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

A. 510(k) Number:

k152422

B. Purpose for Submission:

New Device

C. Measurand:

Free Thyroxine (FT4)

D. Type of Test:

Competitive Fluorescence Immunoassay

E. Applicant:

NanoEnTek, USA Inc.

F. Proprietary and Established Names:

FREND^{TM} Free T4 Test System

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1695, Free thyroxine test system

2. Classification:

   Class II

3. Product code:

   CEC

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   The FREND ™ Free T4 Test System is a rapid indirect competitive immunoassay for the quantitative determination of free thyroxine (FT4) in human serum and lithium heparinized plasma specimens using the FREND™ System. Measurements of free thyroxine (FT4) are in the diagnosis of thyroid disorder. The FREND™ Free T4 Test System is intended for use in clinical laboratories. For in vitro diagnostic use only. The test is not intended for point-of-care facilities.

3. Special conditions for use statement(s):

   For prescription use only

   For in vitro diagnostic use

   Not for use in point-of-care settings

4. Special instrument requirements:

   NanoEnTek FREND™ System

I. Device Description:

The FREND™ Free T4 (reagent cartridge) kit comprise of 20 test cartridges, 20 Gold-T4 antibody tubes, 30 disposable pipette tips, a FREND™ Free T4 code chip and a FREND™ Free T4 package insert.

The FREND™ Free T4 Test cartridge is a disposable plastic device that houses the reagents and contains a port or opening (inlet) where the sample is applied. Cartridges are provided in individually sealed pouches and must be stored between 2-8 °C.

Each Gold-T4 antibody tube is a single-use disposable plastic tube that contains an anti-T4 antibody and gold particles.

The FREND™ System (previously cleared in k124056) is not provided with the kit but is required for utilization with the FREND™ Free T4 cartridge. The FREND™ System is a bench top fluorescence reader containing a touch-screen user interface.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Abbott ARCHITECT Free T4

2. Predicate 510(k) number(s):
   k123379

3. Comparison with predicate:

Similarities / Differences		
Item	FREND™ Free T4 Test System (Candidate Device)	Abbott Architect Free T4 (Predicate device) k123379
Intended Use	For the quantitative determination of Free T4 (FT4) levels in serum and plasma	Same
Assay Methodology	Fluorescent immunoassay	Chemiluminescent immunoassay
Sample Type	Serum and Lithium heparin plasma	Same
Measuring range	0.40 – 6.00 ng/dL	Same
Calibrators	All calibration statistics and information have been electronically stored on the FREND™ Free T4 Code chip included in each box of FREND™ Free T4 cartridges.	6-point calibration 6 levels 0.0, 0.5, 1.0, 2.0, 3.5, 6.0 ng/dL
Antibody	Monoclonal mouse anti-T4	Polyclonal sheep anti-T4

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

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedure

CLSI EP07-A2: Interference Testing in Clinical Chemistry

CLSI EP09-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP14-A3: Evaluation of Commutability of Proceeded Samples

CLSI EP17-A2: Evaluation of Detection Capabilities for Clinical Laboratory Measurement Procedures Quantitation

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents

CLSI EP28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

The FREND™ Free T4 Test utilizes microfluidic technology and detects immune complexes bound to FT4. A 70 µl sample is first incubated during Step 1 in the FT4 Gold AB Tube with monoclonal (mouse) anti-T4 antibody conjugated with gold micro-particles. In Step 2, 35µL of the mixture from Step 1 is manually loaded into the inlet of the cartridge, where it hydrates a T4-BSA fluorescent bead conjugate and migrates along the test strip. During migration the bound FT4 in the sample and the fluorescent bead conjugates of T4-BSA compete to form antigen antibody complex in the test zone. Unbound T4-BSA fluorescent conjugates flow through and bind to the anti-T4 antibody that is fixed on the surface in the reference zone.

FT4 quantification is based upon the ratio of the intensity of the test and reference zones. A lower ratio of fluorescence is indicative of a higher FT4 concentration, in other words, the magnitude of the fluorescent ratio is inversely proportional to the amount of FT4 in the sample.

There is no calibration by the user because each cartridge is coded with the calibration information generated by the manufacturer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The sponsor performed a precision study for the FREND™ Free T4 Test System according to the CLSI EP05-A3. Three serum pools with low (0.917ng/dL), intermediate (1.850 ng/dL), and high (3.979 ng/dL) FT4 levels were assayed in duplicate twice per day for 20 days (80 total measurements) on a single lot of reagent cartridge on a single analyzer. The precision data for the FREND™ Free T4 Test are summarized below:

Sample Pool	Mean ng/dL	Repeatability		Between-run		Between day		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.917	0.067	7.3	0.000	0.0	0.032	3.5	0.074	8.1
2	1.850	0.103	5.6	0.000	0.0	0.069	3.7	0.124	6.7
3	3.979	0.186	4.7	0.152	3.8	0.093	2.3	0.258	6.5

b. Linearity/assay reportable range:

To demonstrate the linearity of the assay, a study design was used based on CLSI

CLSI-EP6-A. A serum base pool with an elevated FT4 (7.5 ng/dL) was prepared and diluted to a total of 11 levels. The range of samples tested was 0.11 – 7.50 ng/dL. At each dilution level, the samples were tested in duplicate to determine the experimental value of FT4. The data was analyzed using least square linear regression. The quadratic terms of the second and third order polynomial fit were statistically insignificant (p value <0.001). Linear regression equation generated was $y = 0.978x – 0.0881$; $r^2 = 0.9938$.

Linearity data support the sponsor's claimed measuring range from 0.4– 6.0 ng/dL.

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

The standards/calibrators are internally prepared according to the guideline of CLSI C45-A Measurement of Free Thyroid Hormones; Approved Guideline – first Edition. This internal standard is manufactured by gravimetric methods based on the L-Thyroxine (from commercial source). At each concentration level, the Free T4 levels of calibrators are confirmed by measurement on ARCHITECT *i* free T4 assay (K123379).

There is no need for calibration by the operator as the calibration information is coded in the individual cartridge.

The sponsor recommends a minimum of two (2) levels of commercially available controls be run at least once per month or once for each new lot, whichever comes earlier. Each laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the FREND™ Free T4 assay was determined in accordance with the CLSI EP17-A2.

<u>LoB Protocol</u>
LoB was determined with five different blank samples (<0.1 ng/dL). Each sample was assayed 12 times over a period of five days using a single reagent lot (n= 60 measurements). The LOB was calculated from N = 60 (4 measurements X 5 blank samples X 3 days = 60), using a parametric analysis for a Gaussian distribution of the blank values, as described in CLSI EP-17A.

<u>LoD Protocol</u>
LoD was determined using five low level samples (0.3 - 0.45). Each sample was assayed in 4 replicates for three days using a single lot of reagent (n = 120 measurements). LoD was reported the lowest level material where the *beta*-percentile was 5%.

LoQ Protocol

The LoQ was determined by performing a functional sensitivity study. Seven levels of a low sample (0.0 – 0.9 ng/dL) were assayed using three lots of reagent for four days. The lowest FT4 concentration giving an imprecision within 20%CV was determined to be the LoQ value.

The detection limits studies results are summarized in the table below:

LoB	0.171 ng/dL
LoD	0.320 ng/dL
LoQ	0.360 ng/dL

The claimed measuring range of the candidate device is 0.4 to 6.0 ng/dL.

e. Analytical specificity:

The sponsor performed studies to evaluate the effect of potential interferents on the performance of the FREND™ Free T4 assay, following the CLSI EP07-A2 guideline. Two levels of serum samples containing approximately 1.0 ng/dL and 2.5 ng/dL of FT4 were spiked with different concentrations of the potential interferents. Each level of FT4 was tested with each interferent in 3 replicates. The sponsor defined non-significant interference as recovery from 90% to 110% of the expected Free T4 concentration. The table below lists the substances tested and the concentration at which no significant interference was observed:

Endogenous substances	Potential Interferent	Highest concentration tested at which no significant interference was observed
	Hemoglobin	500 mg/dL
	Bilirubin, conjugated	20 mg/dL
	Bilirubin, unconjugated	20 mg/dL
	Triglycerides	3000 mg/dL
	Total protein	12 g/dL
	Biotin	2.5 µg/mL
	IgG	2.5 mg/dL
	IgA	60 µg/mL
	IgM	45 µg/mL

	Potential Interferent	Highest concentration tested at which no significant interference was observed
Pharmaceuticals	Acetaminophen	200 µg/mL
	Erythromycin	60 µg/mL
	Diltiazem	6.24 µg/mL
	Verpamil	2 µg/mL
	Acetylcysteine	415 µg/mL
	Acetylsalicylic acid	250 µg/mL
	Amiodarone	6 µg/mL
	Ampicillin-Na	50.3 µg/mL
	Ascorbic Acid	60 µg/mL
	Carbimazole	500 ng/mL
	Cefoxitin	66 µg/mL
	Cyclosporine	3 µg/mL
	Doxycycline	400 ng/mL
	Fluocortolone	400 ng/mL
	Furosemide	12.5 µg/mL
	Heparin	3000 U/L
	Hydrocortisone	1.8 µg/mL
	Ibuprofen	250 µg/mL
	Iodide	380 µg/mL
	Levodopa	4 mg/mL
	Methyldopa	15 µg/mL
	Metronidazole	120 µg/mL
	Octreotide	2 ng/mL
	Perchlorate	16 ng/mL
	Pednisolone	3 µg/mL
	Propranolol	2 µg/mL
	Propylthiouracil	10 µg/mL
	Rifampicin	640 µg/mL
	Theophylline	400 µg/mL
	Thiamazole/Methimazole	500 ng/mL
	Avidin	5 µg/mL
	Au-nanoparticles	**5** µg/mL
Heterophilic Antibodies	RF	1075 IU/mL
	HAMA	70 ng/mL

Cross reactivity study:

The cross-reactivity study was evaluated for potential cross reactants to two levels (low and high) FT4 concentrations.

% cross reactivity = 100 x [(Measured value – true value) / concentration of interferent)]

The cross reactivity results are summarized in the table below:

Cross reactant	Cross-reactant Concentration (ng/dL)	% Cross reactivity
Levothyroxine, T4	1000	99.6
Diiodothyronine, T2	5000	0.0005
Tetraiodothyroacetic Acid	10,000	0.0005
Triiodothyroacetic Acid	1000	0.0157
Triiodothyropropionic acid	5000	0.0055
Diiodotyrosine, DIT	1,000,000	2×10^{-6}
L-Triiodothyronine, T3	1000	0.026
Monoiodotyrosine	1,000,000	0.000019
Reverse T3	10,000	0.0022

The sponsor stated the following limitations in the package insert:

- Specimens from patients with heterophilic antibodies, such as anti-mouse (HAMA), anti-goat (HAGA), or, anti-rabbit (HARA) antibodies, may show falsely elevated or depressed values or may result in the error message "Incomplete Test." Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interferences. If the FT4 level is inconsistent with clinical evidence, additional FT4 or other thyroid testing using a different method is suggested to confirm the results. Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.

- Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

- Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor performed a method comparison study on the NanoEnTek FREND™ FreeT4 assay and the predicate device (Abbott Architect Free T4 assay) using 358 natural serum specimens with the approximate measuring range (0.43 - 5.99 ng/dL). The testing was performed by three operators at a single hospital clinical laboratory.

The regression results of the Ordinary least square fit method are summarized below:

Analyte	n	Range (ng/dL)	Slope (95% CI)	Intercept (95% CI)	r
FT4	358	0.43 – 5.99	1.010 (0.992-1.028)	0.057 (0.021-0.094)	0.986

b. Matrix comparison:

The matrix comparison study was performed using 48 sample pairs, each with serum and lithium heparin plasma. All samples were measured using the FREND™ Free T4 assay. Passing-Bablok regression analysis of serum results (x) compared to lithium heparin plasma results (y) was performed. The results are summarized in the table below:

Slope: 1.017 (95% Cl: 0.991-1.044)	y-Intercept: -0.008 (95% Cl: -0.055-0.0451)
n = 48	Sample range tested: 0.44-5.63 ng/dL
r^2	0.9948

The results from the matrix comparison study support the sponsor claim that lithium heparin samples are acceptable for this assay.

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. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   To determine the reference range for the FREND™ FreeT4 assay, a total of 196 apparently healthy ambulatory adults aged 21 years and older were utilized. All samples were assayed on the FREND™ FT4 to establish the reference range interval according to CLSI EP28-A3. The reference interval for the FREND™ FreeT4 Test System was found to be 0.83 – 1.60 ng/dL based on the central 95% of the frequency distribution.

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.