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

A. 510(k) Number:

k131928

B. Purpose for Submission:

New device

C. Measurand:

Thyroid Stimulating Hormone (TSH)

D. Type of Test:

Quantitative, Fluorescence Immunoassay

E. Applicant:

NanoEnTek Inc.

F. Proprietary and Established Names:

FREND TSH

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1690. Thyroid Stimulating Hormone test system.

2. Classification:

   Class II

3. Product code:

   JLW

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below

2. Indication(s) for use:

   FREND™ TSH is designed for *in vitro* DIAGNOSTIC USE ONLY for the quantitative measurement of Thyroid Stimulating Hormone (thyrotropin or TSH) in human serum and lithium heparin plasma using the FREND™ system.

   FREND™ TSH is indicated for use in clinical laboratories upon prescription by the attending physician as an aid to clinicians in the diagnosis of thyroid disease.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   NanoEnTek FREND™ System.

I. Device Description:

Material Provided - FREND™ TSH, includes the following in the kit

- 25 FREND™ TSH cartridges - The Test Cartridge is a disposable plastic device that houses the reagents and contains an opening where the sample is applied. Sample and reagents interact before being analyzed by the FREND System fluorescence reader. One Cartridge contains:
  - Mouse monoclonal anti-Human Beta-TSH: 450± 45 ng
  - Goat Polyclonal anti- Human Beta-TSH: 480 ± 48 ng
  - Fluorescent particle: 3.96 ± 0.39 μg
- 30 Disposable pipette tips
- 1 FREND™ TSH Code Chip

The FREND System (previously cleared in k124056) is not provided with the kit but is required for the utilization of the FREND TSH cartridge.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ST AIA-PACK TSH (TOSOH Bioscience)

2. Predicate 510(k) number(s):
   k972586

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device	Predicate Device
Intended Use	For the quantitative measurement of Thyroid Stimulating Hormone (thyrotropin or TSH) in human serum and lithium heparin plasma.	Same
Test Vessel	Disposable single-use reaction vessel	Same
Sample Type	Human serum and heparinized plasma	Human serum
Reaction Type	Antibody/antigen complexes	Same
Type of Test	Fluorescent immunoassay detecting TSH	Same
Test Throughput	Single Test - 7 minutes to result. 50 tests would take 350 minutes.	Single test - 18 minutes; 50 tests - 68 minutes.
Calibration Material	WHO International Standard Hormone, NIBSC code: 81/565	WHO 2nd International Reference
Reference Range	0.49 – 3.82 mIU/L	0.5 – 6.0 mIU/L

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

• CLSI EP05-A2; Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition

• CLSI EP06-A – Evaluation of the Linearity of Quantitative Measurement; Approved Guideline

• CLSI EP07-A2 – Interference Testing in Clinical Chemistry; Approved Guideline – second edition

• CLSI EP09-A2 - Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – second edition

• CLSI EP14-A2 – Evaluation of Matrix Effects; Approved Guideline – Second Edition

• CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

• CLSI C28-A3 – Defining, Establishing; and Verifying Reference Intervals in the Clinical Lab; Approved Guideline – Third Edition

• FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable

• FDA Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (Document 337)

• Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (March 13, 2007)

L. Test Principle:

The FREND™ TSH test cartridge is a rapid quantitative "sandwich" immunoassay utilizing fluorescent nanoparticles in microfluidic flow to capture and quantify TSH in serum and heparinized plasma.

The FREND™ TSH uses single-use transparent plastic cartridges in which all required reagents are stored within the cartridge itself. The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been manually loaded to the sample inlet in the cartridge and the cartridge placed into the FREND™ System.

A 35 µL drop of patient serum or lithium heparin plasma is placed in the FREND™ TSH cartridge inlet port, where the sample interacts with a proprietary mix of dry-loaded reagents. One of these reagents includes antibody-conjugated fluorescent nanoparticles, forming immune complexes with TSH in the patient sample. Capillary action moves the sample to the detection region, where capture antibodies grab the TSH-nanoparticle. The concentration of TSH is calculated by the FREND™ System when the ratio of Test/Reference fluorescence in an unknown is compared to that same ratio for standards of known concentration. Total TSH concentration in a sample analyzed with the FREND™ TSH on the FREND™ System correlates directly with the fluorescence intensity - the higher the TSH concentration, the greater the fluorescence ratio. The TSH result is calculated using information stored on the lot specific FREND™ TSH Code Chip and then is displayed on the FREND™ System screen.

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

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

An internal precision study was performed according to CLSI EP5-A2 guideline. Four patient serum samples were analyzed using one lot of FREND TSH cartridge. Samples were analyzed in replicates of two at two separate times per day for twenty days. Results of the precision study are as follows:

Sample	Mean TSH Conc. (mIU/L)	Within-run		Between-run		Between-day		Total precision	
		SD (mIU/L)	CV%	SD (mIU/L)	CV%	SD (mIU/L)	CV%	SD (mIU/L)	CV%
1	0.496	0.043	8.6	0.012	2.4	0.017	3.4	0.047	9.6
2	5.948	0.353	5.9	0.082	1.4	0.031	0.5	0.364	6.1
3	11.989	0.555	4.6	0.375	3.1	0.156	1.3	0.688	5.7
4	23.763	0.846	3.6	0.478	2.0	0.000	0.0	0.972	4.1

b. Linearity/assay reportable range:

A high TSH concentration pool (25.5 mIU/L) was diluted with a low TSH concentration pool (0.017 mIU/L) based on the CLSI EP06-A guideline, resulting in eleven equally-spaced TSH concentrations samples which spanned the assay's measuring range. All samples were run in triplicates using one FREND system. The observed results were plotted against the expected results and the linear regression analysis yielded the following regression equation: Y= 0.977 + 0.17, r = 0.999.

Linearity results:

Sample no.	Expected results (mIU/L)	Observed results (mIU/L)	Difference (mIU/L)	% difference
1	0.0	0.017	0.017	N/A
2	2.6	2.803	0.203	3
3	5.2	5.483	0.283	5.4
4	7.8	8.267	0.467	5.9
5	10.4	9.883	-0.517	5.0
6	13.0	12.627	-0.373	2.9
7	15.6	15.180	-0.420	2.7
8	18.2	17.927	-0.273	1.5
9	20.8	20.780	-0.002	0.01
10	23.4	23.143	-0.257	1.1
11	26.0	25.543	-0.487	1.9

The sponsor states the measuring range of the assay is 0.06 mIU/L to 25.0 mIU/L.

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

Traceability

The FREND TSH assay is traceable to the WHO standard 81/565. Each lot of cartridges has a calibrator code chip which is traceable to WHO International Thyroid Stimulating Hormone, human for Immunoassay, NIBSC code: 81/565. For the end user, there is no need for calibration as the calibration information is coded in the individual cartridge.

Stability

Real time stability studies at 2-8°C and room temperature are ongoing. Real time stability studies were conducted and the shelf life for the FREND™ TSH are good for at least one year from date of manufacturer if stored refrigerated appropriately as directed at 2-8°C. Stability study protocol have been provided and found to be adequate.

d. Detection limit:

The detection limits, (LoB, LoD and LoQ) for the FREND™ TSH were evaluated based on the CLSI EP17-A protocol. The Limit of Blank (LoB) was evaluated by analyzing three lots of TSH-free charcoal-stripped serum samples measured over a period of six days for a total of n=60 observations. The LoD was evaluated by analyzing three low level TSH human serum samples over a period of three days and three different lot cartridges to arrive at a total of n=72 measurements. The LoQ was evaluated by analyzing three low level TSH human serum samples over a period of three days and three different lot cartridges to arrive at a total of n=60 measurements. LoQ is defined as the concentration that generated less than 20%CV. Results are summarized in the table below:

LoB	LoD	LoQ
0.03 mIU/L	0.06 mIU/L	0.06 mIU/L

The measuring range of this assay is 0.06 to 25.0 mIU/L.

e. Analytical specificity:

Interference Studies

An interference study was performed according to CLSI-EP07 to determine whether the presence of bilirubin, triglycerides, hemoglobin, or total protein may interfere with the FREND TSH assay. Human serum pools were used with two different concentrations (low [approx. 0.3 – 0.5 mIU/L] and high [approx. 5-10 mIU/L]) of TSH. Each sample level was spiked with the interferent and then compared to a

sample which contained no interferent. The sponsor considers significant interference as greater than +/- 10%. The results of the highest concentration tested without significant interference are summarized below.

Interferent	TSH level	Recovery (%)
Triglyceride (3g/dL)	low	96.3
	high	106.9
Bilirubin (20 mg/dL)	low	106.2
	high	93.8
Hemoglobin (500 mg/dL)	low	100.0
	high	97.70
Total protein (12 g/dL)	low	100.0
	high	100.8

An interference study was conducted to determine whether the presence of common drugs interfere with the FREND TSH assay. Human serum pools were spiked with a known amount of drug interferent and then compared to the control pools containing no interferent. For each interferent, a single concentration was tested with low (0.3-0.5 mIU/L) and high (10 mIU/L) TSH concentrations. The sponsor considers significant interference as +/- 10%. The results of the concentration tested without significant interference are summarized below.

Interferent	TSH level	Recovery (%)
Acetominophen (1324 uM)	low	92.4
	high	92.2
Diltiazem (15 uM)	low	95.0
	high	105.4
Erythromycin (81.6 uM)	low	101.3
	high	100.6
Verapamil (4.4 uM)	low	96.0
	high	98.7

Interference studies of HAMA (3.5, 52.5 and 70 ng/ml) and RF (53.8, 161.3, and 215 IU/ml) were performed on TSH Low (1.0 mIU/L) and TSH High (7.20 mIU/L) serum. The sponsor considers significant interference as greater than +/- 10%. No significant interference was observed for concentrations at or below 52.5 ng/ml HAMA and 53.8 IU/ml RF.

Hook Effect: TSH was spiked into a human serum sample which had been depleted of TSH. A TSH standard was spiked into the sample to achieve concentrations ranging from 0.31- 2500 mIU/L was analyzed. No hook effect was observed at TSH concentrations up to 2500 mIU/L.

Ambient temperature interference study was conducted and provided. Study demonstrated that ambient temperature between 22°C and 31°C does not significantly interfere with the TSH assay.

Cross-Reactivity

Human serum samples were spiked with potential cross-reactants FSH, LH and hCG and compared to a control pool containing no cross-reactant. Testing was done according to the instructions recommended in CLSI EP07-A guideline. Percent cross-reactivity with various cross-reactants is summarized in the table below.

Sample TSH Conc. (mIU/L)	Interferent	Material added	% Cross Reactivity
0.49	hCG	200,000 mIU/L	2×10^{-8}
0.55	LH	500 mIU/L	2×10^{-4}
0.55	FSH	500 mIU/L	-5×10^{-6}
6.22	hCG	200,000 mIU/L	3×10^{-7}
6.06	LH	500 mIU/L	3×10^{-5}
6.06	FSH	500 mIU/L	1×10^{-4}

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were conducted internally using the FREND TSH assay on the FREND™ system and the predicate device. A total of 438 natural patient samples ranging from 0.09 – 24.96 mIU/L were analyzed. Samples were analyzed in singlicate on both devices. Results are summarized in the table below.

Analyte	n	Range	Slope [95% CI]	Intercept [95% CI]	R^2
TSH	438	0.09 – 24.96	0.951	0.0266	0.9849
			[0.940,0.962]	[-0.0258,0.0790]	

b. Matrix comparison:

To evaluate the effect of anticoagulant, the FREND TSH assay was used to measure the TSH concentrations of matched sets of serum and Lithium-Heparin plasma. A total of 40 matched pair samples were analyzed and compared, giving a linear regression line of $y = 0.995x - 0.320$, $r = 0.992$.

The results from the matrix comparison study support the sponsors 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:

   Serum samples from 386 normal, apparently healthy individuals consisting of 191 females and 195 males were assayed on 3 lots of the FRENDS TSH assay using a single FREND system. The reference range was evaluated based on the CLSI C28-A3 guideline. The reference range for the FREND TSH assay is 0.49 – 3.82 mIU/L 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 determination.