

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

**A. 510(k) Number:**

k133652

**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:**

GSP Neonatal Total Galactose kit

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.1310 - Galactose test system

2. Classification:

Class I, reserved

3. Product code:

JIA

4. Panel:

Chemistry

## H. Intended Use:

1. 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.

2. Indication(s) for use:

See intended use above.

3. Special conditions for use statement(s):

For prescription use only, presumptive positive screening results for Galactosemia require confirmatory diagnostic or follow-up testing.

4. Special instrument requirements:

For use on the GSP instrument only.

## I. Device Description:

The GSP Neonatal Total Galactose kit contains sufficient reagents to perform 1152 assays. The 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. 6 levels of calibrators are included: A – 0.5 mg/dL, B – 2.5 mg/dL, C – 5.0 mg/dL, D – 10.0 mg/dL, E – 20 mg/dL, F – 50 mg/dL
- 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.
- Neonatal Total Galactose Assay Reagent 1 (trehalose, N-(2-acetamido)-2-aminoethanesulfonic acid (ACES), 10-Acetyl-3,7-dihydroxyphenoxazine, 4-hydroxyphenylacetic acid, horseradish peroxidase, and superoxide dismutase from bovine erythrocytes) – 3 lyophilized vials
- Neonatal Total Galactose Assay Reagent 2 (cupric sulfate (< 0.02 %), trehalose, ACES, galactose oxidase from *Dactylium dendroides*, and alkaline phosphatase from bovine intestine.) – 3 lyophilized vials
- Neonatal Total Galactose Assay Buffer (ACES and ProClin® 300) – 3 bottles, 40 ml

- Neonatal Total Galactose Assay Reconstitution Solution (dimethyl sulfoxide and water)– 1 bottle, 20 ml
- Neonatal Extraction Solution (zinc sulfate (< 2 %) and 2-propanol (~40 %)) – 1 bottle, 60 ml

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.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Wallac Oy Neonatal Total Galactose Kit

2. Predicate 510(k) number(s):

k071649

3. Comparison with predicate:

<b>Similarities and Differences</b>		
<b>Item</b>	<b>Proposed Device</b>	<b>Predicate (k071649)</b>
Intended Use/Indications for Use	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.	Same
Test Methodology	Enzymatic assay	Same
Detection Method	Fluorescence – measured at 505 nm and 580 nm wavelengths	Fluorescence – measured at 340 nm and 405 nm wavelengths
Instrument Platform	GSP instrument, automated (originally reviewed under k090846)	Fluorometer, manual
Sample Type	Dried blood spot	Same
Calibrators	A – 0.5 mg/dL B – 2.5 mg/dL C – 5.0 mg/dL D – 10.0 mg/dL E – 20 mg/dL F – 50 mg/dL	A – 0 mg/dL B – 1.5 mg/dL C – 4.0 mg/dL D – 9.0 mg/dL E – 18 mg/dL F – 40 mg/dL
Reportable Range	1.15 – 50 mg/dL	1.3 – 40 mg/dL

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

CLSI document EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*

CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*

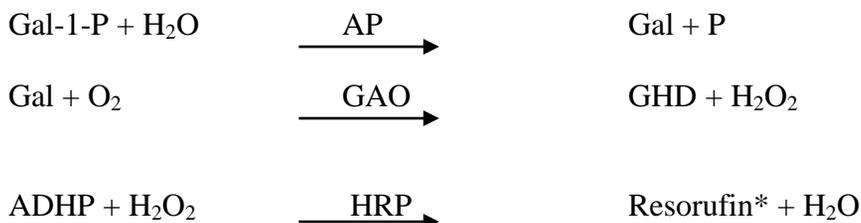
CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*

CLSI Protocol EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

CLSI Protocol EP9-A: *Method Comparison and Bias Estimation Using Patient Samples*

**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 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:



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

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

The study was performed using three lots of the GSP Neonatal Total Galactose kit, three operators, and three GSP instruments in 54 runs on 21 operating days using 7 dried blood spot samples. The samples PS2-PS7 were prepared by spiking galactose and galactose-1-phosphate with equimolar concentrations into heparinized whole blood. In PS1 washed human red blood cell concentrate was used to minimize endogenous total galactose. The hematocrit value of the blood used in the sample preparation was adjusted to 40-55 % and dried blood spots were prepared. The precision of GSP Neonatal Total Galactose kit were calculated with a full calibration curve in duplicate for each plate. Two plates were used with 4 replicates of each sample tested. Precision results are summarized in the tables below:

Sample	n	Mean TGAL (mg/dL)	Within run		Within lot		Between lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
PS1	216	3.13	0.34	10.7	0.37	11.9	0.07	2.2	0.38	12.1
PS2	216	5.15	0.43	8.3	0.48	9.2	0.06	1.1	0.48	9.3
PS3	216	8.61	0.69	8.0	0.81	9.4	0.20	2.4	0.83	9.7
PS4	216	11.8	0.98	8.3	1.16	9.8	0.39	3.3	1.22	10.3
PS5	216	16.8	1.39	8.3	1.73	10.3	0.64	3.8	1.84	11.0
PS6	216	32.2	2.70	8.4	3.35	10.4	1.59	4.9	3.70	11.5
PS7	216	45.6	3.60	7.9	5.85	12.8	2.62	5.8	6.41	14.1

b. *Linearity/assay reportable range:*

The claimed measuring range for the GSP Neonatal Total Galactose kit is 1.15 – 50 mg/dL based on the Limit of Quantitation (see d. below) and Linearity studies.

The linearity was assessed in this study following the principles described in CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. The study used one kit lot and one GSP instrument. A full calibration curve in duplicate was included in the plate. Each sample and control were measured in four replicates, the samples were positioned in a random order on the plate.

The samples used were dried blood spot specimens prepared from heparinized whole blood. Blood drawn from one apparently healthy adult represented a “high activity” sample. The hematocrit value of the blood was adjusted to 40% - 55% to correspond to the hematocrit of neonates. This unspiked blood represented a “low concentration” sample. A part of the blood was separated and equimolar concentrations of galactose and galactose-1-phosphate were added to obtain a “high concentration” total galactose sample. Samples with intermediate concentrations were prepared by mixing the “low concentration” TGAL sample with the “high concentration” sample.

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 second order polynomial had statistically significant non-linear terms ( $\beta_2$ ). Thus, the second order model was compared with the linear model.

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

$$y = 0.96x - 1.11 (R^2 = 0.99)$$

where  $y$  = Measured TGAL concentration (mg/dL)

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

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

Traceability: There is no available internationally accepted reference material or a reference method for total galactose. The calibration of the GSP Neonatal Total Galactose kit has been traceable to the in-house reference calibrator series (primary calibrators). The reference calibrator series include six levels, A-F, as dried blood spots on filter paper. The reference calibrators have been manufactured using adult human blood (endogenous biotinidase activity in serum) and washed red blood cells as blood matrices.

Value assignment: The in-house reference materials of the GSP Neonatal Total Galactose kit include primary calibrators, secondary calibrators, level calibrators 1-4, QA controls Low and High “component” stage calibrators (Component Calibrators) and controls (Component QC) and, final “kit configuration” or combination assignment of the kit calibrators (Kit Cals) and kit controls (Kit QC). The primary calibrators are used to monitor the level of secondary calibrators and level calibrators. An initial primary calibrator lot is prepared adding gravimetrically determined amounts of Galactose to human red blood cell matrix (washed red blood cell concentrate in sucrose solution). The final concentrations of the manufactured GSP Neonatal Total Galactose primary calibrators are based on gravimetrically determined amounts of added galactose taking into account the endogenous level of the blood suspension. Kit calibrators and controls are value-assigned using an internal procedure, with testing using multiple replicates on multiple instruments

Kit calibrator contains 6 levels of calibrators: A – 0.5 mg/dL, B – 2.5 mg/dL, C – 5.0 mg/dL, D – 10.0 mg/dL, E – 20 mg/dL, and F – 50 mg/dL.

Kit control contains 2 levels of controls: Level 1= 4.0 mg/dL, Level 2= 12.0 mg/dL.

Stability: The results of the accelerated and real time stability studies support a shelf life of at least 12 months for all components of GSP Neonatal Total Galactose kit, when stored at +2 to +8°C (Assay Reagent 1, Assay Reagent 2, Assay Buffer, Reconstitution Solution and Extraction Solution) or at -30 to -16°C (kit calibrators and controls). After opening, the Kit Calibrators and Controls should be stable at 2 to 8 °C for 7 days. Neonatal Extraction Solution can be stored on-board in the reagent storage carousel of the GSP instrument for up to 14 days. Assay Reagent 1, Assay Reagent 2 and Assay Buffer can be stored on-board in the reagent storage carousel of the GSP instrument for up to 7 days. Microplates with punched calibrators and controls in wells can be stored on-board in the GSP instrument plate storage for 12 hours.

Stability testing protocols and acceptance criteria for stability testing has been reviewed and found to be acceptable.

Sample stability: The objective of this sample stability study was to determine the short term stability of dried blood spot specimens spiked with galactose and/or galactose-1-phosphate. The test was performed using different storage conditions.

The sample stability study was performed using one GSP Neonatal Total Galactose kit lot and two GSP instruments. At the zero time point each sample was analyzed in 16 replicates (4 replicates per plate) and in the other 5 time points (1, 5, 7, 9, and 14 days) each sample was analyzed in 8 replicates (4 replicates per plate).

The nine samples were dried blood spot specimens prepared from heparinized human whole blood spiked with either a.) galactose b.) galactose-1-phosphate or c.) equal molar concentrations of galactose and galactose-1-phosphate. The zero time point samples were analyzed immediately after the overnight drying while the samples for the other 5 time points were stored after the overnight drying in various storage conditions. The sample stability is better at 2 to 8 °C than 21°C and 35 °C, if samples are protected from moisture.

*d. Detection limit:*

The objective of the study was to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the GSP Neonatal Total Galactose kit. The analysis of the LoB, LoD and LoQ were performed in accordance with CLSI document EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.

The LoB study was performed using two lots, the LoQ / LoD study was performed using three lots of the GSP TGAL kit and three GSP instruments.

Limit of the Blank (LoB): The samples for LoB study were dried blood spots specimens prepared from washed red blood cells in sodium chloride-sucrose solution. The samples were subjected to short term stressful environmental conditions to

decrease the endogenous total galactose concentrations of the samples and stored long term in  $-16^{\circ}\text{C}$  –  $-30^{\circ}\text{C}$ . The hematocrit value was adjusted to 40-55% and dried blood spots were prepared.

The samples were measured with two GSP Neonatal Total Galactose kit lots using two GSP instruments. Each plate included a full calibration curve in duplicate and the results were analyzed using a plate specific calibration curve. LoB was calculated based on 150 repeated measurements of five blank samples per one kit lot. Specifically, repeated measurements ( $n = 60/\text{sample}$ ) were carried out for the blank (analyte free) dried blood spot samples on filter paper. The Kit Controls Low and High, in quadruplicates ( $n = 4$ ), were included in each plate and used for run acceptance. The five LoB samples were assayed with six replicates in ten separate runs performed over five operating days.

The LoB for total galactose is 0.34 mg/dL ( $19\ \mu\text{mol/L}$ ), defined as the 95<sup>th</sup> percentile of a distribution of blank samples.

Limit of Detection (LoD) and Limit of Quantitation (LoQ): The samples for the LoD/LoQ study were prepared from washed human red blood cells in sodium chloride-sucrose solution by spiking with equimolar galactose and galactose-1-phosphate. The hematocrit value was adjusted to 40-55 % to correspond to the hematocrit of neonates. The LoD/LoQ samples were spotted on to Whatman 903 filter paper ( $75\ \mu\text{L}/\text{spot}$ ), dried overnight at room temperature in a fume hood and stored at  $-16^{\circ}\text{C}$  –  $-30^{\circ}\text{C}$  in sealed bags with desiccant. The LoD/LoQ samples were measured with three GSP Total Galactose kit lots using three GSP instruments. Repeated measurements ( $n = 216$ ) were carried out using four low level DBS. Each plate included a full calibration curve in duplicate and the results were analyzed using a plate-specific calibration curve and a calibration curve for the batch of two plates. The Kit Controls Low and High in quadruplicate ( $n = 4$ ) were included in each plate and used for run acceptance. Altogether 27 runs were assayed during 20 operating days using three GSP instruments and three GSP Neonatal Total Galactose kit lots.

The LoQ is 1.15 mg/dL ( $64\ \mu\text{mol/L}$ ), defined as the lowest concentration with a total CV equal to or less than 20 %.

The sponsor's claimed measuring range of the device is 1.15 – 50 mg/dL.

*e. Analytical specificity:*

The objective of the study was to evaluate the effect of potential interfering substances in dried blood spot samples on the measurement of GSP Neonatal Total Galactose kit. The interference study was performed in accordance with the principles described in CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline. The study used one kit lot and one GSP instrument.

The samples were dried blood spot specimens prepared from heparinized whole blood

spiked with equimolar concentrations of galactose and galactose-1-phosphate. Three clinically relevant total galactose concentrations (5, 10 and 15 mg/dL) were used. The hematocrit level was adjusted to 40-55% to correspond to the hematocrit of neonates. 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. A paired-difference test was performed for the potential interfering substances. Both test and control pools were analyzed within one analytical run with 12 replicates for all substances. A bias exceeding  $\pm 15\%$  is considered a significant interference. The tested compounds are listed in the following table:

Tested substance	Concentration with $\leq 15\%$ interference
Acetaminophen	2.75 mg/dL
Ampicillin	152 $\mu\text{mol/L}$
Ascorbate	6 mg/dL
Bilirubin (conjugated)	16.6 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	102 g/L
Intralipid	250 mg/dL
NADH	100 $\mu\text{mol/L}$

The sponsor's packages insert states:

Intralipid was found not to interfere up to added concentrations of 250 mg/dL at 5 and 10 mg/dL total galactose; and up to 375 mg/dL at 15 mg/dL total galactose. When present above these amounts Intralipid may cause a false positive screening result for a specimen with measured total galactose concentration close to the cut-off 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, hemoglobin level at 198 g/L and above in combination with elevated bilirubin level may cause a false positive screening result for a specimen with measured total galactose concentration close to the cut-off value.

Hematocrit levels from 30% to 66% (Hemoglobin levels 102–230 g/L) were found not to interfere at total galactose concentrations of 5, 10 and 15 mg/dL.

*f. Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was performed to compare the candidate device (GSP Neonatal Total Galactose kit) to the predicate device (Neonatal Total Galactose kit). A total of 141 dried blood spot specimens, including newborn patient routine sample and adult whole blood dried specimens spiked with galactose and galactose-1-phosphate were used in the study analysis. The weighted Deming regression of the data yields the following equation:

$$y = 1.16x - 0.49; 95\% \text{ CI: slope } (1.07; 1.26), \text{ intercept } (-0.73; -0.25), N=141$$

The slope showed an approximate bias of 16% between the candidate device and the predicate device; however, the purpose of this device modification was adjust the calibration to better align with the CDC reference sample concentration target levels. Therefore, test results from the candidate device do not, and are not expected to, directly correlate with test results from the predicate device.

A clinical study (see Section M.3.c below) was conducted to evaluate the screening performance between the candidate device and the predicate device using their respective cut-offs values. The screening performance of both devices is similar (see tables below) and found to be acceptable. The clinical screening performance, not the method comparison results, was the basis of the substantial equivalence determination.

b. *Matrix comparison:*

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

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):

Screening study at state public health laboratory: A study to evaluate the screening performance of the GSP Neonatal Total Galactose kit compared to the predicate device was conducted at a U.S. state public health laboratory that routinely performs newborn screening. The sample results for six retrospective samples from newborns

diagnosed with Galactosemia and 2159 leftover routine newborn samples were included in the study. Two GSP instruments and one kit lot of the new device were used.

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	2314	1.15	17.3	2.3	1.6	10.5	8.5	5.6
Predicate	2314	1.3	40	2.9	2.4	11.7	9.4	5.9

Screening results:

Summary of accuracy – 99.5 <sup>th</sup> percentile (All specimens)				
		Predicate device		
		Positive	Negative	Total
GSP	Positive	10*	9	19
	Negative	8	2293	2301
	total	18	2302	2320

Overall percent agreement =  $(10+2293)/2320 \times 100\% = 99.3\%$

Summary of accuracy – 99 <sup>th</sup> percentile (All specimens)				
		Predicate device		
		Positive	Negative	Total
GSP	Positive	16*	14	30
	Negative	14	2276	2290
	total	30	2290	2320

Overall percent agreement = 98.8%

Summary of accuracy – 95 <sup>th</sup> percentile (All specimens)				
		Predicate device		
		Positive	Negative	Total
GSP	Positive	82*	45	127
	Negative	47	2146	2193
	total	129	2191	2320

Overall percent agreement = 96%

\* The six samples from newborns confirmed to have Galactosemia were screen positive with both the predicate and the GSP Neonatal Galactose kit with all three cut-offs (see below). The remaining samples were from unaffected newborns (false positives).

Results for the neonatal samples from confirmed Galactosemia patients:

Specimen no.	Predicate device (mg/dL)	GSP Neonatal Galactose (mg/dL)
1	19.2	29
2	23.9	17
3	20.4	25.2
4	37.5	>50
5	30.8	31.4
6	>40	>50

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

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)	2314	1.15	17.3	2.3	1.6	10.5	8.5	5.6

In the labeling the manufacturer recommends that each laboratory should establish its own reference range and cut-off.

**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.