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

A. 510(k) Number:

k110274

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:

AutoDELFIA® 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 AutoDELFIA 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 1235 AutoDELFIA® automatic immunoassay system.

3. Special conditions for use statement(s):

   For prescription use only.

   The data obtained using the AutoDELFIA® 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 1235 AutoDELFIA® automatic immunoassay system.

I. Device Description:

The AutoDELFIA ® Neonatal IRT kit assay is a solid phase, two-site fluoroimmunometric 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 and 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 the dried blood on filter paper disks. The complete assay requires only one incubation step.

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

The 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.
2. Calibrators consist of 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 filter paper cassettes (Whatman, no. 903), each containing 2 sets of DBS. Level C1 contains 30ng/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 negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies.

The user has the option of buying an AutoDELFIA Neonatal IRT kit that provides enough reagents to run 1152 assays (kit B005-212) or 384 assays (kit B005-204). These kits are identical in composition except for the number of number of reagent bottles and plates packaged in the kit:

Kit number	B005-212	B005-204
Number of assays	1152	384
Neonatal IRT calibrators	8 filter paper cassettes	4 filter paper cassettes
Neonatal IRT Controls	6	3
Anti-IRT-Eu tracer (2.4mL vials)	6 vials	4 vials
IRT assay Buffer	3 Bottles	2 Bottles
Anti-IRT Microtitration strips	12 Plates	4 Plates

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 (Feature)	AutoDELFIA Neonatal IRT kit B005-212/B005-204 (New Device)	AutoDELFIA Neonatal IRT kit B005-112 (Predicate Device)
Intended Use / Indications for Use	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 1235 AutoDELFIA® automatic immunoassay	Same

Similarities		
Characteristic (Feature)	AutoDELFIA Neonatal IRT kit B005-212/B005-204 (New Device)	AutoDELFIA Neonatal IRT kit B005-112 (Predicate Device)
	system.	
Chemical Principle	The AutoDELFIA Neonatal IRT assay is a solid phase, two-site fluorimmunometric 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.	Same
Detection principle	Time-resolved fluorescence	Same
Specimen	Dried blood on filter paper disks with a diameter of approximately 3.2 mm (1/8 inch)	Same
Intended User	Adequately trained laboratory personnel in laboratories performing newborn screening	Same
Antibodies	Two different mouse monoclonal antibodies	Same
Calibrator and Control Matrix	Human blood derivative with a hematocrit of 50-55% and spotted onto filter paper (Whatman, no. 903)	Same
Hook effect	No hook effect has been found with IRT concentrations up to 40,000 ng/mL	Same
Number of Kit Calibrators and Calibrator Values	6 levels; approx. values 0, 25, 50, 100, 250, 500 ng/mL blood.	Same
Number of Kit Controls	3 levels	Same
Calibration	Calibrated using gravimetric methods (In-house calibrators	Same (In-house calibrators

Similarities		
Characteristic (Feature)	AutoDELFIA Neonatal IRT kit B005-212/B005-204 (New Device)	AutoDELFIA Neonatal IRT kit B005-112 (Predicate Device)
	contain protease inhibitors and BSA item 1.)	without protease inhibitors, contain BSA item 2.)
Assay buffer	IRT Assay Buffer, ready for use Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and additives. (BSA different; see differences table below)	Same (BSA different; see differences table below)
Coated Plates	Anti-IRT Microtitration Strips, 8 X 12 wells coated with antibodies directed against a specific site on the IRT molecule (mouse monoclonal)	Same
Tracer	Anti-IRT-Eu tracer stock solution (~50 µg/mL), mouse monoclonal, ready for use.	Same
Instrument	1235 AutoDELFIA Instrument	Same
Dissociation solution	Enhancement Solution	Same
Expected Values	The measurement of IRT from dried blood spots 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. The identification is based on the use of a fixed cut-off value or population percentile. The IRT cut-off levels must be determined by each newborn screening laboratory to meet the	Same

Similarities		
Characteristic (Feature)	AutoDELFIA Neonatal IRT kit B005-212/B005-204 (New Device)	AutoDELFIA Neonatal IRT kit B005-112 (Predicate Device)
	desired sensitivity and specificity of the screen and should be evaluated periodically.	
Antibody Cross-Reactions in the Assay	α2-macroglobulin < 4 ng/ml blood α1-antitrypsin < 4 ng/ml blood Phospholipase A2 < 4 ng/ml blood Chymotrypsin < 4 ng/ml blood Human IgG < 4 ng/ml blood Uropepsinogen < 4 ng/ml blood	Same

Differences		
Characteristic (Feature)	AutoDELFIA Neonatal IRT kit B005-212/B005-204 (New Device)	AutoDELFIA Neonatal IRT kit B005-112 (Predicate Device)
Measuring Range	16 to 480 ng/mL blood	4 to 500 ng/mL blood
Kit Control Concentrations	Approx. values 30, 70 and 110 ng/mL blood	Approx. values 40, 70 and 120 ng/mL blood
Analytical Sensitivity / Limit of Blank, Limit of Detection	Limit of Blank 0.53 ng/mL blood Limit of Detection 2.9 ng/mL blood	Limit of Blank < 4 ng/mL blood
Precision (Total Variation using a full calibration curve on each plate)	16.7 ng/mL blood CV% 8.7 22.5 ng/mL blood CV%	42.6 ng/mL blood CV% 9.3 98.8 ng/mL blood CV%

	9.6 48.0 ng/mL blood CV% 9.1 104 ng/mL blood CV% 8.0 247 ng/mL blood CV% 8.3 401 ng/mL blood CV% 8.4 449 ng/mL blood CV% 9.4	10.0 266 ng/mL blood CV% 9.6
In-house calibrators Tracer and IRT assay buffer	Contains protease inhibitor and BSA Celliance 81-066	Does not contain protease inhibitor; contains BSA Celliance 3160
Kit calibrators and controls	Blood cells in buffer contains protease inhibitor and BSA Celliance 81-066	Blood cells in buffer contains saccharose/saline

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 AutoDELFIA ® Neonatal IRT kit is a solid phase, two-site fluoroimmunometric 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 one incubation step. DELFIA Inducer dissociates europium ions from the labeled antibody into solution where they form highly fluorescent chelates with components of the DELFIA 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*. Samples # 1 – 7 were prepared from heparinized adult whole blood containing some endogenous IRT. The hematocrit value of blood used in sample preparation was measured to confirm that the hematocrit is within the reference range of newborns (45% – 67%), or adjusted as necessary. Samples 8 – 10 were prepared from blood suspension containing washed red blood cells and the hematocrit was adjusted to 52.5% to correspond to the hematocrit of neonates. The dilution was made with saline-saccarose solution. The whole blood and blood cell suspensions were spiked with trypsin. The sample 1 was prepared from unspiked heparinized adult whole blood containing only the endogenous IRT. The concentrations of the samples were chosen to cover the claimed measuring range of the kit (16 – 480 ng/mL blood). The IRT activities of the series of dried blood spot (DBS) samples were measured by three operators with three AutoDELFIA IRT kit lots in 21 runs over 14 operating days using three AutoDELFIA instruments (9 runs per instrument). Four replicates each of three kit controls 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 precision study was performed using one reagent lot only in order to assess the variation between lots. Separate estimations for the precision of AutoDELFIA IRT 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 four plates. Results are summarized below:

Summary of results for Precision. The results are calculated by using a ***full calibration curve in duplicate for each plate.*** *Run = plate*

		RESULTS				
Sample	**n**	**Mean IRT concentration (ng/mL blood)**	**Within-run variation**		**Total within-lot variation**	
			SD	**CV%**	**SD**	**CV%**
1	168	16.7	1.2	7.0	1.5	8.7
2	168	22.5	1.7	7.4	2.2	9.6
3	168	48.0	3.6	7.6	4.4	9.1
4	168	104	7.3	7.1	8.3	8.0
5	168	247	19.0	7.7	20.5	8.3
6	168	401	27.8	6.9	33.8	8.4
7	168	449	34.3	7.6	42.2	9.4
8	168	20.6	1.3	6.2	1.6	7.8
9	168	54.1	3.5	6.5	4.6	8.5
10	168	90.6	5.7	6.3	7.4	8.1

Summary of results and acceptance criteria for Precision. The results are calculated by using a ***full calibration curve in duplicate for every batch of four plates.***

		RESULTS				
Sample	**n**	**Mean IRT concentration (ng/mL blood)**	**Within-run variation**		**Total within-lot variation**	
			SD	**CV%**	**SD**	**CV%**
1	168	16.7	1.3	7.8	1.5	8.8
2	168	22.5	1.8	7.9	2.1	9.4
3	168	48.4	3.7	7.6	4.3	8.9
4	168	104	7.3	7.0	8.3	8.0
5	168	247	19.5	7.9	20.4	8.3
6	168	400	28.7	7.2	32.5	8.1
7	168	448	34.9	7.8	41.2	9.2
8	168	20.6	1.4	6.7	1.6	7.6
9	168	54.5	3.6	6.7	4.3	8.0
10	168	91.1	5.7	6.3	6.9	7.6

Note: An additional study was conducted to validate the between run

variability. The data was reviewed to be adequate.

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 16 – 480 ng/mL blood.

Fresh patient specimens with high IRT concentration were not available. Therefore, blood drown 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 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 mean results for each specimen assayed constituted the data for the statistical analysis. The assumption of constant variance across all levels is not fulfilled in the 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 ($\beta 2$, $\beta 3$) at a 95% significance level (p-value <0.05).

The fitted models are:

Linear: $y=10.7+744.1x$

Second order: $y=10.3+ 796.6x - 132.2 x^2$

Third order: $y= 10.4 + 795.5 x- 123.2 x^2 -11.9 x^3$

where y = IRT Concentration (ng/mL blood) and x = dilution point

The relative difference between the linear model and the measured concentrations of the dilution series ranged between -7.0% and 5.5%. Results of the comparison between the measured and expected values are summarized below:

Comparison of the measured and expected results for narrowed range

Dilution	Measured IRT (ng/mL blood)	Linear model fitted IRT (ng/mL blood)	Absolute difference (ng/mL blood)	Relative difference (%)
0.017	26.2	23.4	2.9	12
0.057	51.6	53.1	-1.53	-2.9
0.102	91.3	86.6	4.72	5.5
0.202	165	161	4.47	2.8
0.302	237	235	2	0.8
0.4	315	308	6.57	2.1
0.5	375	383	-7.86	-2.1
0.602	441	459	-18.1	-4.0

Linear regression analysis results:
$y = 1.06x + 0.16$ ($R^2 = 0.996$)
95% CI: slope 1.02; 1.11
95% CI intercept (-1.61; 1.93)
The labeling states that the measuring range of the kit has been demonstrated to be 16ng/mL- 480ng/mL.

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

The AutoDELFIA ® 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 AutoDELFIA ® Neonatal IRT kit 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.

d. *Detection limit:*

The Limit of the Blank (LoB) for the AutoDELFIA ® Neonatal IRT 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 unaltered blood samples), were tested in six separate runs, replicates of four, over three operating days.

The Limit of the Blank was determined to be 0.53 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 2.9 ng/mL blood based on 576 determinations of 4 low level samples.

e. *Analytical specificity:*

Analytical specificity was deemed to be identical to the predicate and appropriate cross reactive and interfering factors have been tested and cleared under the previous device (k003668).

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 the kit (16.3 - 439 ng/mL) and DBS prepared from spiked samples (N=53) were tested and compared between the new device and predicate device. These studies were performed using three lots of 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 (l, lambda) was estimated to be 1 (i.e. the variances of the methods were considered to be of equal size across the measuring range for the AutoDELFIA IRT). The results are shown below:

Data within measuring range (N=231)	Regression equation	Regression coefficients	95% confidence interval	
			Lower	Upper
Weighted Deming	y=0.99x +0.07	Intercept=0.07	-1.15	1.28
		Slope=0.99	0.95	1.02

Screening performance study: This study was performed in a U.S. state public health laboratory. The initial total number of acquired samples was comprised of 2287 routine screening left-over specimens and 20 specimens diagnosed/confirmed positive for CF. However, 338 samples were excluded because of run rejected unacceptable QC results. Further, 42 specimens were rejected due to the poor quality and missing information. Additionally, one sample was excluded suspecting contamination and 10 more were excluded because the age of the new born was greater than the 31 day limit in the study. Therefore, the final total number of samples tested was comprised of 1916 samples (1896 routine screening left-over specimens + 20 confirmed positive specimens). The specimens were analyzed in singlicate using both the proposed and predicate devices 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: Expected Values/Reference Range 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:

With regard to the diagnosed/confirmed CF specimens (N=20), both the proposed and predicate devices classified all these specimens as test positive with the 95% percentile cut off:

Proposed	Predicate	Total subjects	Diagnosed CF	Normal
+	+	102	20	82
+	-	14	0	14
-	+	14	0	14
-	-	1786	0	1786
Total		1916	20	1896

Distribution of samples into test results categories using 95% percentile cut-off determination:

		Predicate		
		Test positive	Test negative	Total
Proposed	Test positive	102	14*	116
	Test negative	14*	1786	1800
	Total	116	1800	1916

Overall % agreement = (102 + 1786) / 1916 * 100% = 98.5% (CI 97.9% - 99.0%)
Positive % agreement = (102 / 116) * 100% = 87.9% (CI 80.6% - 93.2%)
Negative % agreement = (1786 / 1800) * 100% = 99.2% (CI 98.7% - 99.6%)

*As shown in the table above, there were 28 discrepant samples (14 + 14) when measurements obtained with the proposed assay were compared to the predicate measurements. However, all of these samples were originally classified as screen negative samples. Therefore, of the total 1916 samples tested, only 14 samples are false positive samples.

Screening results based on 99% percentile:

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

Proposed	Predicate	Total subjects	Diagnosed CF	Normal
+	+	35	20	15
+	-	5	0	5
-	+	5	0	5
-	-	1871	0	1871
Total		1916	20	1896

Distribution of samples into test results categories using 99% percentile cut-off determination:

		Predicate		
		Test positive	Test negative	Total
Proposed	Test positive	35	5	40
	Test negative	5	1871	1876
	Total	40	1876	1916

Overall % agreement = (35 + 1871) / 1916 * 100% = 99.5% (CI 99.0% - 99.7%)
Positive % agreement = (35 / 40) * 100% = 87.5% (CI 73.2% - 95.8%)
Negative % agreement = (1871 / 1876) * 100% = 99.7% (CI 99.4% - 99.9%)

A regression analysis was performed to compare the proposed assay to the predicate assay 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 (n=1916)	y=-0.52+1.04x	Intercept=-0.55	-0.83	-0.27
		Slope=1.04	1.03	1.05

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):
   Not applicable.

4. <u>Clinical cut-off</u>**:**
   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 Society [2], Cystic Fibrosis Foundation [3], and U.S. Centers for Disease Control and Prevention [4].
   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., Cameau A.M., Kharrazi M., Rosenfeld M., and Wilfond B.S. (2004): Newborn Screening for Cystic Finbrosis. 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	Mean	Median	Upper percentiles (IRT ng/mL)				
				95%	96%	97%	98%	99%
AutoDELFIA ® Neonatal IRT kit	1896	24.8	21.5	50.3	53.6	59.2	64.3	73.3

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.