

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

A. 510(k) Number:

k070889

B. Purpose for Submission:

Modification of previous device

C. Measurand:

Immunoreactive trypsin (IRT)

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Wallac Oy

F. Proprietary and Established Names:

AutoDELFLIA Neonatal IRT L kit

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1170, chloride test system

21 CFR §862.1725, trypsin test system

2. Classification:

Class II

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

3. Product code:

CGZ, chloride test system

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 AutoDELFLIA Neonatal IRT L 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 AutoDELFLIA automatic immunoassay system.

3. Special conditions for use statement(s):

For prescription use only.

The data obtained using the Neonatal IRT L 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:

For use on the 1235 AutoDELFLIA automatic immunoassay system.

I. Device Description:

Assay: The anti-IRT-Eu tracer is a stock solution of approximately 50 ug/mL of mouse monoclonal antibodies. There are three sets of two 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 molecular (mouse monoclonal).

Standards (calibrators) consist of 3 sets of 6 vials (levels) of lyophilized material (trypsin in Tris-HCl buffered salt solution with bovine serum albumin and protease inhibitors).

Controls consist of three sets of 3 vials (levels) of lyophilized material Level CL contains 40ng/mL of blood, level CM contains 95 ng/mL of blood, and level CH contains 200 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.)

J. Substantial Equivalence Information:

1. Predicate device name(s):
AutoDELFLIA Neonatal IRT kit
2. Predicate 510(k) number(s):
k003668
3. Comparison with predicate:

| Similarities | | |
|-----------------|---|-----------------|
| Characteristic | Predicate device (k003668) | Proposed device |
| 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 |
| Assay principle | Time-resolved fluoroimmunoassay | Same |

| Similarities | | |
|-----------------------------|--|-----------------|
| Characteristic | Predicate device (k003668) | Proposed device |
| Antibodies | Mouse monoclonal antibodies | Same |
| Standards: number of levels | 6 levels | Same |
| Controls: number of levels | 3 levels | Same |
| Kit components | Anti-IRT-Eu tracer, Neo IRT assay buffer, Anti-IRT microtitration strips | Same |

| Differences | | |
|--------------------------------------|--|---|
| Characteristic | Predicate device (k003668) | Proposed device |
| Procedure for Standards and Controls | Standards and controls on filter paper are punched into wells. | Liquid standards and controls are dispensed into wells. |
| Standards and Controls matrix | Blood spots on filter paper | Buffer based solutions, lyophilized |

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

CLSI Documents:

- *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline (EP6-A2)*
- *Interference Testing in Clinical Chemistry: Approved Guideline (EP7-A2)*
- *Method Comparison and Bias Estimation using Patient Samples: Approved Guideline (EP9-A2)*
- *Protocols for Determination of Limits of Detection and Limits of Quantitation (EP17-A)*

L. Test Principle:

The AutoDELFI A Neonatal IRT L assay is a solid phase, two-site fluorimmuno metric assay based on the direct sandwich technique in which two mouse monoclonal antibodies are directed against two separate antigenic determinants on the IRT molecule. Standards, 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 spots on the filter paper discs. 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision: imprecision of the kit was determined using spiked, dried blood spot samples created by pooling heparinized whole blood samples collected from three donors and spiking to three analyte levels. Three kit lots were tested in 18 runs consisting of 4 plates with 4 replicates and with 3 AutoDELFIA instruments.

Results using a full calibration curve on each plate:

| Sample | Total mean value ng/mL blood | Within-plate variation (% CV) | Between-lot variation (% CV) | Within-lot variation (% CV) | Total variation (% CV) |
|--------|------------------------------|-------------------------------|------------------------------|-----------------------------|------------------------|
| 1 | 49.0 | 8.8 | 1.6 | 11.0 | 11.1 |
| 2 | 74.8 | 7.5 | 1.4 | 9.4 | 9.5 |
| 3 | 118 | 8.8 | 0.9 | 11.0 | 11.0 |

Results using one calibration curve for each batch of 4 plates:

| Sample | Total mean value ng/mL blood | Within-plate variation (% CV) | Between-lot variation (% CV) | Within-lot variation (% CV) | Total variation (% CV) |
|--------|------------------------------|-------------------------------|------------------------------|-----------------------------|------------------------|
| 1 | 48.5 | 8.8 | 1.7 | 11.1 | 11.2 |
| 2 | 74.0 | 7.4 | 1.7 | 9.3 | 9.4 |
| 3 | 117 | 8.8 | 1.2 | 11.1 | 11.2 |

b. *Linearity/assay reportable range:*

Linearity was evaluated using CLSI Document EP6-A2 as a guideline. The measuring range for this device is 13 – 370 ng/mL.

Unprocessed fresh heparin whole blood was used as a “low concentration” pool. Trypsin dissolved in 10 mM Glycine-HCl, pH 3.0, was added to heparinized whole blood to obtain a “high concentration” pool. Samples with intermediate concentrations were prepared by diluting the high concentration pool with the low concentration pool to various concentrations across the measuring range before spotting on filter paper. Trypsin concentrations of the series of samples were measured with the Neonatal IRT L kit during a single run. The samples were analyzed in 6 replicates.

Linear Regression statistics:

$$y = 0.98x + 0.31$$

$$r^2 = 0.998$$

In addition, observed IRT concentration at each dilution was within $\pm 10\%$ of the expected IRT concentration.

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

Human pancreas trypsin is purchased as a frozen liquid (there is no international reference preparation or reference method for immunoreactive trypsin IRT) and used to manufacture a primary calibrator for the IRT L kit

calibration (see below). Secondary calibrators are manufactured and value assigned based on direct comparison to primary calibrators. Level calibrators (dried blood spots, stored at -20°C) and kit controls and calibrators (lyophilized and stored at 4°C) are assigned values based on the secondary calibrators.

Stability:

In-use and on-board stability: Reconstituted stability of the IRT L standards and controls is 1 week at 2-8°C. Wash solution for the plate processor is stable for 2 weeks at 2-25°C in a sealed container. Wash solution for the sample processor is stable for 1 week at 2-25°C in a sealed container. On-board stability of all the reagents, except the controls, is 24 hrs. Study protocols, summary data and acceptance criteria were reviewed and found to be acceptable.

Real-time and accelerated stability: Study protocols, preliminary data and acceptance criteria for stability testing were provided for the Neonatal IRT L kit for real-time studies at recommended storage temperatures (2-8°C) and accelerated temperatures and found to be acceptable. Based on the accelerated stability data, nine month stability at 2-8°C is claimed for the kit. Real-time studies will continue to confirm and extend the dating.

Specimen stability: As noted in the package insert, specimens stored in ambient temperature should be analyzed for IRT within two weeks of collection as IRT levels decrease 6-9% per week. For longer storage, place specimens in plastic bags and store frozen^{1,2}. Seasonal variation in IRT levels have been reported³. The cause may be due in part to the instability of IRT during transport and storage.

¹ Kirby, L.T. et al. "Use of a dried blood spot in immunoreactive trypsin assay for detection of cystic fibrosis in infants." *Clin. Chem.* 27: 678-680 (1981).

² Borgstrom, A. et al. "Immunoreactive trypsin screening for cystic fibrosis." *Acta Paediatr. Scand.* 71: 621-624 (1982).

³ Nadeau, F.L., et al. "Neonatal cystic fibrosis screening: Implications for apparent seasonal variations in immunoreactive trypsinogen (IRT) concentration." *Proc 11th National Neonatal Screening Symp.*, 200-201 (1995).

d. *Detection limit:*

The Limit of the Blank (LoB) for the AutoDELFI A 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*. Multiple (72) measurements of two sample types, the zero calibrator and dried human blood spots (with low levels of analyte), were performed with the Neonatal IRT L kit. The Limit of the Blank was determined to be 0.18 ng/ml, the 95th percentile of the distribution of the test values.

The low limit of the claimed assay measuring range (13 ng/mL) was determined based on the linearity study described above.

e. *Analytical specificity:*

The Neonatal IRT L kit was evaluated for interference consistent with CLSI Guideline EP7-A2.

Heparinized whole blood samples taken from three donors were pooled and spiked with two medically relevant trypsin concentrations (60 and 100 ng/ml). Unconjugated bilirubin (up to a concentration of 200 mg/l) and hemoglobin (5000 mg/l) were added to the pools before creating dried spots on filter paper and analyzed in six replicates in parallel with unspiked samples. Testing of these concentrations showed no interference in the measurement of the two analyte levels. Testing of lipemic (triglycerides \leq 5000 mg/l) and icteric (bilirubin \leq 30 mg/dl) samples was evaluated with a previous device (k003668) that uses the same assay reagents and found not to interfere with the measurement of IRT.

As noted in the package insert: if the specimen is not applied directly onto the filter paper, do not use EDTA or citrate tubes or capillaries to collect blood, as these anticoagulation reagents will affect the assay by chelating the europium label.

Hook effect: The potential for hook effect was evaluated with a previous device (k003668) that uses the same assay reagents and showed no hook effect for the Neonatal IRT L kit when concentrations up to 40,000 ng/ml IRT were tested.

Carry over: A study was performed to determine the extent of carry-over in the sample pipetting probes when the Neonatal IRT L kit is used with the 1235 AutoDELFIA automatic immunoassay system. The aim was to determine whether IRT-containing samples in liquid form (standards and controls) transfer between the wells by the pipetting probes. The patient samples are dried blood spots, therefore there is no possibility of carry-over of those samples. Carry-over was determined by pipetting first the IRT standard F (500ng/ml) and thereafter the analyte-free calibrator A. This was repeated three times with each sample probe. Carry-over percentage was defined as the average amount of IRT transferred from the high sample to the analyte-free sample well, as a percentage of the high sample concentration. The study consisted of four different assays performed with two different instruments, repeated once for a total of eight assays in the study. The observed carry-over result observed in all of the tests performed ranged from 0.002% - 0.272%.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The AutoDELFIA Neonatal IRT L kit was compared to the predicate device

using CLSI Document EP9-A2 as a guideline for the method comparison study.

The AutoDELFIA Neonatal IRT L kit was compared to the predicate device (AutoDELFIA Neonatal IRT kit) using spiked (heparinized whole blood samples spiked with trypsin) and routine screening blood spot specimens. Samples were tested over 5 operating days. 99 specimens with an IRT range of 13 - 366 ng/mL blood were tested. The correlation was found to be:

$$y = 0.883x - 0.655$$

$$r^2 = 0.963$$

Clinical data was provided to establish clinical performance of the device (see section 3c. below) since the assay does not analytically align well with the predicate device.

b. *Matrix comparison:*
Not applicable.

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 168622 newborn screening specimens tested in a U.S. state laboratory are presented in the following tables. The numbers reported in this section are from initial screens only, unless otherwise noted. All initial screens were performed in singlicate using the AutoDELFIA Neonatal IRT L kit. The screening strategy was as follows: Samples with the highest 1.6% (> 62 ng/mL) of the IRT results were tested by DNA analysis of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. Samples having a CF-related genotype in the DNA analysis were tested using a diagnostic sweat test. The CF-positive cases detected (see chart below) include 10 sweat test positive cases and 14 cases with intermediate sweat test result (suspect CF).

Summary

| | | CF Positive | CF Negative | Total |
|---------------------------|-----------------|--------------------|--------------------|--------------|
| IRT Initial Result | Positive | 24 | 2614 | 2638 |
| | Negative | 0 | 165984 | 165984 |
| | Total | 24 | 168598 | 168622 |

Range of IRT values from the CF positive patients.

| | | |
|-------------------------|-----|-----|
| | Min | Max |
| IRT Concentration ng/mL | 68 | 350 |

4. Clinical cut-off:

Each laboratory should establish 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 for second tier testing.

Do not use a cut-off that is based on data collected with another product and do not use a fixed cut-off.

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

5. Expected values/Reference range:

IRT patient values by percentile in the Neonatal IRT L kit study data. (The unit of IRT concentration is ng/mL blood.)

| n | Mean | Median | Percentile | | |
|--------|------|--------|------------|-----|-----|
| | | | 90% | 95% | 99% |
| 168622 | 22 | 18 | 38 | 47 | 68 |

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.