510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K162378

B. Purpose for Submission:

Modification of Previously Cleared Device

C. Measurand:

Total Prostate Specific Antigen

D. Type of Test:

Fluorescence Immunoassay, Quantitative

E. Applicant:

NanoEnTek, Inc.

F. Proprietary and Established Names:

FREND™ PSA Plus

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.6010 – Tumor-Associated Antigen Immunological Test System

2. Classification:

   Class II

3. Product code:

   LTJ – Prostate Specific Antigen (PSA) for Management of Prostate Cancers

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   The NanoEnTek FREND™ PSA Plus is designed for *in vitro* DIAGNOSTIC USE ONLY for the quantitative measurement of total Prostate Specific Antigen (PSA) in human serum, Li-heparinized plasma, and K3-EDTA plasma using the FREND™ System. This device is indicated for the serial measurement of total PSA to be used as an aid in the management of patients with prostate cancer.

2. Indication for use:

   Same as Intended Use above

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   NanoEnTek FREND^{TM} System (K124056)

I. Device Description:

FREND™ PSA Plus is comprised of the following materials (to perform 25 tests):

- FREND™ PSA Plus cartridges (25): each cartridge contains monoclonal anti-PSA1, monoclonal anti-PSA2, and fluorescent particle.
- Disposable pipette tips (30)
- FREND™ PSA Plus Code Chip
- FREND™ PSA Plus Package Insert

J. Substantial Equivalence Information:

1. Predicate device name:

   NanoEnTek FREND PSA Plus,

2. 510(k) number:

   K124056

3. Comparison with predicate:

Similarities		
Item	**Device** Modified FREND™ PSA PLUS	**Predicate** FREND™ PSA Plus
Intended Use	The NanoEnTek FREND™ PSA Plus is designed for *in vitro* DIAGNOSTIC USE ONLY for the quantitative measurement of total Prostate Specific Antigen (PSA) in human serum, heparinized plasma, and EDTA plasma using the FREND™ System. This device is indicated for the serial measurement of total PSA to be used as an aid in the management of patients with prostate cancer.	Same
Analyte	Total PSA	Same
Type of Test	Fluorescent immunoassay	Same
Instrument	FREND system	Same
Sample type	Serum, heparinized plasma, EDTA plasma	Same
Interpretation of results	Interpolation from a lot-specific calibration curve	Same
Calibration standard	WHO International Standard Prostate Specific Antigen (90:10) NIBSC code: 96/670	Same

Differences		
Item	**Device** Modified FREND™ PSA PLUS	**Predicate** FREND™ PSA PLUS
Sample size	35 µL	30 µL
LoD/LoQ	LoD: 0.03 ng/mL LoQ: 0.08 ng/mL	LoD: 0.1 ng/mL LoQ: not determined
Assay measuring range (AMR)	0.08–25 ng/mL	0.10–25 ng/mL

K. Standard/Guidance Document Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP6-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline.
- CLSI EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition—Second Edition
- CLSI EP09-A2, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition

- 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—Second Edition
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guidelines
- CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

L. Test Principle:

The FREND™ PSA Plus is a fluorescence immunoassay. The FREND™ Test Cartridge utilizes microfluidic technology and detects sandwich immune-complexes bound to PSA. Each FREND™ PSA Plus Test Cartridge contains a test zone and as well as a reference zone. The specimen is added by the operator to the sample inlet with a transfer pipet, allowing the appropriate volume of sample to be delivered into the FREND™ PSA Plus Test Cartridge. The Cartridge containing the sample is placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. The PSA quantification is based on the amount of fluorescence detected by the FREND™ System at the FREND™ PSA Plus Test Cartridge window. The magnitude of the fluorescent signal is directly proportional to the amount of total PSA in the sample.

M. Performance Characteristics:

1. Analytical performance: The test results met the sponsor's pre-determined acceptance criteria for all analytical performance studies.

   a. Precision/Reproducibility:

   The precision and lot-to-lot reproducibility of the assay was evaluated per CLSI EP05-A3 by testing four serum sample pools containing various concentrations of PSA. Each sample was run in duplicate, in two runs per day, for 20 days with three lots of cartridge on one FREND Plus system by one operator (n=240 per sample). The results are summarized in the table below.

Sample	Mean (ng/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.08	0.01	14.1	0.00	2.6	0.00	2.4	0.00	0.0	0.01	14.6
2	0.10	0.01	12.3	0.00	0.0	0.00	2.0	0.00	0.0	0.01	12.5
3	4.00	0.29	7.2	0.06	1.6	0.00	0.0	0.00	0.0	0.30	7.4
4	21.40	1.20	5.6	0.51	2.4	0.00	0.0	0.09	0.4	1.30	6.1

The site-to-site reproducibility of the assay was evaluated by testing three serum samples at three sites. Each sample was tested in replicates of five per run, one run

per day, for five days using one lot of reagent (n=75 per sample). The results are summarized in the table.

Sample	Mean (ng/mL)	Within-Run		Between-Day		Within-Site		Between-Site		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.26	0.03	10.1	0.01	4.4	0.03	11.0	0.00	0.0	0.03	11.0
2	2.87	0.22	7.6	0.03	1.2	0.22	7.7	0.05	1.8	0.23	7.9
3	11.49	1.14	9.9	0.49	4.2	1.24	10.8	0.22	1.9	1.26	10.9

b. *Linearity/assay reportable range:*

Linearity:

Linearity was evaluated according to the CLSI guideline EP6-A by preparing two dilution series. The first dilution series was made by diluting a serum sample containing a PSA concentration above the upper limit of measuring range with a serum sample containing a low concentration of PSA. Each dilution was tested in replicates of five using three lots of cartridges. The second dilution series was made by diluting a serum sample with a PSA concentration at 1.0 ng/mL with a serum sample with a PSA concentration below the lower limit of measuring range. Each dilution was tested in replicates of four using three lots of cartridge to determine the observed value of PSA. Percent recovery and linear regression analysis was determined by comparing the observed PSA result with the expected value. The results are summarized as follows:

Dilution Range (ng/mL)	Slope (95% CI)	Y-intercept (95% CI)	R^2	% Recovery
0.10–25.53	1.00 (0.98–1.02)	0.04 (−0.26–0.35)	0.99	96.0–102.0
0.05–1.00	0.99 (0.96–1.03)	-0.00 (−0.02–0.02)	0.98	97.2–100.0

The results support that the assay is linear for the claimed measuring range, 0.08–25 ng/mL.

Hook effect:

The presence of high dose hook effect was tested using a sample with PSA concentration of 1200 ng/mL. No high dose hook effect was observed in the sample with a PSA concentration as high as 1200 ng/mL.

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

Traceability:

The FREND™ PSA Plus assay is traceable to the WHO International Standard Prostate Specific Antigen (90:10) NIBSC code: 96/670.

Value assignment: The internal standard is manufactured by gravimetric methods based on the PSA Ag ACT complex (Scripps P0624-90010) or WHO standard material (NIBSC 96/670). The concentrations of calibrators are confirmed based on the measurement on ARCHITECT *i* total PSA assay (P910007) or TOSOH AIA ST PA Test (P910065). The calibration information is then coded in the individual cartridge.

Stability:

The stability of the cartridges for FREND™ PSA Plus was established previously per K124056. The cartridges are stable for one year from the date of manufacture if stored refrigerated as directed.

d. Detection limit:

Limit of Blank (LoB) was determined by assaying five PSA free serum samples. Each sample was tested in replicates of two per day for six days with three lots of cartridges to generate 60 data points. The LoB was calculated based on the 95th percentile of 60 measurements and determined to be 0.02 ng/mL.

The Limit of Detection (LoD) was determined by assaying five samples with PSA concentrations close to the lower limit of measuring range. Each sample was tested in replicates of two per day for six days with three lots of cartridges to generate 60 data points. The LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples and was determined to be 0.03 ng/mL.

The Limit of Quantification (LoQ) was evaluated using the precision profile approach and the data from the precision study following CLSI EP05-A3. Within-laboratory precision was determined using four samples with PSA concentration at 0.025, 0.05, 0.075 and 0.1 ng/mL. The LoQ was calculated as the sample concentration at which the pre-determined goal of within-lab imprecision was met. The LoQ was determined to be 0.08 ng/mL.

e. Analytical specificity:

Endogenous Interference: Two serum base samples (1 ng/mL and 4 ng/mL) were spiked separately with endogenous interferents. The control samples were prepared for each interfering substance by spiking with the same volume of diluents. Each sample was tested in three replicates and the percent recovery was calculated by comparing to a corresponding control sample. No interference was detected in the samples using interferents up to the concentrations listed in the table below.

Potential Interfering Substances	Concentration Tested	Range of % Recovery
Hemoglobin	500 mg/dL	86.79–106.68
Bilirubin (unconjugated)	200 µg/mL	89.52–108.14
Triglycerides	3 g/dL	89.10–116.30
Total Protein	50 mg/mL	88.89–112.83
Rheumatoid Factor	1075 IU/mL	102.99–118.48
HAMA	70 ng/mL	87.18–96.46

Exogenous Interference: The same protocol used for evaluation of endogenous interference was used to evaluate the potential interference of common drugs. No interference was observed for each drug at the concentrations listed below.

Name of Agent	Concentration Tested	Range of % Recovery
Acetaminophen	250 ng/mL	87.47–114.99
Aspirin	600 µg/mL	94.23–109.28
Ibuprofen	500 µg/mL	88.62–115.63
Flutamide	10 µg/mL	88.68–99.47
Diethylstilbestrol (DES)	5 µg/mL	89.42–110.00
Goserelin	40 ng/mL	94.90–110.20
Leuprolide	275 ng/mL	89.15–106.38
Finasteride	250 ng/mL	89.52–108.14
Tamsulosin	100 ng/mL	89.71–120.05
Docetaxel	10 µg/mL	90.83–105.50
Prostatic acid phosphatase	10 ng/mL	87.71–110.42

f. Assay cut-off:

See clinical cut-off

2. Comparison studies:

a. Method comparison with predicate device:

A total of 64 serum samples were tested with the modified FREND™ PSA Plus using a sample volume of 35 µL and the predicate device using a sample volume of 30 µL. The concentrations of PSA for these samples covered the measuring range for both assays. Passing-Bablok regression analysis was performed by comparing the results obtained from the modified FREND™ PSA Plus (y) and the predicate (x). The results are summarized below:

N	Range (ng/mL)	Slope (95% CI)	Y-Intercept (95% CI)	Correlation Coefficient
64	0.11–20.23	0.98 (0.96–1.01)	−0.09 (−0.09–0.07)	0.99

b. Matrix comparison:

A study was performed using the modified FREND™ PSA Plus to compare PSA values obtained for 40 matched samples each with serum, lithium heparin plasma and K3-EDTA plasma. The concentrations of PSA for these matched serum/plasma samples covered the measuring range of the assay. Passing-Bablok regression analysis was performed by comparing the results of samples from different plasma samples (y) to the results of corresponding serum samples (x). The results are summarized below:

N=40	Range (ng/mL)	Slope (95% CI)	Y-Intercept (95% CI)	Correlation Coefficient
Li-heparin plasma vs. Serum	0.08–23.59	0.96 (0.89–1.03)	0.03 (−0.15–0.24)	0.99
K3-EDTA plasma vs. Serum	0.08–23.59	1.03 (0.99–1.09)	−0.08 (−0.37–0.08)	0.99

c. Other supporting comparison studies:

A total of 207 samples were tested with the modified FREND™ PSA Plus on FREND™ System and Abbott ARCHITECT total PSA (P910007) assay on the Abbott ARCHITECT *i* System. The PSA concentrations of these samples covered the measuring range for both assays. The results generated from the modified FREND™ PSA Plus (y) were compared to the results generated from ATCHITECT total PSA assay (x). Results from the Passing-Bablok regression analysis are summarized in the following table:

N	Range (ng/mL)	Slope (95% CI)	Y-Intercept (95% CI)	Correlation Coefficient
207	0.08–23.56	0.98 (0.94–1.01)	−0.05 (−0.101–0.00)	0.98

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The effectiveness of the modified FREND PSA Plus assays as an aid in monitoring of disease status in prostate cancer subjects was determined by assessing changes in PSA levels in serial serum samples from 63 subjects diagnosed with prostate cancer compared to changes in their disease status. The sample inclusion and exclusion criteria are as follows:

Inclusion criteria:

- Sample must be serum and is entered into the study only once.
- Sufficient volume for the sample exists to allow testing on both the predicate and the experimental device.

- Serial sets must include a minimum of three draws. Samples are to be from blood draws performed at or after definitive diagnosis throughout the clinical course of the disease.
- Clinical information that details the status of the subject's disease must be available for each sample as must the treatment regimen being used at the time of the blood collection.
- Sample may be fresh or stored for one week at 2–8 °C. Samples stored for more than one week must be held at −20 °C or colder.

Exclusion criteria:

- Samples that appear to be contaminated, turbid, lipemic, icteric or hemolyzed will be excluded.
- Samples failing to meet all the inclusion criteria will be excluded.

Among 63 subjects, the majority of subjects (n = 49) were Caucasian, the remaining subjects included nine African American, one Asian, and four Hispanics. 62 out of 63 subjects had staging information of initial diagnosis based on the recommendations by the American Joint Committee on Cancer (AJCC) TNM system: three were stage I; 25 were stage II; 22 were stage III; and 12 were stage IV. Of 63 subjects, 40 subjects were treated, including prostatectomy, radical perirectal prostatectomy, bilateral scrotal orchiectomy, lymphadenectomy, bilateral pelvic lymphadenectomy, prostate curettage, crainal resection mets, cryoblation, seminal vesiculectomy, and transurethral resection of the prostate (TURP). A total of 257 values consisting of 63 baseline values and 194 monitoring observations were obtained with the mean number of sample draws of 4.08 per subject.

Results:

In this study, all 257 serum samples (including 63 baseline and 194 follow-up measurements) from 63 subjects were measured using both the modified FREND PSA Plus on the FREND system and the predicate FREND PSA Plus which was previously cleared under K124056. At each follow-up visit, the percentage change of the PSA was calculated by comparing the test result to the results obtained from the previous visit. The performance of the FREND PSA Plus as an aid in the management of patients with prostate cancer subjects was evaluated by assessing the changes in PSA levels correlated to the clinical assessment at the time of assay.

For both modified and predicate FREND™ PSA Plus assays, a positive change in the PSA concentration was defined as measurable increase in the value that is 20% greater than the previous value of the test for samples with the PSA concentration > 1 ng/mL, and increase >0.2 ng/mL for sample with the PSA concentration ≤ 1 ng/mL.

For each pair of samples in a serial set, the change in the PSA concentration was compared to the change in the clinical status of the patients as determined by the physician based on other laboratory tests, patient interviews, physical examinations, and medical imaging. The following definition was used to categorize the patient's

disease status:

1. No Evidence of Disease (NED) – All detectable tumors had disappeared.
2. Stable – The tumors stayed the same size. To account for measurement errors on scans and to discount "insignificant" changes, stable disease includes either a small amount of growth (typically less than 20%–25%) or a small amount of shrinkage (typically less than 25%).
3. Responding – Significant (≥50%) amount of decrease in the total tumor volume responding to the treatment, with evidence of some residual disease still remaining.
4. Progression – Clinical evidence of growth in the primary tumor or new tumors have appeared (*see reference below).

    * Costelloe CM, Chuang HH, Madewell JE, Ueno NT. (2010). Cancer response criteria and bone metastases: RECIST 1.1, MDA and PERCIST. *J. Cancer*. 1:80-92.

The following table represents the summary of all follow-ups (n=194) for subjects at which a clinical evaluation occurred. A positive percentage change of the subjects at these clinical evaluations was defined by the cut-off of >20% change for samples with PSA concentration ≥1 ng/mL or > 0.2 ng/mL for samples with PSA concentrations <1 ng/mL

		Clinical Status				
		No-Progression			**Progression**	**Total**
		NED	**Stable**	**Responding**		
Change in PSA value	**Positive**	16	6	1	46	69
	Negative	80	9	9	27	125
	Total	96	15	10	73	194

Based on clinical disease status using progression or no-progression (NED, stable, responding), the performances of the modified FREND PSA Plus are summarized in the following table:

Performance Measurement	**Value**	**95% CI**
Sensitivity	63.0%	51.5%–73.2%
Specificity	81.0%	73.1%–87.0%
Positive Predictive Value (PPV)	66.7%	57.1%–75.0%
Negative Predictive Value (NPV)	78.4%	72.7%–83.2%
Positive Likelihood Ratio	3.32	2.23–5.01
Negative Likelihood Ratio	0.46	0.33–0.61

In this study, 62 out of 63 subjects had staging information from the initial diagnosis. The summary statistics of the change of the FREND PSA Plus results from the preceding sample for all follow-up samples by disease stage are shown in the following tables:

Change in FREND PSA Plus	Clinical Status				
	No-Progression			Progression	Total
	NED	Stable	Responding		
Stage I (N=8 from 3 subjects)					
Positive	0	0	0	2	2
Negative	4	0	1	1	6
Total	4	0	1	3	8
Stage II (N = 85 from 25 subjects)					
Positive	5	2	0	22	29
Negative	39	2	4	11	56
Total	44	4	4	33	85
Stage III (N=60 from 22 subjects)					
Positive	7	2	0	15	24
Negative	21	3	4	8	36
Total	28	5	4	23	60
Stage IV (N=37 from 12 subjects)					
Positive	3	2	1	7	13
Negative	13	4	0	7	24
Total	16	6	1	14	37
Stage unknown (N = 4 from 1 subject)					
Positive	1	0	0	0	1
Negative	3	0	0	0	3
Total	4	0	0	0	4

As 40 out of 63 subjects had surgery, the following tables summarized the change of the FREND PSA Plus results from the preceding sample for all follow-up samples and clinical performance of the FREND PSA Plus values over successive visits by surgery status:

Change in FREND PSA Plus	Clinical Status				
	No-Progression			Progression	Total
	NED	Stable	Responding		
Non-Surgery Group					
Positive	5	5	0	19	29
Negative	26	6	4	10	46
Total	31	11	4	29	75
Surgery Group					
Positive	11	1	1	27	40
Negative	54	3	5	17	79
Total	65	4	6	44	119

Performance Measurement	Non-Surgery (N=78)	Surgery (N=116)
Sensitivity (95 % CI)	66.7 % (47.2–82.7%)	60.5% (44.4–75.0%)
Specificity (95% CI)	79.2 % (65.0–89.5%)	82.2% (71.5–90.2%)
PPV (95% CI)	66.7% (47.2–82.7%)	66.7% (49.8–80.9%)
NPV (95% CI)	79.2% (65.0–89.5%)	77.9% (67.0–86.8%)
Positive likelihood ratio	3.20 (1.74–5.87)	3.40 (1.96–5.88)
Negative likelihood ratio	0.42 (0.25–0.71)	0.48 (0.33–0.71)

b. Other clinical supportive data:

The clinical performance of the modified FREND PSA Plus was evaluated against that of the predicate, FREND PSA Plus (K124056). The table below compared the clinical sensitivity and specificities between these two assays using the same sample cohort in the clinical study described above.

	N=257 (63 subjects)	
	The Modified FREND PSA Plus	**The FREND PSA Plus (K124056)**
Sensitivity	63.0% (51.5–73.2%)	65.8% (54.3–75.6%)
Specificity	81.0% (73.1–87.0%)	81.0% (73.1–87.0%)
Total Concordance	74.2% (67.4–80.2%)	75.3% (68.6–81.2%)
PPV	66.7% (57.1–75.0%)	67.6% (58.2–75.8%)
NPV	78.4% (72.7–83.2%)	79.7% (73.8–84.5%)
Positive Likelihood Ratio	3.32 (2.23–5.01)	3.46 (2.34–5.21)
Negative Likelihood Ratio	0.46 (0.33–0.61)	0.42 (0.30–0.57)

4. Clinical cut-off:

An absolute cutoff is not applicable for monitoring. The significance of PSA increase was defined as a change greater than 0.2 ng/mL for samples with PSA concentration ≤ 1 ng/mL and the percentage increase >20% for samples with PSA concentration >1 ng/mL.

5. Expected values/Reference range:

The reference range for the modified FREND™ PSA Plus was determined by testing serum samples from a total of 121 apparently healthy males aged 50 to 88 years (average age of 63, median age of 63). The results are summarized in the table below:

N	121
Mean	1.27 ng/mL
Median	1.01 ng/mL
95th percentile	2.92 ng/mL
97th percentile	3.19 ng/mL
99th percentile	4.59 ng/mL

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.