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

A. 510(k) Number:

k162754

B. Purpose for Submission:

New Device

C. Measurand:

Total 25-hydroxyvitamin D (25-OH vitamin D)

D. Type of Test:

Quantitative, Fluorescence Immunoassay

E. Applicant:

NanoEnTek, Inc.

F. Proprietary and Established Names:

FREND Vitamin D Test System

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System

2. Classification:

   Class II

3. Product code:

   MRG-Vitamin D Test System

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The FREND Vitamin D Test System is a rapid indirect competitive fluorescence immunoassay designed for the quantitative measurement of 25-hydroxy Vitamin D and related hydroxylated metabolites in human serum and plasma (K_3EDTA, lithium-heparin and citrate) specimens using the FREND System, and the FREND AP System. Measurements of total 25-hydroxy Vitamin D and related hydroxylated metabolites are used to aid in the assessment of Vitamin D sufficiency.

   The FREND Vitamin D microfluidic flow cartridge is designed for use in the FREND System fluorescent immunoassay reader, and the FREND AP System. The FREND Vitamin D Test System is intended for use in clinical laboratories. For *in vitro* diagnostic use only. The test is not intended for use in point-of-care settings.

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 and FREND AP System

I. Device Description:

The FREND Vitamin D test kit comprise of 20 FREND™ Vitamin D test cartridges, 20 sample dilution tubes, 20 FREND™ Vitamin D gold antibody pretreatment tubes, 30 disposable pipette tips, a FREND Free Vitamin D code chip and a FREND Vitamin D package insert.

The FREND Vitamin D test cartridge is a single-use 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. The sample dilution tube contains perfluorohexanoic acid. The FREND Vitamin D gold antibody pretreatment tube is a single-use disposable plastic tube that contains Vitamin D gold nanoparticle-conjugated antibody. The Vitamin D code chip allows lot information and calibration data loaded into the software for each reagent kit.

Each cartridge contains:

- Monoclonal mouse anti- Vitamin D 1.6 ± 0.16 ng
- 25-hydroxyvitamin D 136 ± 13.6 ng
- Fluorescence particles 2.4 ± 0.24 µg

Each sample dilution tube contains:

- Perfluorohexanoic acid 9.5 ± 0.95 µg

Each Gold antibody pretreatment tube contains:

- Gold nano-particle conjugation antibody 7.0 ± 0.7 µg

The FREND System (previously cleared in k124056), which is a bench top fluorescence reader containing a touch-screen user interface, is not provided with the kit but is required for the FREND Vitamin D test. The FREND AP System is an "Advanced Preparing" device that performs automated pre-analytical steps of mixing, timed heating, and pipetting of the sample from the Gold Antibody Pretreatment tube into the FREND Vitamin D test cartridge. A sample is diluted in the sample dilution tube first and then transferred to the pretreatment tube prior to being inserted into the FREND AP System for sample mixing, incubation and pipetting into the test cartridge. The test cartridge is then inserted into the FREND System to be analyzed.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Abbott ARCHITECT 25-OH Vitamin D

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

   k110619

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate device FREND Vitamin D Test System (K162754)	Predicate device ARCHITECT 25-OH Vitamin D (k110619)
Intended Use	For quantitative measurement of 25-hydroxy Vitamin D in human serum and plasma.	Same
Sample Type	Serum, K_3EDTA, Lithium Heparin and citrate plasma	Serum, Sodium Heparin and Lithium Heparin plasma
Assay Methodology	Fluorescent immunoassay	Chemiluminescent immunoassay
Assay Range	13.0-96.0 ng/mL	Same
Sample Size	35 µL	60 µL (priority) or 150 µL (≤3h)

Similarities and Differences		
Item	Candidate device FREND Vitamin D Test System (K162754)	Predicate device ARCHITECT 25-OH Vitamin D (k110619)
Test Cartridge	Disposable single-use cartridge	No single-use cartridge

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

CLSI EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline

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

CLSI EP14-A3, Evaluation of commutability of Processed Samples; Approved Guideline

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
CLSI C28-A3C: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

L. Test Principle:

The FREND Vitamin D Test System is a competitive immunoassay with gold microparticles labeled with Vitamin D-specific monoclonal anti-Vitamin D-antibody (mouse), Vitamin D-biotin labeled with fluorescence nanoparticles and fluorescence detection by the FREND System. The concentration of the analyte of interest in an unknown sample is calculated using the ratio of the fluorescent intensity of the test zone and the reference zone. The magnitude of the fluorescent ratio is inversely proportional to the amount of 25-OH Vitamin D in the sample. 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. Analytical performance:

a. Precision/Reproducibility:

An internal precision study was performed in accordance with CLSI EP5-A3 in which three serum samples with low, intermediate and high 25-OH Vitamin D levels were assayed in duplicate in two runs per day for 20 days using one reagent lot for a total of 80 results per sample. The precision results are summarized in the table below:

ID #	N=80	Repeatability		Between run		Between day		Total	
	Mean (ng/mL)	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	20.1	1.76	8.7%	0.46	2.3%	0.22	1.0%	1.83	9.1%
2	50.6	2.26	4.5%	1.31	2.6%	0.59	1.2%	2.67	5.3%
3	80.5	4.53	5.6%	1.13	1.4%	0.74	0.9%	4.72	5.9%

b. Linearity/assay reportable range:

Linearity was evaluated based on CLSI EP-6A. A linearity sample set was prepared by diluting a serum sample with high 25-OH vitamin D concentration with a serum sample with low 25-OH vitamin D concentration to obtain nine (9) concentration levels (8.0, 20.2, 32.4, 44.6, 56.8, 69.0, 81.2, 93.4, 105.6ng/mL) across the measuring range of the candidate assay. Samples were run in replicates of 4 on one FREND system using one reagent lot. The ordinary least squares linear regression of the observed mean concentrations versus the expected concentration was calculated and yielded the following linear regression equation:

$y = 0.9567x + 1.9626$, $r^2 = 0.9894$

The results of the linearity study support the sponsor's claim that the assay is linear from 13 – 96 ng/mL.

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

Traceability:

The FREND Vitamin D Code chip calibration information is traceable to the manufacturer's primary calibrators. The primary calibrators are prepared gravimetrically from a certified standard material and confirmed by measurement on the ARCHITECT *i* 25-OH Vitamin D assay (k110619). All calibration statistics and information have been electronically stored on the FREND Vitamin D Code chip included in each box of FREND Vitamin D cartridges. There is no need for calibration by the operator as the calibration information is coded in each code chip, which is lot-specific.

Quality Control:

The sponsor recommends the use of at least two levels of commercially available quality control material that contains 25-OH Vitamin D as the measurand. The sponsor used Bio-Rad Lyphocheck Immunoassay Controls (cleared under k133960) during performance characteristic studies.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) studies were performed according to CLSI EP-17A2.

To establish LoB, six blank serum samples were assayed 10 times per day per sample over 6 days using 3 reagent lots on one FREND system. The Limit of Blank was calculated from N = 60 results using a non-parametric analysis and was determined to be 3.3 ng/mL.

To determine LoD, five low level serum samples were assayed 2 times per day over 6 days using 3 reagent lots on one FREND system. The LoD was calculated from LoB using the following formula LoD = LoB+ C_β x SD and was determined to be 6.3 ng/mL.

The LoQ was determined by performing a functional sensitivity study. Six levels of 25-OH vitamin D serum samples (2.5– 40.0 ng/dL) were assayed using 3 lots of reagent for 4 days on one FREND system. The 25-OH vitamin D concentration giving an imprecision of 20%CV was determined to be the LoQ value.

Detection limits results:

Limit of Blank	Limit of Detection	Limit of Quantitation
3.3 ng/mL	6.3 ng/mL	9.9 ng/mL

The reportable range of the assay is 13-96 ng/mL.

e. *Analytical specificity:*

Interference:

The sponsor performed studies to evaluate the effect of potential interferents on the performance of the FREND Vitamin D Test, following the CLSI EP07-A2 guideline. Two levels of serum sample pools containing approximately 20 ng/mL and 40-50 ng/mL of Vitamin D were spiked with different concentrations of the potential interferents. Each level of Vitamin D sample was tested with each interferent in 3 replicates. The sponsor defined non-significant interference as recovery from 90% to 110% of the expected Vitamin D concentration. The table below lists the substances tested and the concentration at which no significant interference was observed:

	Potential Interferent	Highest concentration of substance tested that demonstrated no significant interference
Endogenous substances	Conjugated bilirubin	30 mg/dL
	Unconjugated bilirubin	30 mg/dL
	Hemoglobin	500 mg/dL
	Triglycerides	1500 mg/dL
	Total Protein	12 g/dL
	Biotin	6 µg/mL
	Cholesterol	500 mg/dL

	Potential Interferent	Highest concentration of substance tested that demonstrated no significant interference
	Rheumatoid factor	536 IU/mL
	HAMA	1000 ng/mL

Cross reactivity:

Cross-reactivity with the FREND Vitamin D was evaluated by spiking potential cross reactants to two levels of (low and high) 25-OH Vitamin D serum pools. The cross-reactivity was calculated using the following equation:

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

Results from this study are summarized in the tables below:

Cross-reactant	Conc. of cross-reactant (ng/mL)	% Cross-reactivity	
		Low	High
Vitamin D2 (Ergocalciferol)	500	0.5	0.8
Vitamin D3 (Cholecalciferol)	500	0.5	0.7
1,25-(OH)2-Vitamin D2	100	2.2	0.7
1,25-(OH)2-Vitamin D3 (Calcitriol)	100	6.9	1.9
3-epi-25-(OH) Vitamin D3	100	2.1	2.0
25-(OH) Vitamin D2	25	97.2	97.3
25-(OH) Vitamin D3	25	107.3	92.2
Paricalcitol	25	15.8	14.7

The sponsor includes the following limitations in the labeling:

- Patients taking the drugs containing Paricalcitol (e.g., Zemplar) should not be tested by this assay.
- 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.

Hook effect study:

The sponsor performed a hook effect study by testing Vitamin D-depleted serum spiked with 25-OH Vitamin D3 using a single lot of reagents on one FREND system. The Vitamin D concentrations ranged from 20 to 1600 ng/mL. The results supported the following statement in the labeling:

The FREND Vitamin D assay has been evaluated and no high dose hook effect was observed for Vitamin D concentrations up to 1600 ng/mL.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the candidate device (FREND Vitamin D Test System) to the predicate device (ARCHITECT Vitamin D assay, k110619). A total of 133 native serum samples were tested by two operators in singlicate at one clinical laboratory. Passing-Bablok regression analysis was performed, as summarized below:

N	Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	r
133	13.3-91.3	1.069 (1.001-1.117)	-0.182 (-1.561 – 1.106)	0.971

b. Matrix comparison:

The matrix comparison study was performed using 36 paired samples in the control tube type (serum separator tube) and in the following tube types: lithium heparin plasma, K_3EDTA plasma and citrate plasma tubes. The Passing-Bablok analysis for results from each plasma tube type in comparison to serum separator tube results are summarized below:

Lithium heparin plasma: y= 0.958x +0.067, r=0.995

K_3EDTA plasma: y= 0.991x -0.062, r=0.993

Citrate plasma: y=0.937x + 0.700, r=0.995

The results of the matrix comparison study support the sponsor claims that all the tube types tested above are acceptable for use with the FREND Vitamin D 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:

   The reference range study was conducted with reference to the CLSI C28-A3 guideline. A total of 300 unaltered serum specimens from 139 females and 161 males, ages 21 to 90 years, were collected from geographically diverse locations in the United States in Summer (June to August) and Fall/Winter (October to February). These samples were tested in singlicate using two FREND analyzers and 2 lots of reagents in one clinical laboratory. No more than 50% of the subjects were taking vitamin D supplements. None of the subjects were taking vitamin D supplements of more than 2000 IU vitamin D. Specimens with abnormal PTH, TSH, and Calcium levels were excluded from the data. The 95% reference interval was calculated using a non-parametric method and the following range was obtained:

   Normal Adults: <13.0 – 48.4 ng/mL (N=300)

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.