

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

A. 510(k) Number:

k103484

B. Purpose for Submission:

New Device

C. Measurand:

Thyroxine (T4) from dried blood spots

D. Type of Test:

Quantitative Time-resolved Fluoroimmunoassay

E. Applicant:

Wallac Oy

F. Proprietary and Established Names:

GSP Neonatal Thyroxine (T4) kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
KLI, Enzyme Immunoassay, Non-Radiolabeled, Total Thyroxine	Class II	21 CFR § 862.1700, Total thyroxine test system.	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The GSP Neonatal Thyroxine (T4) kit is intended for the quantitative determination of human thyroxine (T4) in blood specimens dried on filter paper as an aid in screening newborns for congenital (neonatal) hypothyroidism using the GSP instrument.

3. Special conditions for use statement(s):

For prescription use only.

The sponsor states in the labeling that the data obtained using the GSP Neonatal

T4 blood spot immunoassay should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician. Samples giving T4 values below the cut-off limit should be repeated and confirmed. hTSH determination in combination with T4, FT4 or T3 can be used in confirming the presence or absence of congenital hypothyroidism.

Heterophilic antibodies in the sample may interfere with the assay. Hematocrit values in the blood samples may affect the measured thyroxine concentration.

Screening for congenital hypothyroidism by measurement of T4 concentrations requires a decision of the screening policy, definition of tiers, cut-off values and follow-up. The laboratory needs to establish its own cut-off values, which can be based on a percentile, or on the basis of a normal range. The cut-off values should be monitored and reassessed when necessary. Interpretation of results and the recommended follow-up algorithm for congenital hypothyroidism screening is described by American Academy of Pediatrics/American Thyroid Association American Academy of Pediatrics (*Pediatrics*. 2006 **117**:2290-2303).

4. Special instrument requirements:
For use on the GSP instrument only.

I. Device Description:

The GSP Neonatal Thyroxine (T4) kit is comprised of the following components.

Reagent	Description
Neonatal Thyroxine (T4) calibrators and controls	Prepared from human blood with a hematocrit value of 50-55% and calibrated against the L-Thyroxine (IRMM-468). The six calibrators contain concentrations of added T4 at approximately 0, 2, 4, 8, 16 and 30 µg/dL serum. The three controls contain approximate T4 concentrations of 3, 7 and 12 µg/dL serum.
T4-Eu tracer	The tracer is in Tris-HCl buffered (pH 7.8) salt solution containing bovine serum albumin, mouse IgG, and < 0.1% sodium azide as preservative.
T4 antibody	The mouse monoclonal antibody is in Tris-HCl buffered (pH 7.8) salt solution containing bovine serum albumin and < 0.1% sodium azide as preservative.
T4 Assay Buffer	Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, bovine globulin, Tween 40, 8-anilino-1-naphthalenesulfonic acid (ANS), sodium salicylate, rabbit IgG, an inert red dye, and < 0.1% sodium azide as preservative.
Anti-Mouse IgG Microtitration Strips	Plates coated with anti-mouse IgG antisera.

This kit contains calibrators and controls manufactured from human blood components. The human blood has been tested using FDA approved methods or equivalent and found to be negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies.

J. Substantial Equivalence Information:

Predicate device name	Predicate 510(k) number
AutoDELFLIA Neonatal Thyroxine T4	k943416

Comparison with predicate:

Similarities and Differences		
Item	Proposed Device	Predicate Device (k943416)
Intended Use	Same	The quantitative determination of human thyroxine (T4) in blood specimens dried on filter paper as an aid in screening newborns for congenital (neonatal) hypothyroidism.
Test Principle	Same	Solid phase time-resolved fluoroimmunoassay
Specimen	Same	Newborn Dried Blood Spots on filter paper
Calibrators	Same	Six Levels
Controls	Same	Three Levels
Analyzer	GSP Instrument	1235 AutoDELFLIA Instrument

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods.

CLSI EP7-A2: Interference Testing in Clinical Chemistry.

CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples.

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

EN13640: Stability Testing of In Vitro Diagnostic Reagents.

L. Test Principle:

The GSP Neonatal T4 assay is a solid phase time-resolved fluoroimmunoassay based on the competitive reaction between europium-labeled T4 and sample T4 for a limited amount of binding sites on T4 specific monoclonal antibodies (derived from mice). The use of 8-anilino-1-naphthalenesulfonic acid (ANS) and salicylate in the T4 Assay Buffer facilitates the release of T4 from the binding proteins. Thus the assay measures the total amount of T4 in the test specimen. A second antibody, directed against mouse IgG, is coated to the solid phase, and binds the IgG thyroxine complex, giving convenient separation of the antibody bound and free antigen.

DELFLIA Inducer dissociates europium ions from the labeled antibody into solution where they form highly fluorescent chelates with components of DELFLIA Inducer. The fluorescence in each well is then measured. The fluorescence of each sample is inversely proportional to the concentration of T4 in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Three GSP Neonatal Thyroxine (T4) kit controls (C1, C2, C3) and five precision samples (PS1 - PS5) were used for determining the precision. The three controls (C1, C2, C3) and two precision samples (PS1 and PS2) were prepared from a suspension of washed red blood cells and filtered serum. These samples were spiked with thyroxine. Three precision samples (PS3 – PS5) were prepared from a whole blood specimen drawn from one apparently healthy adult and spiked with thyroxine. The hematocrit of the samples was adjusted to correspond to the hematocrit of neonates (approximately 50-55%). Dried blood spot samples for testing were prepared and analyzed in duplicate with one GSP Neonatal Thyroxine (T4) kit lot and one GSP Instrument over ten days.

The within-run and between-run variations were calculated as instructed in the CLSI EP5-A2 for single-run per day experiments. The total variation is the sum of the within and between-run variations.

Summary of Precision Study Data

Sample	n	Mean µg/dL	Within Run		Between Run		Total	
			SD	CV%	SD	CV%	SD	CV%
C1	20	3.3	0.32	9.5	0.00	0.00	0.32	9.5
C2	20	7.4	0.47	6.3	0.13	1.7	0.48	6.6
C3	20	12.9	0.68	5.3	1.02	7.9	1.23	9.5
PS1	20	2.2	0.31	13.7	0.08	3.4	0.31	14.1
PS2	20	4.5	0.38	8.3	0.30	6.7	0.48	10.6
PS3	20	8.7	0.81	9.3	0.30	3.5	0.86	9.9
PS4	20	11.5	0.99	8.6	1.04	9.0	1.44	12.5
PS5	20	25.5	2.13	8.4	0.08	0.3	2.13	8.4

b. *Linearity/assay reportable range:*

A low and high concentration blood suspension samples were prepared using washed red blood cell and filtered serum. The high concentration sample was spiked with thyroxine. The hematocrit of the samples was adjusted to correspond to the hematocrit of neonates (approximately 50-55%). A dilution series of 12 samples, ranging from 0 to 36 µg/dL thyroxine, was prepared from the low and high concentration samples. Dried blood spot samples for testing were prepared and dried overnight. The thyroxine concentrations of the dilution series of dried blood spot samples were measured with two GSP Neonatal Thyroxine (T4) kit lots and two GSP Instruments. The samples were

analyzed in three replicates.

A polynomial evaluation of linearity was used for the data analysis. The assumption of constant variance across all levels is not fulfilled in the GSP Neonatal Thyroxine (T4) kit. Rather, the variance is proportional across different measurement levels. Therefore, weighted regression models were used. A linear regression line and second and third order polynomials were fitted to the data and the results of regression analyses were compared. The significance of the second and third order polynomials were evaluated by performing a t-test. The second order regression had a statistically significant nonlinear term (β_2) at a 95% significance level (p-value 0.08). while the third order regression was not statistically significant. For Thyroxine (T4) concentrations $> 2 \mu\text{g/dL}$ serum, the maximum observed difference between the linear regression models was 5.4 %. For concentrations $\leq 2 \mu\text{g/dL}$ serum, the observed absolute difference between the models was 0.13 $\mu\text{g/dL}$.

The linearity study data supports a claimed measuring range of 1.6 to 30 $\mu\text{g/dL}$ serum.

In the labeling the sponsor recommends that samples that result in values below 1.6 $\mu\text{g/dL}$ serum are to be reported as "< 1.6 $\mu\text{g/dL}$ ". Likewise, samples that result in values above 30 $\mu\text{g/dL}$ serum are recommended to be reported as "> 30 $\mu\text{g/dL}$ serum".

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

Calibrators and Controls

The Neonatal Thyroxine (T4) kit calibrators and controls are dried blood spots (DBS) on filter paper in sheet format prepared from T4 reference material and washed red blood cells diluted in filtered human serum (with a final hematocrit value of 50-55%). The six calibrators contain concentrations of added T4 at approximately 0, 2, 4, 8, 16 and 30 $\mu\text{g/dL}$ serum. The three controls contain approximate T4 concentrations of 3, 7 and 12 $\mu\text{g/dL}$ serum. The calibrators and controls are stored at 2 to 8 °C.

Certified reference material for L-Thyroxine (IRMM-468) is used to prepare primary calibrators for GSP Neonatal Thyroxine (T4) kit. The reference material is diluted in 80 mM NaOH solution and the concentration of the stock solution is gravimetrically determined. The stock solution is added to T4-free human serum and washed red blood cells. The secondary calibrators, which are prepared gravimetrically from the primary calibrator, are dried blood spots on Whatman paper prepared from the L-Thyroxine solution, T4-free human serum and washed red blood cells.

The level calibrator concentrations are assigned against secondary calibrators using the GSP instrument. A two-stage calibration procedure is used for kit calibrators and controls. The initial concentration values for the kit calibrators and controls are assigned against the secondary calibrators using another commercially available instrument. The final, kit lot concentrations for the kit calibrators and controls are assigned in the final release test using the level calibrators and GSP instrument.

Stability:

The performance of three different lots of GSP Neonatal Thyroxine T4 kit were tested in real time stability studies at the following time points (0, 1, 2, 3, 6, 9, 12, 13, 18 and 19 months). The kit components were stored at 2 to 8°C during the real time studies. Based upon the results of the studies, the sponsor claims a shelf life of 18 months.

A shipping stability study was performed using two different lots of GSP Neonatal Thyroxine T4 kit. The kit components were sequentially exposed to the following shipping conditions (18 hours at -20°C, 6 hours at 35°C, 6 days at room temperature and 6 hours at -20°C) and then the performance was evaluated at the following time points (0, 1, 2, 3, 6, 9, 12, 13, 18 and 19 months). Based upon the results of the shipping condition studies, the sponsor claims a shelf life of 18 months.

In-use and on-board stability studies were performed using a single lot of GSP Neonatal Thyroxine T4 kit. Different components of the kit have different stabilities as described below. Once opened, the calibrators, controls and plates can be stored for 14 days at 2 to 8°C. The on-board stability of tracer, antibody and assay buffer is 14 days at 10°C. The anti-mouse IgG microtitration strips with punched calibrators and controls in wells can be stored on-board for 12 hours at 2 to 8°C.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was determined in accordance with the CLSI Guideline EP 17-A.

Limit of Blank: Three blank pools were prepared using washed red blood cell and filtered serum. The hematocrit of the specimens was adjusted to correspond to the hematocrit of neonates (approximately 50-55%). Blank dried blood spot samples for testing were prepared and dried overnight. Repeated measurements (n = 216, n = 72 / specimen, n = 24 / specimen / kit lot) were carried out using the samples. 18 separate runs were performed using three kit lots and three GSP instruments over six days. The Limit of Blank, which was estimated non-parametrically as the 95th percentile of the measurements, was determined to be 0.457 µg/dL.

Limit of Detection and Limit of Quantitation: A blood suspension made of washed red blood cells and filtered human serum was spiked with thyroxine to obtain four different low concentrations (approximately 0.5, 1, 1.5, and 2 µg/dL). The hematocrit of the specimens was adjusted to correspond to the hematocrit of neonates (approximately 50-55%). Dried blood spot samples for testing were prepared and dried overnight. Repeated measurements (n = 216 / specimen) were carried out using the four dried blood spot specimens. The runs were performed using three kit lots and three GSP instruments over 27 days. The Limit of Detection (LoD) was calculated using the formula $LoD = LoB + c_{\beta} SD_s$ and determined to be 0.99 µg/dL.

In absence of a recognized reference method, a functional sensitivity study was used to define Limit of Quantitation (LoQ). LoQ is the lowest concentration of thyroxine that can be measured with acceptable total variation of the assay ($CV\% < 20\%$). Limit of Quantitation was calculated using the formula $LoQ = SD/CV$ and determined to be 1.61 µg/dL.

e. *Analytical specificity:*

The effect of potential interfering substances on the measurement of thyroxine concentrations in dried blood spot samples with the GSP Neonatal Thyroxine (T4) kit was evaluated in accordance with the CLSI EP7-A2. Potential interference was measured using the paired-difference method. According to this method the tested substance is added to the sample. The effect of the substance on the results is compared with the results of the control sample with no additions other than the solvent used to dissolve the substance. If a high concentration of the added substance does not demonstrate interference, no further testing is needed. If the added substance is shown to cause interference, a dilution series of the tested substance is analyzed and the effect of the concentration of the interfering factor is assayed (dose-response method). Based upon the study results, unconjugated bilirubin (up to 20 mg/dL in serum), conjugated bilirubin (up to 20 mg/dL in serum), hemoglobin (up to 15 g/L) and intralipids (up to 15 mg/mL) did not interfere with the assay.

Cross-reactivity was determined by the method of Guy Abraham known as 50% displacement method which is typical used for competitive assays. A calibrator curve was calculated for the dilution series of each substance and the thyroxine concentration at the 50% displacement level was compared to the concentrations of the tested substance at the 50% displacement level of their calibrator curves. The results are presented in the following table:

Substance	Cross reactivity %
3,3',5-Triiodo-L-thyronine (LT ₃)	1.67
3,3, 5-Triiodothyroacetic acid	0.14
3,5-Diiodo-L-thyronine	< 0.1
3,5-Diiodotyrosine (DIT) dihydrate	< 0.1

5,5-Diphenylhydantoin	< 0.1
3-iodo-L-tyrosine (MIT)	< 0.1
Phenylbutazone	< 0.1
6-N-Propyl-2-thiouracil	< 0.1
Methimazole	< 0.1
L-Tyrosine	< 0.1
3,3-Methylene-bis (4-hydroxycoumarin)	< 0.1
Acetylsalicylic acid	< 0.01

f. *Assay cut-off:*
Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

The thyroxine (T4) concentration of 90 newborn screening samples and 9 blood samples spiked with thyroxine were analyzed on the proposed and predicate devices. The T4 concentration ranged from 1.94 to 27.7 µg/dL serum. Weighted Deming regression analysis of the data yielded the following.

Regression Equation	95% Confidence Interval
$y = 1.07x - 0.23$	Slope: (1.00, 1.14) Intercept: (-0.98, 0.52)

b. *Matrix comparison:*

Not applicable since this assay uses only neonatal dried blood spots.

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):

Clinical data from newborn screening specimens tested at two U.S. state laboratories are presented below. At both sites, the routine screening samples were obtained from the samples submitted to the laboratories for testing by order of a physician. The confirmed positive samples were acquired from retrospective, banked samples in the possession of the laboratories.

All assayed specimens were categorized as test positive and test negative according to the cutoff recommendations for T4 measurements found in the

2006 Update of Newborn Screening and Therapy of Congenital Hypothyroidism by the American Academy of Pediatrics (*Pediatrics*. 2006 **117**:2290-2303). A test positive result is defined as a T4 concentration of the lowest 10% percentile for the assay during a predetermined time interval.

Assay	Lowest 10% percentile	
	Study 1	Study 2
GSP T4	10.5 µg/dL serum	10.6 µg/dL serum
AutoDELFIAT4	11.4 µg/dL serum	11.6 µg/dL serum

Study 1: A total of 2,337 routine left-over neonatal DBS specimens were assayed in the study including 16 retrospective Congenital Hypothyroidism (CH) positive samples. All 16 CH positive samples tested as screen positive with the GSP T4 method.

		AutoDELFIAT4		
		Screening Negative	Screening Positive	Total
GSP T4	Screening Negative	2028	57	2085
	Screening Positive	56	196	252
	Total	2084	253	2337

The overall percent agreement was 95.2%, the positive percent agreement was 77.5% and the negative percent was 97.3%.

Study 2: A total of 1,975 routine left-over neonatal DBS specimens were assayed in the study including 20 retrospective Congenital Hypothyroidism (CH) positive samples. All 20 CH positive samples tested as screen positive with the GSP T4 method

		AutoDELFIAT4		
		Screening Negative	Screening Positive	Total
GSP T4	Screening Negative	1701	56	1757
	Screening Positive	60	158	218
	Total	1761	214	1975

The overall percent agreement was 94.1%, the positive percent agreement was 73.8% and the negative percent was 96.6%.

4. **Clinical cut-off:**

Screening for congenital hypothyroidism by measurement of T4 concentrations requires a decision of the screening policy, definition of tiers, cut-off values and follow-up. In the labeling the sponsor recommends that the laboratory establish its own cut-off values, which can be based on a percentile, or on the basis of a

normal range. The cut-off values should be monitored and reassessed when necessary.

5. Expected values/Reference range:

The T4 patient values by percentile from the testing completed with the GSP Neonatal Thyroxine (T4) kit at the two U.S. state laboratories described above (3c) are presented in the table below:

Study	n	T4 $\mu\text{g/dL}$ serum		Lower Percentiles T4 $\mu\text{g/dL}$ serum			
		Mean	Median	10%	5%	2%	1%
1	2,321	15.5	15.2	10.5	8.7	6.3	4.9
2	1,955	14.5	14.3	10.6	9.6	8.1	6.6

Since cut-off values of T4 in dry blood spots may vary between different tests and different populations, the sponsor recommends in the labeling that each laboratory establishes its own reference range and cut-off limit from a representative sample population.

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.