

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

A. 510(k) Number:

k102419

B. Purpose for Submission:

New Device

C. Measurand:

Immunoreactive trypsin (IRT)

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Wallac Oy

F. Proprietary and Established Names:

GSP Neonatal IRT kit

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1725, trypsin test system

2. Classification:

Class I exempt, exceeds the limitation to exemption in 862.9(c)(2)

3. Product code:

JNO, trypsin test system

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

The GSP Neonatal IRT kit is intended for the quantitative determination of human immunoreactive trypsin(ogen) (IRT) in blood specimens dried on filter paper as an aid in screening newborns for cystic fibrosis using the GSP instrument.

3. Special conditions for use statement(s):

For prescription use only.

The data obtained using the GSP Neonatal IRT kit should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician.

The measurement of IRT is used as a means of identifying a population of newborns who are at increased risk of having CF and should be selected for 2nd tier testing.

4. Special instrument requirements:

Only for use on the GSP Instrument

I. Device Description:

The GSP Neonatal IRT kit consists of the following reagents:

1. The anti-IRT-Eu tracer is a stock solution of approximately 50 ug/mL of mouse monoclonal antibodies. There are three vials, each containing 2.4 mL. The tracer is in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, mouse IgG and less than 0.1% sodium azide (preservative). The Neo IRT Assay buffer is provided in three 120 mL bottles containing Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin bovine globulin, Tween 40, polyethyleneglycol 6000, an inert red dye and less than 0.1% sodium azide (preservative). The Anti-IRT microtitration strips come as three sets of four plates. These strips contain 8x12 wells coated with antibodies directed against a specific site on the IRT molecules (mouse monoclonal).
2. Calibrators consist of 7 filter paper cassettes (Whatman, no. 903) each containing 1 set of dried blood spots (DBS) with the following concentrations of IRT: 0 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, 250 ng/mL and 500 ng/mL of blood.
3. Controls consist of 5 filter paper cassettes (Whatman, no. 903), each containing 2 sets of DBS. Level C1 contains 30 ng/mL of blood, level C2 contains 70 ng/mL of blood, and level C3 contains 110 ng/mL of blood. (Note that 1ng/mL blood equals 2.22 ng/mL serum assuming 55% hematocrit). (The lyophilized controls are produced from trypsin in Tris-HCl buffered salt solution with bovine serum albumin and protease inhibitors.)

These calibrators and controls were prepared from human blood components tested using FDA approved methods and shown to be free from hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Wallac Oy AutoDELFIA Neonatal IRT kit
2. Predicate K number(s):
k003668
3. Comparison with predicate:

Similarities		
Characteristic	Proposed device	Predicate device (k003668)
Intended use	Quantitative determination of human immunoreactive trypsin(ogen) (IRT) in blood specimens dried on filter paper as an aid in screening newborns for cystic fibrosis	Same
Chemical Principle	The GSP Neonatal IRT assay is a solid phase, two-site fluorimetric assay based on direct sandwich technique in which two monoclonal antibodies (derived from mice)	Same
Detection principle	Time-resolved fluorescence	Same
Specimen	Filter paper disk with a diameter of approximately 3.2 mm (1/8 inch)	Same
Assay buffer	IRT Assay buffer, ready for use	Same
Calibrators	6 levels A-F (approx. values 0, 25, 50, 100, 250, 500 ng/mL)	Same
Calibration	Calibrated using gravimetric method	Same
Calibrator and Control matrix	Human blood with a hematocrit of 50-55% and spotted onto filter paper cassettes (Whatman, no. 903)	Same
Coated plates	Anti-IRT microtitration strips, 8x12 wells coated with antibodies directed against a specific site on the IRT molecule (mouse monoclonal)	Same

Differences		
Characteristics	Proposed device	Predicate device (k003668)
Instrument	GSP Instrument	1235 AutoDELFLIA Instrument
Dissociation solution	Delfia Inducer	Enhancement Solution
Antibody Cross-Reactions in Assay	α 2- macroglobulin 0% α 1-antitrypsin 0% Phospholipase A2 0.014% Chymotrypsin 0.959% Human IgG 0% Pepsinogen -0.056% Complement Factor I 0%	α 2- macroglobulin < 4ng/mL blood α 1-antitrypsin < 4ng/blood Phospholipase A2 < 4ng/mL blood Chymotrypsin < 4ng/mL blood Human IgG < 4ng/mL blood (Uro) Pepsinogen < 4ng/mL blood Complement Factor I NA
Measuring Range	9 to 500 ng/mL blood	4 to 500 ng/mL blood
Tracer	Anti-IRT-Eu tracer stock solution, approximate 40 μ g/mL mouse monoclonal, ready for use	Anti-IRT-Eu tracer stock solution, approximate 50 μ g/mL mouse monoclonal, ready for use
Analytical Sensitivity	LoB = 0.76 ng/mL blood LoD = 2.2 ng/mL blood LoQ = 2.2 ng/mL	LoB = 0.53 ng/mL blood LoD = 2.9 ng/mL blood LoQ = 2.9 ng/mL blood
Precision	10.9 ng/mL blood CV% 7.3 22.2 ng/mL blood CV% 7.2	16.7 ng/mL blood CV% 8.7 20.6 ng/mL blood CV% 7.8

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

CLSI EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*

CLSI EP6-A2, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*

CLSI EP9-2A, *Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline*

CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline..*

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

CEN 13640, *Stability Testing of In Vitro Diagnostic Reagents*

L. Test Principle:

The GSP Neonatal IRT assay is a solid phase, two-site fluoroimmuno-metric assay based on the direct sandwich technique in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the IRT molecule. Calibrators, controls or test specimens containing IRT are reacted simultaneously with immobilized monoclonal antibodies directed against a specific antigenic site on the IRT molecule and europium-labeled monoclonal antibodies (directed against a different antigenic site) in assay buffer. The assay buffer elutes IRT from dried blood on filter paper disks. The complete assay requires only one incubation step.

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

sample is proportional to the concentration of IRT in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was evaluated according to the CLSI Document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*. Heparinized whole blood samples (1- 9) were used in these studies to prepare DBS.

Natural (1-3) samples with endogenous IRT, samples spiked with exogenous IRT (4-9) and a control (10) sample were chosen to cover the measuring range of the GSP Neonatal IRT kit, which is from 9 to 500 ng/mL. The hematocrit of the samples was adjusted to 50 to 55% to correspond to the hematocrit of neonates. The IRT activities of the series of DBS samples were measured by three operators with three GSP Neonatal IRT kit lots in 27 runs over 21 operating days using three GSP instruments (9 runs per instrument). Three kit controls in four replicates were included in each run (In this study one run means a run consisting of two plates tested 20-24 hours apart on the same instrument with stored calibration.) The within run study was performed using one reagent lot only in order to assess the variation between lots. Separate estimations for the precision of GSP Neonatal IRT assays were calculated with two options: a full calibration curve in duplicate for each plate and a full calibration curve for every batch of two plates. Results are summarized below:

Sample	Results (using a full calibration curve on each plate)				
	n	Total mean value ng/mL blood	Within-run variation (% CV)	Within-lot variation (% CV)	Total Variation (%CV)
1	216	10.9	5.1	7.2	7.3
2	216	22.2	4.5	7.2	7.2
3	216	28.5	5.0	7.0	7.0
4	216	40.0	6.9	7.9	8.2
5	216	50.2	5.2	7.7	8.0
6	216	61.6	5.5	7.6	7.8
7	216	93.5	4.7	6.7	7.2
8	216	302.3	6.0	7.2	7.4
9	216	449.1	5.9	7.4	7.5
10	216	55.0	5.9	7.3	7.6

Sample	Results (using one calibration curve valid for 20-24 hours)				
	n	Total mean value ng/mL blood	Within-run variation (% CV)	Within-lot variation (% CV)	Total Variation (%CV)
1	216	10.8	5.2	7.4	7.5

2	216	22.1	5.1	7.0	7.0
3	216	28.3	5.5	7.2	7.3
4	216	39.8	7.1	8.1	8.5
5	216	49.9	6.7	8.1	8.5
6	216	61.1	6.6	7.2	7.5
7	216	92.9	5.2	6.4	7.0
8	216	300.0	6.2	7.1	7.4
9	216	446.0	6.3	7.5	7.7
10	216	54.7	6.2	7.1	7.5

b. *Linearity/assay reportable range:*

Linearity was evaluated according to the CLSI Document EP6-A2, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*. The claimed measuring range for this device is 9 – 500 ng/mL blood.

Fresh patient specimens with high IRT concentration were not available. Therefore, blood drawn from one healthy adult was split into two aliquots, one representing the “low concentration pool” and a second aliquot used to prepare the high concentration pool. Purified human trypsin (Scripps Laboratories) was added to a second pool to obtain a “high concentration” sample. Samples with intermediate concentrations were prepared by mixing the “low concentration” sample with the “high concentration” sample. The hematocrit of the samples was adjusted to 50 to 55% to correspond to the hematocrit of neonates. The series of samples were used to prepare DBS by dispensing the prepared samples onto filter paper and dried overnight. There is no international reference material or reference method for IRT that could be employed to determine targets for the linearity samples.

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 IRT 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. The results of regression analyses were compared. The significance of the second and third order polynomials were evaluated by performing a t-test. Both, the second and third order regressions have no statistically significant nonlinear terms (β_2 , β_3) at a 95% significance level (p-value <0.05). The fitted models are:

Linear: $y = 9.18 + 622x$

Second order: $y = 9.19 + 621x + 3x^2$

Third order: $y = 9.21 + 616x + 30x^2 + -26x^3$

Where y = IRT concentration (ng/mL) and x = dilution point.

The relative difference between the linear model and the measured

concentrations of the dilution series ranged between -2.9% and 9.0%. Results of the comparison between the measured and expected values are summarized below:

Dilution	Measured GSP IRT (ng/mL blood)	Linear Model predicted GSP IRT (ng/mL blood)	Absolute difference (%)	Relative difference (%)
0	8.99	9.18	-0.19	-2.1
0.017	21.5	19.18	1.77	9.0
0.057	43.4	44.6	-1.30	-2.9
0.102	71.9	72.7	-0.74	-1.0
0.202	133	135	-1.53	-1.1
0.302	198	197	0.56	0.3
0.4	261	258	3.28	1.3
0.5	325	320	4.21	1.3
0.602	383	384	-0.064	-0.2
0.701	443	445	-1.98	-0.4

The measured vs. expected linear regression analysis for samples within the measuring range of 9 – 500 ng/mL is provided below:

$$y = 1.00x + 0.15 \text{ (} r^2 = 0.999 \text{)}$$

95% CI: slope (0.98; 1.03)

95% CI: intercept (-0.59; 0.90)

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

The GSP Neonatal IRT kit also includes calibrators and controls which need to be employed in assay calibration and quality control (QC) testing. The kit calibrators and controls are DBS prepared from blood cell suspension consisting of washed human red blood cells and artificial serum. The blood used to prepare the kit calibrators is spiked with purified human trypsin to cover the range of 0 – 500 ng/mL blood, whereas the kit controls are spiked at the clinically relevant concentration area (control levels 30, 70, 110 ng/mL blood).

Traceability: There is no international reference material that can be used as the primary calibrator and no reference method that can be used to assign values. The IRT concentration is traceable to the in-house primary calibrators prepared from Human Pancreas Trypsin. The primary calibrators are a dilution series of trypsin made gravimetrically with Tris-HCl buffered solution with bovine serum and protease inhibitors. Protein concentration of the trypsin raw material has been determined spectrophotometrically using an Extinction Coefficient (A289, 0.1% solution, 1 cm pathway) of 1.45. The possible change of trypsin immunoreactivity occurring during the lyophilization step performed during primary calibrator manufacturing is

corrected by measuring the IRT concentration of the lyophilized calibrators against non-lyophilized calibrators. The primary calibrators are stored at -60 to -80°C.

The primary calibrators are used to monitor the level of the secondary calibrators.

Value assignment: The primary and secondary calibrators are in house materials that are used for the A-F level specific calibrators. The acceptance criteria for value assignment were reviewed and found to be adequate.

Stability: Real time stability studies evaluating shelf-life, shipping stability and on-board stability for the entire kit were performed, including the calibrators and controls. The protocols and acceptance criteria were reviewed and found to be adequate. The GSP Neonatal IRT assay reagents, controls and calibrators are stable for 12 months when stored unopened at 2 – 8° C and 14 days on-board at 2-8 °C.

Detection limit:

The Limit of the Blank (LoB) for the GSP Neonatal IRT L kit was determined in accordance with the CLSI guideline EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*. 120 measurements of two sample types, the zero calibrator and dried human blood spots (3 samples of “zero” calibrator and 2 unspiked blood samples), were tested in six separate runs, replicates of four, over three operating days.

The Limit of the Blank was determined to be 0.76 ng/mL, the 95th percentile of the distribution of the test values.

The Limit of Detection (LoD) is defined as the sample concentration where the 95% of measurements exceeds LoB. The Limit of Detection (LoD) is estimated to be 2.2 ng/mL blood based on 504 determinations of 7 low level samples.

Limit of Quantitation (LoQ) is the lowest concentration of IRT that can be measured with acceptable total variation and is equal or higher than LoD. The observed LoQ of the GSP Neonatal IRT assay is 2.2 ng/mL blood, when the acceptable total variation is defined as CV<20%.

d. *Analytical specificity:*

The sponsor performed a study to evaluate the effect of potential interfering substances in dried blood samples (such as unconjugated and conjugated bilirubin, lipemia and hemoglobin) on the performance of the GSP Neonatal IRT assay. These studies were performed in accordance with CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline*. The blood was spiked with trypsin to obtain two different clinically relevant IRT concentrations (approximately 50 and 80 ng/mL blood). The IRT responses of

the DBS interference samples were analyzed with one kit lot in 12 replicates. Three kit controls in duplicate were included in each run. The pool without any additions, except equal volume of solvent used to dissolve the substance, was used as a control. The table below lists all substances tested at concentrations with no significant interference. The no significant interference (acceptance criteria) was defined as $\leq 15\%$ interference for samples in clinically relevant range of 25 to 500 ng/mL and $\leq 20\%$ interference for samples below 25 ng/mL:

Compound	Concentration added to samples
Bilirubin (conjugated)	16.6 mg/dL in blood equivalent to 197 $\mu\text{mol/L}$
Bilirubin (unconjugated)	10 mg/dL in blood or 171 $\mu\text{mol/L}$
Hemoglobin	10 g/dL in blood
Intralipid (triglycerides)	1500 mg/dL in blood

Additional study was performed to evaluate the potential cross-reactivity of the GSP Neonatal IRT kit with substances structurally similar to IRT. The list of the compounds tested and results are summarized below:

Compound	Tested concentration	Cross-reactivity (%)
$\alpha 2$ -microglobulin	5 mg/mL blood	< 0.1
$\alpha 1$ -antitrypsin	2 mg/mL blood	< 0.1
Phospholipase A2	500 ng/mL blood	< 0.1
Chymotrypsin	800 ng/mL blood	1.0
Human IgG	15 mg/mL blood	< 0.1
Pepsinogen	150 ng/mL blood	< 0.1
Complement factor 1	35 $\mu\text{g/mL}$ blood	< 0.1

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

2. Comparison studies:

a. *Method comparison with predicate device:*

Internal and external method comparison studies were evaluated according to the CLSI Document EP9-A2, *Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline*.

Internal studies: Routine newborn screening DBS leftover samples (N=201 retrospective specimens) in the range of 10.4 – 322 ng/mL and DBS prepared from spiked samples (N=47) in the range of 322 – 500 ng/mL were tested and compared between the new device (GSP Neonatal IRT) and predicate device (AutoDELFIA IRT). These studies were performed using three lots of both the GSP Neonatal IRT and AutoDELFIA IRT kits. The samples were run in singlicate.

The assumptions of ordinary least squares regression analysis are not fulfilled in the IRT assay because the standard deviation across the measuring range is not constant. Therefore, weighted Deming analysis was applied and the variance ratio (λ) was estimated to be 1 (i.e. the variances of the methods were considered to be of equal size across the measuring range for both GSP IRT and AutoDELFIA IRT). The results are shown below:

Data within measuring range (N=248)	Regression equation	Regression coefficients	95% confidence interval	
			Lower	Upper
Weighted Deming	$y=0.99x - 1.03$	Intercept=-1.03	-1.92	-0.14
		Slope=0.99	0.96	1.02

External studies or Screening performance: These studies were performed in a U.S. state newborn screening laboratory. The initial total number of acquired samples was comprised of 2216 routine screening left-over specimens and 20 specimens diagnosed/confirmed positive for CF. However, 3 GSP runs and 3 AutoDELFIA runs were rejected due to unacceptable QC results (total of 255 rejected specimens). Further, additional 36 specimens were rejected due to the poor quality and missing information. Therefore, the final total number of samples tested was comprised of 1945 samples (1925 routine screening left-over specimens + 20 confirmed positive specimens). The specimens were analyzed in singlicate using both the GSP Neonatal IRT and the AutoDELFIA Neonatal IRT kits according to the respective package inserts. These studies were conducted by two laboratory technicians. The confirmed CF positive specimens were dispersed in a blinded fashion throughout the multiple assay runs. Three levels of kit controls were run in duplicate at the beginning on each plate to validate the assay runs for both the tested and the predicate method. None of the assays were outside the QC acceptance criteria. (See section 5 below for more information about the IRT cut off values.)

Results of the agreement between the proposed device and the predicate device are summarized in the tables below:

Screening results based on 95% percentile:

Of the diagnosed/confirmed CF specimens (N=20) both methods classified all these specimens as test positive with the 95% percentile cut off:

GSP	Auto-DELFI A	Total subjects	Diagnosed CF	Non diagnosed CF
+	+	96	20	76
+	-	21	0	21

-	+	21	0	21
-	-	1807	0	1807
Total		1945	20	1925

Distribution of samples into test results categories using 95% percentile cut-off determination: GSP Neonatal IRT vs. AutoDELFIA Neonatal IRT:

		AutoDELFIA		
		Test positive	Test negative	Total
GSP	Test positive	96	21*	117
	Test negative	21*	1807	1828
	Total	117	1828	1945

Overall % agreement = $(96 + 1807) / 1945 * 100\% = 97.8$ (CI 97.1% - 98.4%)

Positive % agreement = $(96 / 117) * 100\% = 82.1\%$ (CI 73.9% - 88.5%)

Negative % agreement = $(1807/1828) * 100\% = 98.9 \%$ (CI 98.2%-99.3%)

*As shown in the table above, there were 42 discrepant samples (21 + 21) when measurements obtained with the GSP IRT assay were compared to the AutoDELFIA measurements. However, all of these samples were originally classified as screen negative samples. Therefore, of the total 1945 samples tested, only 21 samples or 1.1% are false positive samples.

Screening results based on 99% percentile:

Furthermore, of the confirmed CF specimens (N=20) both methods classified all these specimens as test positive with the 99% percentile cut off:

GSP	Auto-DELFIA	Total subjects	Diagnosed CF	Non diagnosed CF
+	+	35	20	15
+	-	5	0	5
-	+	5	0	5
-	-	1900	0	1900
Total		1945	20	1925

Distribution of samples into test results categories using 99% percentile cut-off determination: GSP Neonatal IRT vs. AutoDELFIA Neonatal IRT:

		AutoDELFIA		
		Test positive	Test negative	Total
GSP	Test positive	35	5	40
	Test negative	5	1900	1905
	Total	40	1905	1945

Overall % agreement = $(35 + 1900) / 1945 * 100\% = 99.5$ (CI 99.1% - 99.8%)

Positive % agreement = $(35/40) * 100\% = 87.5\%$ (CI 73.2% - 95.8%)

Negative % agreement = $(1900/1905) * 100\% = 99.7 \%$ (CI 99.4%-99.9%)

A regression analysis was performed to compare the GSP Neonatal IRT to the AutoDELFIA Neonatal IRT method for the external method comparison study. The assumptions of ordinary least squares regression analysis are not fulfilled in the assays. Namely, the standard deviation across the measuring range is not constant. Therefore, weighted Deming regression is more appropriate regression method and is shown in the table below:

Data	Regression equation	Regression coefficients	95% confidence interval	
			Lower	Upper
Weighted Deming	$y=0.93x + 1.54$	Intercept=1.54	0.92	0.95
		Slope=0.93	1.23	1.84

b. *Matrix comparison:*

Not applicable. The device should be used only with neonatal whole blood from heel prick dried 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):

Specimen Stability Study: This study was performed at Perkin Elmer to monitor the stability of IRT in DBS previously stated in literature. Samples were prepared from two heparinized blood specimens obtained from healthy adult donors. The hematocrit was adjusted to 45% to 55%, four specimens were aliquoted (two per sample) and one sample from each specimen was additionally spiked with trypsin. Specimens were spotted onto filter paper, dried overnight and put at five different storage conditions (i.e., temperature and humidity levels) with two different sample container options: a) sealed bags with a desiccant and b) unsealed foil bags without desiccant. Results are provided in the package insert. The study confirmed that the elevated temperature and humidity are detrimental to IRT in DBS. Therefore, specimens stored in ambient temperature (+18 to +25 °C) should be analyzed for IRT within two weeks of collection as IRT levels decrease 6-9% per week. For longer storage, specimens should be placed in plastic/foil bags with desiccant(s) and store frozen at -16 to -30 °C.

4. Clinical cut-off:

The quantitative determination of human IRT from DBS allows identification of a population of newborns with elevated IRT who are at an increased risk of having CF. The identification is based on the use of a cut-off value or a percentile, which distinguishes between the unaffected and affected individuals.

The screening strategy for CF varies within different laboratories, the most common being the two-tier strategy IRT/DNA. It is known that IRT values and the frequency of CF vary according to geographic and local demographics. Therefore, it is important that each laboratory establishes its own reference range and cut-off value from a representative sample population. Some laboratories choose to set the cut-off at the top 1% to 5%¹. Selection of the cut-off percentile depends on the sensitivity and specificity objectives of the screening program. Choice of a cut-off e.g. 5% of the daily IRT results increases screening sensitivity, whereas selection of a cut-off e.g. 1% minimizes the number of samples referred to second tier testing. More information about cystic fibrosis screening algorithms can be found in reports by European Cystic Fibrosis Society², Cystic Fibrosis Foundation³, and U.S. Centers for Disease Control and Prevention⁴.

The positive predictive value of the assay may vary depending on the prevalence of the disease in the population being tested. The phenomenon of transient (non-CF) neonatal hypertrypsinemia, predominately occurring in the first few days of life, is the primary cause of false high IRT screening results. High IRT results may also occur in neonates with low APGAR scores and in African-American infants. IRT levels decline with age of specimen collection. The use of a second level testing, either DNA or repeat IRT on a second specimen, has been shown to reduce the number of false positives. Healthy carriers of CF mutations may produce a false positive IRT results at a rate about three times that in the general population. False negative IRT values are known to occur in some CF newborns that present with *meconium ileus*. IRT screening in combination with mutation analysis as a second-tier test will result in the identification of CF carriers. Follow local requirements for follow-up testing.

1. Kaye, C.I. and the Committee on Genetics. "Newborn Screening Fact Sheets." *Pediatrics* 118, 934-963 (2006).

2. Castellani C., Souther K.W., Brownlee K., Dankert Roelse J., Duff A., Farrell M., Mehta A., Munck A., Pollitt R., Sermet-Gaudelus I., Wilcken B., Ballmann M., Corbetta C., de Monestrol I., Farrell P., Ferec C., Gartner S., Gaskin K., Hammermann J., Kashirskaya N., Loeber G., Macek M. Jr., Mehta G., Reiman A., Rizzotti P., Sammon A., Sands D., Smyth A., Sommerburg O., Torresani T., Travert G., Vernooij A., and Elborn S. (2009): European best practice guidelines for cystic fibrosis neonatal screening. *J. Cyst. Fibros.* 8, 153-173.

3. Comeau A.M., Accurso F.J., White T.B., Campbell P.W., Hoffman G., Parad R.B., Wilfond B.S., Rosenfeld M., Sontag M.K., Massie J., Farrell P.M., and O'Sullivan B.P. (2007): Guidelines for Implementation of Cystic Fibrosis Newborn Screening Programs: Cystic Fibrosis Foundation Workshop Report. *Pediatrics* 119, 495 – 518.

4. Grosse S.D., Boyle C. A., Botkin J.R., Comeau A.M., Kharrazi M., Rosenfeld M., and Wilfond B.S. (2004): Newborn Screening for Cystic Fibrosis. Evaluation of Benefits and Risks and Recommendations for State Newborn Screening Programs.

5. Expected values/Reference range:

IRT patient values by percentile in the method comparison study data:

	N	Min	Max	Mean	Median	Upper percentiles					
						90%	95%	96%	97%	98%	99%
GSP Neonatal IRT (ng/mL blood)	1925	4.5	174.9	24.5	21.5	39.6	48.3	51.7	54.3	59.3	66.9

Cut-off values of IRT in DBSs may vary between different tests and different populations. Therefore, each laboratory should establish its own reference range and cut-off value 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.