and control samples.

Bilirubin at concentrations ranging from 10-40 mg/dL, hemoglobin at 20 and 25 g/dL, and Intralipid at concentrations ranging from 0.25-1.0 g/dL were separately added to whole blood specimens containing galactose, spotted onto filter paper, dried, and tested. Specimens without added interferant were also tested. Specimens containing the highest levels of bilirubin and hemoglobin did not interfere with the galactose test using the sponsor's acceptance criteria. Conversely, specimens containing lipid interfered with the accurate measurement of galactose across all levels tested and resulted in an over-recovery of galactose up to 30%. This observation is likely due to the physical nature of lipid in that it interferes with the transmission of light. The results are summarized in the package insert.

The following substances were tested and found not to interfere at the levels stated: glucose (1200 mg/dL), mannose (1–10 mg/dL), fructose (25 mg/dL), ascorbate (0.1–3 mg/dL), protein BSA (12 g/dL), and acetaminophen (0.1–1 mg/dL). Glutathione at concentrations up to 6 mg/dL did not interfere, however a concentration of 60 mg/dL, which far exceeds the expected concentration in newborns, caused under-recovery of approximately 40%. This is likely due to glutathione's chemical properties and interference in the assay's enzymatic reaction. The results are summarized in the package insert.

- f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 2109 specimens from newborns were obtained prospectively from a state public health laboratory's routine newborn screening program. Additionally, seven (7) specimens were also tested that were retrospective high galactose samples of which three (3) were from newborns with diagnosed galactosemia. Dried blood spots were assayed using the Perkin Elmer total galactose kit and a commercially available comparator method. The presumptive positive cut-off was determined from the results using each kit's 95th and 99th percentiles. For the Perkin Elmer kit these values were 5.1 mg/dL and 8.3 mg/dL respectively, while the values for the comparator method were 4.2 mg/dL and 9.4 mg/dL, respectively. The results are summarized in the tables below.

95th Percentile Results

Screening summary using the 95 th percentile		Commercially available kit (4.2 mg/dL)		
		+	-	Total
PerkinElmer kit (5.1 mg/dL)	+	73	46	119
	-	41	1956	1997
	Total	114	2002	2116

Screening summary using the 95 th percentile				
Commercially available kit	PerkinElmer kit	Total	Diagnosed galactosemia	No diagnosed galactosemia
+	+	73	3	70
+	-	41	0	41
-	+	46	0	46
-	-	1956	0	1956
Total		2116	3	2113

The overall percent agreement using the 95th percentile is 2029/2116 or 95.9%.

99th Percentile Results

Screening summary using the 99 th percentile		Commercially available kit (9.4 mg/dL)		
		+	-	Total
PerkinElmer kit (8.3 mg/dL)	+	21	9	30
	-	8	2078	2086
	Total	29	2087	2116

Screening summary using the 99 th percentile				
Commercially available kit	PerkinElmer kit	Total subjects	Diagnosed galactosemia	No diagnosed galactosemia
+	+	21	3	18
+	-	8	0	8
-	+	9	0	9
-	-	2078	0	2078
Total		2116	3	2113

The overall percent agreement using the 99th percentile is 2099/2116 or 99.2%.

The results of the three confirmed true galactosemia samples are shown below.

Total Galactose concentrations of the true positive specimens		
Specimen no.	PerkinElmer kit (mg/dL)	Commercially available kit (mg/dL)
1	16.6	23.9
2	12.1	14.6
3	22.2	39.1

b. Matrix comparison:

Not applicable. The device is to be used only with neonatal whole blood.

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):

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The summary of the results from the 2109 newborn specimens tested with this kit is shown in the table below:

	$\mu\text{mol/L}$	mg/dL
Median	94	1.7
95th percentile	283	5.1
97.5th percentile	355	6.4
99th percentile	461	8.3
99.5th percentile	549	9.9

The package insert includes precautionary language that each laboratory should establish their own reference range and cut-off values and that cut-offs from another galactose screening test should not be used. Also included in the labeling is the recommendation that presumptive positive samples be tested with a confirmatory diagnostic method and to follow local requirements for follow up testing.

N. Proposed Labeling:

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

O. Conclusion:

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