DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

NANOENTEK USA, INC.
C/O MAUREEN GARNER
NEW WORLD REGULATORY SOLUTIONS, INC
1983 HAZELWOOD ROAD
TOMS RIVER NJ 08753

January 12, 2017

Re: K162754
Trade/Device Name: FREND™ Vitamin D Test System
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D test system
Regulatory Class: II
Product Code: MRG
Dated: November 23, 2016
Received: November 28, 2016

Dear Maureen Garner:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*

K162754

Device Name

FREND™ Vitamin D Test System

Indications for Use *(Describe)*

The FREND™ Vitamin D test is a rapid indirect competitive fluorescent 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

As required by the Safe Medical Devices Act (SMDA) of 1990 and in accordance with 21 CFR §807.92, a 510(k) summary is provided for K162754.

A. Applicant

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B. 510(k) Preparer Information (Contact Person)

Company Name: New World Regulatory Solutions, Inc.
Address: P.O. Box 5374
Toms River, New Jersey 08754, USA
Contact Person: Maureen Garner
Phone Number: (732) 779-7422
Facsimile Number: (732) 270-4829
Email: NWRSinc@gmail.com

Date Prepared:
January 6, 2017

C. Purpose for Submission:

New Analyte on FREND™ System

D. Measurand:

Vitamin D

E. Type of Test:

Quantitative, Fluorescence Immunoassay

F. Proprietary and Established Device Name:

FREND™ Vitamin D Test System

G. Regulatory Information:

	Test Kit
Classification Name:	Vitamin D Test System
Classification:	Class II
Generic Name:	Competitive Immunoassay, Vitamin D
Proprietary Name:	FREND™ Vitamin D (reagent, cartridge assay)
Regulation Number:	21 CFR §862.1825
Product Code:	MRG
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 fluorescent 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.

4. Special instrument requirements:
   NanoEnTek FREND™ System and FREND™ AP System

I. Device Description

The FREND™ Vitamin D Test is a rapid fluorescence immunoassay designed to quantify the concentration of total 25-hydroxy (OH) vitamin D and related hydroxylated metabolites in human serum and plasma (K_3 EDTA, lithium-heparin and citrate) samples using the FREND™ System.

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 FREND™ Vitamin D Cartridge is a single-use disposable rapid lateral flow fluorescence immunoassay designed to quantify the concentration of total 25-OH vitamin D and related hydroxylated metabolites in human serum and plasma (K3 EDTA, lithium-heparin and citrate) samples. Each test cartridge contains reagents to perform one test and bears a bar code with test type and lot information. Each FREND™ Vitamin D Cartridge contains a test zone and a reference zone (internal control). The fluorescence measured in the test and reference zones corresponds to the amount of fluorescent conjugates of Vitamin D-biotin that is bound to each zone. Cartridges are provided in individually sealed pouches and must be stored between 2 and 8 °C.

In addition to the QC Code Chip there is a Vitamin D Code Chip. Lot information and calibration data are loaded into the software via the Vitamin D Code Chip provided with each reagent kit. Up to three different Vitamin D Code Chip lots can be loaded and used at the same time. Each reagent cartridge bears a barcode that the FREND™ System uses to identify the test type and lot number and links it to the appropriate analytical

program and calibration information. The FREND™ System will not output results if the Vitamin D Code Chip lot does not match the lot of the cartridge used.

The Vitamin D Pretreatment Tube is a small single-use disposable plastic tube that contains Vitamin D gold nano-particle conjugation antibody. The sample is diluted in the dilution tube containing perfluorohexanoic acid and transferred to the pretreatment tube prior to being inserted into the FREND™ AP System to be mixed and then added to the Vitamin D Test Cartridge. The test cartridge is then inserted into the FREND™ System to be analyzed.

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.

The FREND™ System utilizes microfluidic technology and detects immune-complexes bound to Vitamin D. The test cartridge is placed on the warming platform of the FREND™ AP System. A 35 µL patient sample is added to the dilution tube and 70 µL of diluted sample is pipetted into the pretreatment tube containing monoclonal anti-Vitamin D antibody conjugated with gold nanoparticles(Step 1). The tube is then loaded into the FREND™ AP System (Step 2). The tube is mixed and loaded into the test cartridge automatically. In the AP System (Step 3), mixing, reaction, sample addition, hydration of the Vitamin D-biotin fluorescent bead conjugate, and migration along the cartridge channel takes approximately 15 minutes, after which the cartridge is ready to be read on the FREND™ System. During migration, the bound Vitamin D in the sample and the fluorescent bead conjugates of Vitamin D-biotin compete to form antigen-antibody complex in the test zone. Unbound Vitamin D-biotin fluorescent conjugates flow through and bind to the anti-Vitamin D antibody that is fixed on the surface in the reference zone. The fluorescent signals in the test and reference zones are measured, typically within 4 minutes.

Vitamin D 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 Vitamin D concentration; in other words, the magnitude of the fluorescent ratio is inversely proportional to the amount of Vitamin D in the sample.

The measuring range of the FREND™ Vitamin D Test System is 13.0 to 96.0 ng/mL. Results are determined via a lot-specific calibration curve which is generated by the manufacturer using a four-point calibration determined from values averaged from 6 replicates at each level. The established curve is uploaded to the FREND™ System via the Vitamin D Code-chip and is valid until the lot expiration date.

The FREND™ System is a portable, automated FREND™ cartridge reader. The FREND™ System is based on quantitative immunoassay technology capable of quantifying single or multiple analytes by measuring laser-induced fluorescence in a single-use disposable reagent cartridge. The test cartridge is a disposable plastic device that contains a port or opening (inlet) where the sample is applied. Once the sample is applied, it will mix with the reagents and travel towards the detection area via capillary action.

The FREND™ cartridge utilizes microfluidics lateral flow technology where the analyte of interest in the sample forms immune complexes while moving through the fluidics

pathway in the cartridge. 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 FREND™ System is a bench top fluorescence reader containing a touch screen user interface. The System has a slot that accepts the FREND™ Vitamin D Test Cartridge (which contains the reagents and sample), and is programmed to analyze the Test when the sample has fully reacted with the on-board in-cartridge reagents. Results of the test are displayed on the screen and can be printed on an optional printer.

The FREND™ System software controls the graphical user interface, communication with hardware, database management and data analysis. The software also controls the functions of the mechanical components including the motor, laser, printer control and acquisition of data from the sensor. The user can set the time and date and enter patient ID through the graphic user interface. The user cannot make any changes to the software.

The FREND™ Vitamin D Test System includes the following in the reagent kit:

- 20 FREND™ Vitamin D Test Cartridges
- 20 FREND™ Sample Dilution Tubes
- 20 FREND™ Vitamin D Gold Antibody Pretreatment Tubes
- 30 Disposable pipette tips
- 1 FREND™ Vitamin D Code Chip
- 1 FREND™ Vitamin D Package Insert

One 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

One sample dilution tube contains:

- Perfluorohexanoic acid 9.5 ± 0.95 μg

One Gold antibody pretreatment tube contains:

- Gold nano-particle conjugation antibody 7.0 ± 0.7 μg

The FREND™ System (previously cleared in K124056 (FREND™ PSA), K131928 (FREND™ TSH), K152422 (FREND™ Free T4), and K153577 (FREND™ Testosterone) is not provided with the kit but it is required for the use of the FREND™ Vitamin D Test cartridge.

J. Substantial Equivalence Information:

An overview of the similarities and differences between the FREND™ Vitamin D Test System and the predicate is provided in the table below:

Similarities		
Item	**FREND™ Vitamin D Test System**	**Abbott ARCHITECT *i* 25-OH Vitamin D Assay (K110619)**
Intended Use	The FREND™ Vitamin D test is a rapid indirect competitive fluorescent immunoassay designed for the quantitative measurement of 25-hydroxy vitamin D and related hydroxylated metabolites in human serum and plasma (K_3 EDTA, 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.	The ARCHITECT 25-OH Vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 25-hydroxy vitamin D (25-OH Vitamin D) in human serum and plasma. The ARCHITECT 25-OH Vitamin D assay is to be used as an aid in the assessment of Vitamin D sufficiency. The ARCHITECT 25-OH Vitamin D Calibrators are for the calibration of the ARCHITECT i System when used for the quantitative determination of 25-hydroxy Vitamin D (25-OH Vitamin D) in human serum and plasma. The ARCHITECT 25-OH Vitamin D Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System when used for the quantitative determination of 25-hydroxy Vitamin D (25-OH Vitamin D) in human serum and plasma. For *in vitro* diagnostic use only.
Sample Type	Human serum and K_3EDTA, lithium heparin, and citrate plasma	Human serum, lithium heparin and sodium heparin
Analyte	25-OH Vitamin D	Same
Type of Test	Fluorescent immunoassay determination of Vitamin D	Chemiluminescent immunoassay determination of Vitamin D
Quality Control	Internal procedural/instrument quality controls; commercially-available external positive and negative assay controls	Internal procedural/instrument quality controls; external positive and negative assay controls cleared with test kit
Interpretation of Results	Inversely proportional Interpolation of fluorescence against a standard curve	Same
Dynamic Range	13.0 ng/mL to 96.0 ng/mL	Same

Differences		
Item	**FREND™ Vitamin D Test System**	**Abbott ARCHITECT *i* 25-OH Vitamin D Assay (K110619)**
Sample Size	35 µL for the dilution step and 70 µL for running the test	60 µL (priority) or 150 µL (≤3h) for the first test plus 10 µL for each additional test from the same test cup
Test Cartridge	Disposable single-use cartridge	No single-use cartridge
Random Access / Degree of Automation	No random access, manual manipulation	Random access, semi-automated

K. Performance Characteristics (if/when applicable)

1. Analytical performance

a. *Precision/Reproducibility*

A single lot imprecision study was performed at the NanoEnTek laboratory as described in the CLSI protocol EP5-A3. Three serum pools with low, intermediate and high Vitamin D levels were assayed in duplicate twice per day for 20 days (80 total measurements per level). The results are summarized below:

FREND™ Vitamin D Single Site Single Lot Precision

Sample Pool	Mean Vitamin D Concentration (ng/mL)	Repeatability		Between-run		Between-day		Within-laboratory	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	20.1	1.76	8.8%	0.46	2.3%	0.22	1.1%	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 established according to CLSI-EP6-A using nine levels of serum Vitamin D tested in quadruplicate. Linearity was demonstrated across the measuring range of the FREND™ Vitamin D (13.0 ng/mL ~ 96.0 ng/mL).

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

The calibrators are for factory calibration. The standards/calibrators are prepared gravimetrically 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 in each code chip, which is lot-specific.

d. Stability

Real-time stability testing for the Vitamin D reagent kit was performed according to CLSI EP25-A, *Evaluation of Stability of* In Vitro *Diagnostic Reagents*. Reagent stability studies based on procedures and criteria in the NanoEnTek quality system showed that the cartridges for FREND™ Vitamin D will meet performance acceptance criteria for one year from the date of manufacturer if stored refrigerated appropriately as directed.

e. *Detection Limit:*
The Limit of Quantitation (LoQ) for the FREND™ Vitamin D Test System was established at 9.9 ng/mL according to the CLSI EP17-A2 protocol. The analytical sensitivity of the FREND™ Vitamin D Test System is claimed at 13.0 ng/mL.

f. *Analytical specificity:*

Interference Studies
Interference was determined according to CLSI EP07-A2 using triplicate determinations and two concentration of Vitamin D. No interference was specified if recoveries were between 90% to 110% of the expected Vitamin D value. Results are summarized in the table below.

Class	Interferent (Concentration Tested)	% Recovery Vitamin D Low	% Recovery Vitamin D High
Endogenous substances	Hemoglobin (500 mg/dL)	107.0	98.4
	Bilirubin, conjugated (30 mg/dL)	95.6	95.7
	Bilirubin, unconjugated (30 mg/dL)	100.0	94.9
	Triglyceride (1500 mg/dL)	99.6	94.0
	Cholesterol (500 mg/dL)	93.0	96.1
	Total protein (12 g/dL)	103.4	98.8
	Biotin (6 μg/mL)	99.9	102.7
Heterophilic Antibodies	HAMA (1000 ng/mL)	97.4	108.2
	Rheumatoid Factor (536 IU/mL)	101.4	96.8

Cross Reactivity
The following substances were evaluated in triplicate for potential cross-reactivity with the FREND™ Vitamin D Test System at two Vitamin D concentrations. Testing was done according to the CLSI protocol EP07-A2. No significant cross-reactivity was found, with the exception of Paricalcitol. Drugs containing Paricalcitol (e.g., Zemplar) will interfere with the assay.

Cross-reactant	Cross-reactant Concentration (ng/mL)	% Cross-reactivity	
		Vitamin D Low	Vitamin D 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

% Cross-reactivity = 100 x ((Measured value - true value)/concentration of interferent)

g. *Assay cut-off:*

Not applicable

2. Comparison studies

   a. *Method comparison with predicate device:*
   Comparison studies with 133 de-identified leftover samples were performed in a CLIA-certified hospital laboratory. The reference method was the Abbott ARCHITECT *i* 25-OH Vitamin D Assay (K110619) run on the Abbott ARCHITECT *i* System. The samples spanned the measuring range of the FREND Vitamin D Test System. All samples were assayed using sera split between the applicant device and the reference method.

   Results from the FREND™ Vitamin D on the FREND™ System (y) were compared with the reference results (x) by Passing-Bablok regression analysis, giving a slope of 1.069, a y-intercept of -0.182, and a correlation (R) of 0.971.

   b. *Matrix comparison:*
   The matrix comparison study was performed at the NanoEnTek laboratory according to CLSI EP14-A3. Vitamin D concentrations from 36 paired samples, covering the analytical measuring range, were measured in serum, K_3EDTA, lithium-heparin and sodium citrate plasma samples with the FREND™ Vitamin D Test System, giving equivalent results.

3. Expected values/Reference range:
   A study was conducted to establish a Reference Interval for the FREND™ Vitamin D Test System. It is also recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical season, patient, dietary, or environmental factors.

   The study was conducted based on CLSI C28-A3. A total of 300 serum specimens from apparently healthy individuals residing in north, central and southern regions of the US were collected during summer and winter months and tested in the FREND™ Vitamin D Test System. The reference interval is provided below.

Reference Interval	Lower reference limit 90% CI	Upper reference limit 90% CI
<13.0 to 48.4	<13.0 to <13.0	47.7 to 68.0

These limits should be considered as guidelines only. It is important for each laboratory to establish its own reference range, representative of its typical population.

L. Proposed Labeling

The labeling is sufficient and it satisfies the requirements of 21 CFR §809.10.

M. Conclusion

The submitted information in this premarket notification is complete and supports a substantial equivalence determination.