

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

A. 510(k) Number:

k153577

B. Purpose for Submission:

New Device

C. Measurand:

Testosterone

D. Type of Test:

Quantitative, Fluorescence Immunoassay

E. Applicant:

NanoEnTek, Inc.

F. Proprietary and Established Names:

FREND™ Testosterone Test System

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1680 Testosterone Test System

2. Classification:

Class I, Reserved

3. Product code:

CDZ

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below

2. Indication(s) for use:

The FREND™ Testosterone test is a fluorescent nanoparticle immunoassay designed for *in vitro* quantitative measurement of total testosterone in human serum and plasma (K3EDTA and lithium heparin). Measurements of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and, in females, hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

The FREND™ Testosterone microfluidic flow cartridge is designed for use in the FREND™ System fluorescent immunoassay reader. The FREND™ Testosterone 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

Not for use in Point-of-Care settings

4. Special instrument requirements:

NanoEnTek FREND™ System

I. Device Description:

The FREND™ Testosterone Test System includes the following in the kit:

- FREND™ Testosterone cartridges (20)
- Testosterone Gold Antibody Pretreatment Tubes (20)
- Disposable pipette tips (30)
- FREND™ Testosterone Code Chip (1)
- FREND™ Testosterone Package Insert (1)

One cartridge contains:

- Monoclonal mouse anti-testosterone antibody: 32 ± 3.2 ng
- Testosterone-Bovine Serum Albumin (BSA): 482.0 ± 48.2 ng
- Fluorescent nanoparticles: 3.6 ± 0.36 µg

One pretreatment tube contains:

- Gold antibody conjugates: $30 \pm 3.0 \mu\text{g}$

The FREND Testosterone Code Chip contains all the calibration information stored electronically, and is specific for each lot of FREND Testosterone cartridge.

The FREND™ System was previously cleared in k124056 and is not provided with the kit but is required for the use of the FREND™ Testosterone test cartridge. The FREND System is a portable, bench top fluorescence reader.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Abbot ARCHTECT 2nd Generation Testosterone

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

k120009

3. Comparison with predicate:

Similarities		
Item	Candidate Device: FREND™ Testosterone Test System (k153577)	Predicate Device: Abbott ARCHTECT 2 nd Generation Testosterone (k120009)
Intended Use	In vitro diagnostic test for the quantitative measurement of total testosterone in human serum and plasma (K ₃ EDTA and Lithium heparin)	Same
Sample Type	Human serum and plasma (K ₃ EDTA and Lithium heparin)	Same
Type of Test	Immunoassay	Same

Differences		
Item	Candidate Device: FREND™ Testosterone Test System (k153577)	Predicate Device: Abbott ARCHTECT 2 nd Generation Testosterone (k120009)
Methodology	Fluorescent	Chemiluminescent

Differences		
Item	Candidate Device: FREND™ Testosterone Test System (k153577)	Predicate Device: Abbott ARCHITECT 2 nd Generation Testosterone (k120009)
Sample Size	70 µL for the first incubation step and 35 µL for running the test	150 µL for the first Testosterone test and 100 µL for each additional test from the same test cup
Test Cartridge	Disposable single-use cartridge	No single-use cartridge
Measuring Range	20 – 1500 ng/dL	4.33 – 1500 ng/dL

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

CLSI EP05-A3, Evaluation of precision Performance of Quantitative Measurement Methods; Approved Guideline

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline

CLSI EP09-A3, Method 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 Guidelines

CLSI EP28-A3C, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

L. Test Principle:

The FREND™ Testosterone Test is a single-use rapid “competitive” immunoassay utilizing fluorescent nanoparticle in microfluidic flow to capture and quantify total testosterone levels in human serum and plasma (K3EDTA and lithium heparin) specimens using the FREND™ system.

The FREND™ Testosterone Test System is a two-step competitive immunoassay with gold nanoparticles labeled with mouse monoclonal anti-testosterone antibody, testosterone-biotin

labeled with fluorescence nanoparticles and fluorescence detection by FREND™ System.

The FREND™ Testosterone test utilizes microfluidic technology and detects immune-complexes bound to testosterone. Firstly, a 70 µL patient sample is incubated in the Testosterone Gold Antibody pretreatment tube, where the sample interacts with a proprietary mix of a pretreatment solution. Secondly, the Test Cartridge is placed on the warming platform of the heating block and 35 µL of the mixture from Step 1 is manually loaded into the inlet of the cartridge. The cartridge remains on the warming platform for 30 seconds, while the sample hydrates the testosterone-biotin fluorescent bead conjugate and migrates along the test strip. During migration the bound testosterone in the sample and the testosterone-biotin fluorescent bead conjugates compete to form antigen-antibody complexes in the test zone. Unbound testosterone-biotin fluorescent conjugates flow through and bind to the anti-testosterone antibody that is immobilized on the surface in the reference zone. The cartridge is inserted into the FREND instrument for analysis where fluorescent signals in the test and reference zones are measured.

Testosterone quantification is based upon the ratio of the intensity of the test and reference zones. The magnitude of the fluorescent ratio is inversely proportional to the amount of testosterone 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:

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

Sample Pool	Mean Conc. (ng/dL)	Repeatability		Within Laboratory	
		SD	CV%	SD	CV%
1	39.723	4.5	11.2	4.7	11.8
2	202.965	16.7	8.2	17.3	8.5
3	1012.208	54.7	5.4%	57.5	5.7

b. Linearity/assay reportable range:

Linearity was established according to CLSI-EP6-A in three reagent lots using seven levels of serum testosterone tested in quadruplicate. The range for analyte concentration was tested from 17 ng/dL to 1650 ng/dL using low and high serum pools. The low and high pools were mixed to make five intermediate levels. A total of seven concentrations were tested (17.5, 180.8, 527.2, 850.2, 1203.6, 1509.0, and 1736.6 ng/dL). The following linear regression equation was obtained:

$$Y=1.038X - 4.818, R^2 = 0.994$$

Linearity data supports the FREND™ Testosterone reportable range of 20 ng/dL to 1500 ng/dL.

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

The standards/calibrators are prepared gravimetrically and confirmed by measurement on the ARCHITECT i 2nd Generation Testosterone assay (k120009), which is traceable to the USP testosterone. The calibrators are factory calibration.

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

Controls: The sponsor recommends the use of commercially available quality control material that contains Testosterone as the measurand.

d. Detection limit:

LoB, LoD and LoQ studies were performed according to the CLSI EP17-A guideline using one lot of reagent.

LoB was performed with five different steroid depleted serum samples with 12 replicates each, which generated a total of 60 replicates.

The Limit of Detection (LoD) for the FREND™ Testosterone was established using five different low level samples. All samples were each assayed 12 times over a period of five days using a single reagent lot. Each sample has a total of 60 replicates.

LoQ was determined based on five low samples with concentration between 17 to 20 ng/dL. The sponsor obtained a LoQ value based on <20%CV at sample concentration of 20 ng/dL.

LoB	LoD	LoQ
9.46 ng/dL	14.33 ng/dL	20.0 ng/dL

The claimed measuring range of this assay is 20 ng/dL to 1500 ng/dL.

e. Analytical specificity:

Interference Studies

Potential interference from common endogenous substances was evaluated in accordance with CLSI EP7-A2. Interferents were added to normal human sera spiked with two levels of testosterone, and controls with no interferent were included. The sponsor defines significant interference as > ±10% difference. No significant interference was found if recoveries were between 90% to 110% of the expected

testosterone value. Results with non-significant interference substances are summarized in the table below.

Class of substances tested	Interferent (Concentration Tested)
Endogenous Substances	Hemoglobin (500 mg/dL)
	Bilirubin, conjugated (30 mg/dL)
	Bilirubin, unconjugated (30 mg/dL)
	Triglyceride (3000 mg/dL)
	Total protein (12 g/dL)
	Biotin (30 ng/mL)
	Sex Hormone Binding Globulin (100 nmol/L)
	Acetylcystein (415 µg/mL)
Pharmaceuticals	Ampicillin-Na (50.3 µg/mL)
	Ascorbic acid (60 µg/mL)
	Ca-Dobesilate (40 µg/mL)
	Cyclosporine (3 µg/mL)
	Cefoxitin (66 µg/mL)
	Heparin (3,000 U/L)
	Levodopa (4 mg/mL)
	Methyldopa (15 µg/mL)
	Metronidazole (120 µg/L)
	Doxycycline (30 µg/mL)
	Acetylsalicylic Acid (250 µg/mL)
	Rifampin (640 µg/mL)
	Acetaminophen (200 µg/mL)
	Ibuprofen (250 µg/mL)
	Theophylline (400 µg/mL)
Heterophilic Antibodies	Rheumatoid Factor (RF) (1075 IU/mL)
	Human Anti-mouse antibodies (HAMA) (70 ng/mL)

Sponsor has included the following limitation in the labeling:

“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 an incomplete test. Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interference.”

Cross Reactivity

The following substances were evaluated for potential cross-reactivity with the FREND™ Testosterone at two concentrations (low and high testosterone levels). Testing was done according to the CLSI protocol EP07-A2. Results of the percent cross reactivity are summarized in the table below.

Cross-reactant	Cross-reactant Conc. (ng/dL)	% Cross-reactivity	
		Testosterone Low	Testosterone High
Androstenedione	50 nmol/L	0.0032	0.0337
Androsterone	1,000 nmol/L	0.0004	0.0158
Cortisone	1,000 nmol/L	0.0077	0.0300
Danazol	1,000 nmol/L	0.0007	0.0496
Estradiol	200 nmol/L	0.0033	0.5160
Estrone	500 nmol/L	0.0144	0.0378
17a-Ethynyl estradiol	1,000 ng/mL	0.0033	0.0081
Progesterone	2,000 nmol/L	0.0007	0.0167
Dexamethasone	5 µmol/L	0.0255	0.0122
Ethisterone	20 nmol/L	0.0437	0.3840
D(-) Norgestrel,	20 ng/mL	0.0125	0.1667
Prednisolone	2,000 nmol/L	0.0001	0.0175
Prednisone	2,000 nmol/L	0.0031	0.0040
Spirolactone	500 ng/mL	0.0039	0.0172
Cortisol	10,000 nmol/L	0.0004	0.0070
DHEA	50 nmol/L	0.0178	1.0463
DHEAS	50 µmol/L	0.0002	0.0009
Dihydrotestosterone	40 nmol/L	0.2040	0.4134
Epitestosterone	100 nmol/L	0.0467	0.1502
Ethinodiol diacetate	50 ng/mL	0.0274	0.1280

% Cross-reactivity = 100 x ((Spiked value – unspiked value)/concentration of Cross-reactant)

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Comparison studies using 155 serum samples were performed externally. The comparator method was the Abbott ARCHITECT 2nd Generation Testosterone Assay (predicate device) run on the Abbott ARCHITECT *i* System. The samples spanned the measuring range of the FREND Testosterone Test System (20.84 to 1420.33 ng/dL). All samples were assayed using serum samples split between the candidate device and the comparator method.

Results from the FREND™ Testosterone on the FREND™ System (y) were compared with the comparator results (x) by Passing-Bablok regression analysis, giving a slope of 0.983 (95%CI: 0.944 to 1.00), an intercept of -2.353 (95%CI: -12.0 to 14.36) and a correlation coefficient of 0.977.

b. Matrix comparison:

The matrix comparison study was performed internally. Testosterone concentrations in the serum, lithium heparin plasma and K₃EDTA plasma from 40 individuals were measured using the FREND™ Testosterone Test System. Regression analysis results when compared against the serum samples and are summarized in the table below.

Parameter	K ₃ EDTA Plasma	Lithium Heparin Plasma
Slope: (95% CI)	1.006 (0.962 to 1.066)	1.010 (0.968 to 1.051)
y-Intercept: (95% CI)	-8.185 (-46.842 to 9.627)	1.264 (-14.078 to 22.488)
Correlation, r	0.989	0.993
Sample range tested	23.5 to 1356.2 ng/dL	20.3 to 1343.6 ng/dL

Sponsor has the following recommendation in the labeling:

Patient samples are “recommended to use the same specimen matrix when following patients because the results may not be interchangeable”.

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 a total of 488 normal, apparently healthy adult male and female individuals were assayed on 3 lots of the FREND™ Testosterone assay using two FREND™ Systems according to CLSI C28-A3 guidelines. The central 95th percentile range of each sample category was calculated by finding the 2.5th

ranked sample on the low end and the 97.5th ranked sample on the high end. The reference interval using 95% interval for the FRENDSTM Testosterone Test System, stratified by age range and gender, is provided in the table below.

Gender (Age Range)	Testosterone, ng/dL	Number Tested
F (21 – 49)	<20 – 107.5	120
F (50 – 90)	<20 – 150.3	124
M (21 – 49)	170.1 – 1263.6	123
M (50 – 88)	152.4 – 1095.2	121

It is recommended that each laboratory establish its own expected values for testosterone as performed on the FRENDS System.

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.