

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION**

A. 510(k) Number:

k124056

B. Purpose for Submission:

New device

C. Measurand:

Total prostate specific antigen

D. Type of Test:

Fluorescent immunoassay

E. Applicant:

NanoEnTek, Inc.

F. Proprietary and Established Names:

FREND PSA Plus cartridge on the FREND System

FREND System - Fluorometer for clinical use

G. Regulatory Information:

1. Regulation section:

21 CFR 866.6010 Tumor-associated antigen immunologic test system

21 CFR 862.2560 Fluorometer for clinical use

2. Classification:

Class II for assay

Class I for analyzer system

3. Product code:

LTJ, prostate-specific antigen (psa) for management of prostate cancers (for assay)

KHO, fluorometer, for clinical use (for analyzer system)

4. Panel:

Immunology (82)

Chemistry (75)

H. Intended Use:

1. Intended use(s):

The FREND™ PSA Plus as performed on the FREND™ system, is a quantitative in vitro diagnostic test which measures total Prostate Specific Antigen (PSA) in human serum and plasma. 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 in serum, heparinized plasma and EDTA plasma to be used as an aid in the management of patients with prostate cancer.

The FREND™ PSA Plus is indicated for use in clinical laboratories upon prescription by the attending physician as an aid to clinicians in managing patients with prostate cancer.

The information provided from this test may supplement decision-making and should only be used in conjunction with routine monitoring by a physician and the use of other diagnostic procedures. Because of the variability in the effects of various medications used in the treatment of prostate cancer, clinicians should use professional judgment in the interpretation of PSA results as an indicator of disease status.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

FREND™ System

I. Device Description:

Material Provided - FREND™ PSA Plus - Catalog Number FRPS 025

- 25 FREND™ PSA Plus 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. The shelf-life of FREND PSA Plus cartridges is 12 months. One Cartridge contains:

Monoclonal anti-PSA1 48 ± 98.6 ng

Monoclonal anti-PSA2 114 ± 28.8 ng

Fluorescent particle 2.4 ± 0.48 µg

- 30 Disposable pipette tips (micro-pipettor provided)
- 1 FREND™ PSA Plus Code Chip - The QC Code Chip contains data to ensure the performance of the FREND System's power, optical, and software systems when the QC Cartridge is inserted into the System. Each time a new lot is used, the PSA Code Chip that corresponds to that new lot must be inserted into the instrument and the previous PSA Code Chip must be removed. If the PSA Code Chip and the PSA Cartridge are not from the same lot, an error message will appear.

- 1 FRENDS™ PSA Plus Package Insert
- 1 Product Certificate
- QC Case – Storage box for the QC Cartridge and QC Code Chip
- FRENDS™ System Pipettor – device sheathed in a disposable single use plastic tip used to transfer samples to the Cartridge.
- Adaptor & Power cable – used to supply power to the System
- FRENDS™ System User Manual
- FRENDS™ System Quick Manual
- USB drive 1.1 – Installation File on drive
- Optional printer -

Commercially available controls from a variety of manufacturers are available that contain total PSA as a measured analyte. These controls are not provided with the assay cartridge.

The FRENDS™ System is not provided with the kit but is required for utilization of the FRENDS™ PSA Plus assay cartridge.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Tosoh ST AIA-PACK PA
2. Predicate 510(k) number(s):
P910065/S004
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	To aid in the management of patients who have been diagnosed previously with prostate cancer	Same
Contraindication (s)	Should not be used to measure PSA in patients who have received therapeutic doses of HAMA	Same
Type of Test	Fluorescent immunoassay detecting PSA	Same
Sample Type	Serum and lithium heparin plasma	Same
Quality control	Internal procedural/instrument quality controls; External positive and negative assay controls	Same
Interpretation of Results	Comparing fluorescence against a standard curve	Same
Calibration Standardization	WHO International Standard Prostate Specific Antigen (90:10) NIBSC code: 96/670	Same

Differences		
Item	Device	Predicate
Capture and detection anti-PSA antibodies	Monoclonal Anti-PSA1 and Anti-PSA2	Different monoclonal anti-PSA antibodies
Test Cartridge	Disposable single-use cartridge.	No single-use cartridge
Random Access / Degree of Automation	No random access, manual manipulation	Random access, semi-automated
Test Throughput	Single Test 7 minutes to result	Single test 18 minutes

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

Standards No.	Standards Organization	Standards Title
EP05-A2	CLSI	Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
EP06-A	CLSI	Evaluation of the Linearity of Quantitative Measurement Procedures; Approved Guideline
EP07-A2	CLSI	Interference Testing in Clinical chemistry; Approved Guideline
I/LA19-A	CLSI	Primary Reference Preparations used to standardize calibration of Immunochemical assays for PSA

L. Test Principle:

The FREND™ PSA Plus is a rapid fluorescence immunoassay that measures prostate specific antigen (PSA) in human serum and in lithium heparin and EDTA plasma using the FREND™ system. The FREND™ PSA Plus has a measuring range ranging from 0.1 ng/mL to 25.0 ng/mL. The FREND™ PSA Plus is intended for monitoring quantitative levels of PSA for the management (assessing disease progression) of patients with prostate cancer.

The specimen is added by the operator to the sample inlet with a transfer pipet, allowing the appropriate volume of sample (30 µL) to be delivered into the FREND™ PSA Plus Test Cartridge. The Cartridge is then placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. The reaction and analysis time is approximately 6 minutes. 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.

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. If the fluorescent signal measured in the reference zone

is outside parameters set by the manufacturer, an error message will be displayed and no result will be output.

FREND™ System is a bench top fluorescence reader containing a touch-screen user interface. The FREND™ System includes a simple computerized user interface to order tests, display results and operate the mechanical functions of the instrument. All reactions occur in a self-contained plastic cartridge and the reading is done in the cartridge as well. Cartridges are loaded manually by the operator. Results of the test are displayed on the screen and can be printed on an optional printer. A high-level schematic and process diagram of the FREND™ system are included in the in the User Manual.

The FREND™ System is a closed instrument system. The user does not have access to configuration parameters that could affect the assay process, test analysis or result calculation, or any other parameter that could affect test result outcomes. All software and control parameters are verified by the system’s redundancy check which is established at the time of software release.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision data was determined as described in the CLSI protocol EP5-A2. Four control materials were assayed in two replicates on two separated times per day for twenty days using a single lot cartridge. Results are shown below in Table 5.

Accepted Within-run precision on samples with measured concentrations from 0.1 to 1.0 ng/mL was designated as %CV less than 15%. At concentrations >1.0 ng/mL but < 25 ng/mL, %CV was designated as less than 10%.

Preliminary Imprecision Testing

Sample	Mean PSA Conc. (ng/mL)	Repeatability		Between-run		Between-day		Within-laboratory	
		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
1	0.098	0.013	12.80	0.005	5.5	0.004	3.7	0.014	14.4
2	4.321	0.248	5.7	0.054	1.2	0.089	2.1	0.269	6.2
3	12.735	0.636	5.0	0.405	3.2	0.102	0.8	0.761	6.0
4	25.462	1.278	5.0	0.668	2.6	0.321	1.3	1.477	5.8

A complex precision analysis over a five day period was performed at three different sites each using three different reagent lots. Each site performed the assay utilizing randomized samples based on CLSI EP5-A2 for 5 days, 2 runs of the assay per lot per day, 4 replicates of each precision material per run. Precision material concentrations were chosen in such a way as to estimate imprecision across critical portions of the assay range. Concentrations were targeted at < 1.0 ng/mL (actual mean = 0.29 ng/mL), approximately 4 ng/mL (actual mean = 3.67 ng/mL), and a concentration above 10 ng/mL but within assay linearity (actual mean = 18.33 ng/mL). Statistical analysis was performed using the 40 data points per precision material per lot that were obtained from each site.

The table below is a summary of the findings. The largest contributor to the imprecision was the cartridge itself and at concentrations below 1 ng/mL.

Variation Source	MAT A (0.29 ng/ml)	MAT B (3.67 ng/ml)	MAT C (18.33 ng/ml)	QC 1 (0.30 ng/mL)	QC 2 (2.93 ng/ml)	QC 3 (20.25 ng/ml)
Site-to-Site	3.50%	1.57%	1.67%	3.47%	1.61%	2.06%
Day- to- Day	0.00%	0.99%	1.21%	0.00%	0.00%	0.00%
Lot- to- Lot	9.12%	3.16%	7.01%	6.08%	4.30%	6.00%
Inter- cartridge	18.45%	6.81%	7.94%	20.03%	6.17%	7.49%
Total	20.87%	7.74%	10.79%	21.22%	7.69%	9.81%

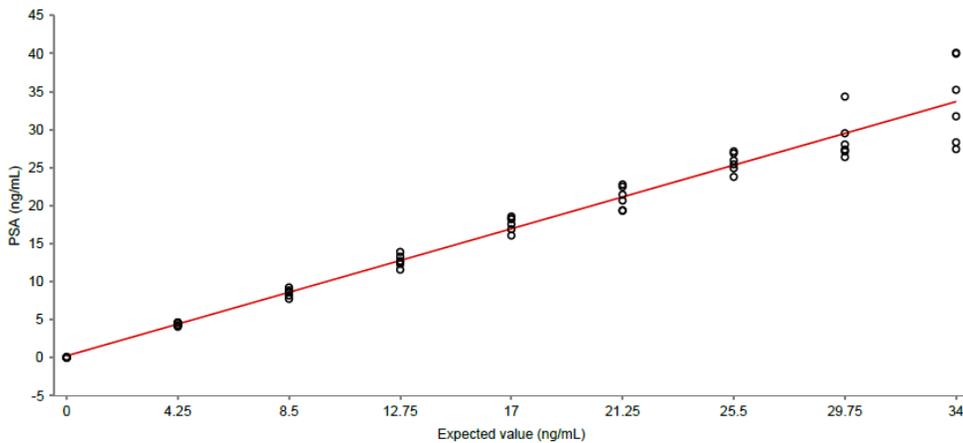
b. Linearity/assay reportable range:

To demonstrate the linearity of the assay, a serum sample pool with an elevated total PSA (34 ng/mL) was diluted to a total of seven levels according to the dilution protocol outlined in CLSI-EP6-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach). In addition, a “neat” sample (no dilution) and a “zero” sample were also be run. At each dilution level, the samples were tested in 5 replicates to determine the observed value of PSA. Percent recovery was calculated by comparing the observed PSA result with the expected value. Recoveries within $\pm 10\%$ of the expected results for the overall mean recovery at concentrations above 1.0 ng/mL for each sample were considered acceptable proof of this performance goal. The correlation coefficient when expected values were compared to actual was $R^2=0.9992$. The results are within the acceptable limits for dilution linearity studies.

Dilution	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN	SD	CV%	Expected Value	% Recovery
0.2	3.11	3.66	3.27	3.55	3.61	3.44	0.24	6.96	4.08	84.2
0.4	7.44	8.11	7.53	7.28	8.73	7.82	0.60	7.62	8.16	95.8
0.6	12.39	11.96	13.28	11.78	11.77	12.24	0.64	5.20	12.25	99.9
0.8	14.53	15.37	14.12	15.86	16.01	15.18	0.83	5.44	16.33	93.0
1	20.76	18.18	19.82	18.38	18.63	19.15	1.10	5.75	20.41	93.8

Scatter plot

CLSI guideline EP06-A section 5.1



A linear relationship fits the data better than a non-linear relationship over the measurement interval. Nonlinearity for PSA values is 10% up to 1 ng/mL. Nonlinearity from 1 to 25 ng/mL is 10%. The measurement interval for this assay is proposed as 0.1 ng/mL to 25 ng/mL.

A separate linearity study in the range between 0.11 ng/mL and 3.84 ng/mL was performed. A linear relationship fits the data better than a nonlinear relationship over the measuring limited interval. Nonlinearity is less than allowable nonlinearity: 0.04 ng/mL up to 0.4 ng/mL; then 10%.

Accuracy

As an accuracy determination, a serum pool from females (initial tPSA concentration 0.01 ng/mL) was spiked with three (3) different known levels of PSA across the range of the assay. After testing in the assay, the percentage of tPSA recovered was compared to the theoretical amount spiked into the samples. Recoveries within $\pm 10\%$ of the expected result for the overall mean recovery for a given sample were considered acceptable proof of this performance goal.

Concentration Added (ng/mL)	Observed Concentration (ng/mL)	Recovery (%)
1.08	1.06	98.3
	1.09	100.7
	1.04	96.7
4.34	4.42	101.8
	4.35	100.3
	4.27	98.4

Concentration Added (ng/mL)	Observed Concentration (ng/mL)	Recovery (%)
12.81	13.58	106
	12.04	94
	11.82	92.3
25.53	24.26	95
	24.67	96.6
	26.90	105.4

The percentage recovery ranged from 92% to 106%. The results are within the acceptance region.

High dose hook effect

The presence of high dose hook effect was tested by analyzing a concentrated sample of purified PSA antigen both neat and on dilution. Claims of “no high dose hook effect up to 1200 ng/mL” used in the product labeling were verified.

Equimolarity

The sponsor does not claim the assay is equimolar or has established equimolarity.

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

Traceability

Calibration of the assay is linked to the World Health Organization International Standard Prostate Specific Antigen (90:10) NIBSC code: 96/670. This standard reference material for PSA is a mixture of 90% complexed PSA and 10% free PSA (unconjugated to other proteins). Complexed PSA is a complex composed of free PSA conjugated to alpha-1-antichymotrypsin. Alpha-1-anti-chymotrypsin is a serine protease inhibitor commonly found in human serum.

Each lot of reagent cartridges contains a separate calibration curve which is traceable to a PSA standard curve.

Device Stability

Real time stability studies of the FRENDS™ PSA Plus Test Cartridge were conducted over 15 months on three different lots (007, 008, 009). The Test Cartridges were either stored at 2 – 8°C or at room temperature. At each three month interval, the Test Cartridge was assayed with five standard specimens. The results using both the refrigerated and the room temperature stored cartridges for the five standard specimens yielded results with a CV of less than 10% which meets the acceptance

criteria. The cartridges are stable for 12 months from the date of manufacture when stored at 2 – 8°C.

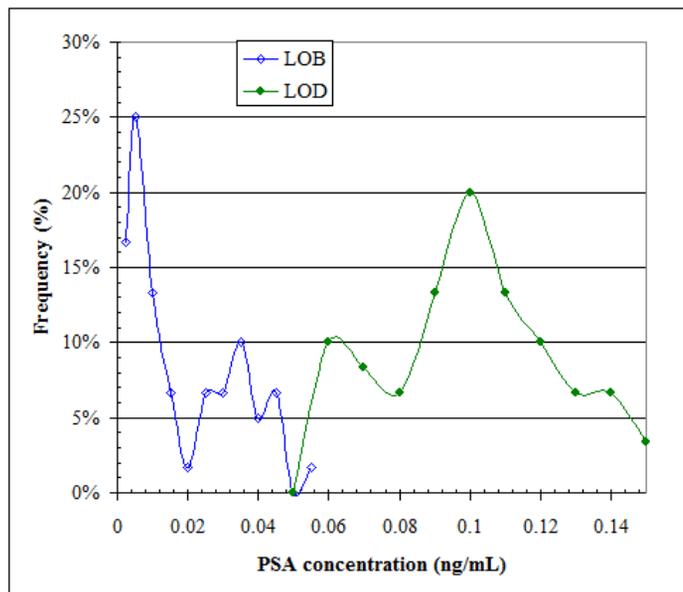
Sample Storage Studies

To demonstrate that analyzing the same sample over a variety of storage conditions returns the same result, fresh serum samples from six subjects demonstrating varying levels of total PSA across the measuring range of the assay were split into aliquots. The first set of six was analyzed with the proposed assay immediately. The second set was placed into refrigerated storage at 2 - 8°C immediately after it was prepared and then was analyzed with the assay after 6 hours in the refrigerator. The last three aliquots were frozen at -70°C immediately after collection and were analyzed with the assay after 1 week, 2 weeks, and 3 weeks. This study was performed at NanoEnTek. Results showed all recoveries were within 10% of the initial concentrations, hence support the conclusion that samples may be stored at 2-8°C for 6 hours or at -70°C for 3 weeks.

d. Detection limit:

Determination of the limit of detection/limit of quantitation was performed according to procedures outlined in CLSI-EP17-A (Protocols for Determination of Limits of Detection and Limits of Quantitation).

In the determination 5 samples without PSA (blank samples) and 5 samples with a low PSA concentration were tested in 12 replicates for each sample. The distribution of all values for the blank samples (60 values) and for the low PSA samples (60 values) is the following:



The 95th percentile value for the blank samples was 0.04 ng/mL. The 5th percentile value for the low PSA samples was 0.055 ng/mL. At a PSA value of 0.1 ng/mL, there were no blank samples (without PSA) detected by the assay and 50% of low PSA sample values (LOD samples) had a PSA value below 0.1 ng/mL. The claimed

limit of detection/quantitation is 0.1 ng/mL for the proposed assay.

e. Analytical specificity:

Prostatic acid phosphatase and kallikrein (not otherwise described) were evaluated for potential cross-reactivity with the FRENDSTM PSA Plus at 10 ng/mL and 15 ng/mL, respectively, utilizing the instructions recommended by CLSI protocol EP7-A. No significant cross-reactivity was found.

Interference studies on endogenous serum substances were performed according to the recommendations in the CLSI protocol EP7-A. Interference is defined, for purposes of this study, to be recovery $\pm 15\%$ of the known specimen mean concentration. Recovery from 85% to 115% of the expected mean is considered acceptable performance. The following describes the interferents tested and the maximum concentration without interference at the total PSA concentrations evaluated:

- Added hemoglobin (up to 500 mg/dL) does not interfere with the assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 97.3%.
- Added conjugated bilirubin (up to 20 mg/dL) does not interfere with the assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 98.2%.
- Added gamma globulin (Total Protein) up to 5.0 g/dL does not interfere with the assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 106.3%.
- Added triglyceride up to 3 grams/dL does not interfere with this assay. Average recover when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 101.5%.

Three different HAMA concentrations (human anti-mouse antibody) and RF concentrations (rheumatoid factor – a surrogate for heterophile antibody) were prepared and added to 2 different PSA concentrations (0.75 ng/mL and 8.2 ng/mL). Additionally Control PSA samples (of the same 2 concentrations levels) without added HAMA or RF were prepared. All samples were tested in 3 replicates using a single lot of the FRENDSTM PSA Plus assay on the FRENDS system. The percent recovery is calculated from the mean observed total PSA concentration obtained for each HAMA/RF sample compared to the expected total PSA concentration of that sample without added interferent. The predetermined acceptance criterion for percent recovery was $100 \pm 15\%$ for the mean recovery of each HAMA/RF level.

No significant interference effects of HAMA/RF were found in the FRENDSTM PSA Plus assay up to the levels of 52.5 ng/mL HAMA and 161 IU/mL RF. The mean %recovery of the 2 PSA concentrations at 35 ng/ml and 52.5 ng/mL HAMA ranged from 85.7% recovery to 94.8% recovery. The mean recovery of the 2 PSA concentrations at 53.8 IU/mL and 161 IU/mL RF ranged from 95.2% recovery to 113.1% recovery.

Interference studies were performed to evaluate various drugs that might be found in the serum/plasma of men diagnosed with prostate cancer which could interfere. The chemotherapeutic drugs were added to the test samples and controls at base

concentrations of tPSA of 1.0 ng/mL and 4.0 ng/mL. A modified CLSI EP7-A2 protocol was utilized in this testing process. The testing does not appear to be substantially different from recommendations in EP7. Acceptable recoveries are defined as those within $\pm 15\%$ of the expected value.

Interference Study Results for FRENDS™ PSA Plus

Substance	Concentration tested	Average % Recovery
flutamide	10 $\mu\text{g/mL}$	94.5%
Diethylstilbestrol (DES)	5 $\mu\text{g/mL}$	103.8%
goserelin	40 ng/mL	103.2%
tamsulosin	100 ng/mL	98.85%
acetaminophen	250 ng/mL	100.45%
acetylsalicylic acid	600 $\mu\text{g/mL}$	95.85%
leuprolide	275 ng/mL	101.5%
ibuprofen	500 $\mu\text{g/mL}$	102.25%
finasteride	250 ng/mL	93.6%
docetaxel	10 $\mu\text{g/mL}$	114.45%

There was no significant interference from the tested drugs that would affect the interpretation of a tPSA result as assayed using the assay.

f. Assay cut-off:

There is no analytical assay cut-off established in the literature for a generic total PSA assay when used for serial surveillance monitoring of prostate cancer subjects. This assay has certain performance characteristics at the cutoff noted in the analysis of serially monitored prostate cancer subjects described below.

2. Comparison studies:

a. Method comparison with predicate device:

Deming (or Passing-Bablok) regression analysis was performed on the data pairs obtained from prostate cancer samples taken at a single point ($n = 85$) and the earliest sample from each of 75 subjects in the serially monitored subjects. For a total of 160 samples ($85 + 75$), all samples were sampled from men diagnosed with prostate cancer. Any samples reading above either of the linearity limits of the predicate or

proposed assay was diluted and the final result determined by multiplying the dilution factor by the answer obtained on that dilution. Only results within the linear limits of both the assays will be compared in this analysis. Performance will be considered acceptable if the Passing-Bablok statistics show a slope between 0.90 and 1.10 when the predicate device is compared to the experimental one.

Deming Regression analysis was performed on a set of 160 samples (dilutions included) and then the samples exceeding the linearity of the FRENDS™ system were excluded and the analysis run again on the remaining 143 samples.

Age Statistics for Prostate Cancer Samples (n=160)

Sample size	160
Lowest value (y)	47
Highest value (y)	90
Arithmetic mean (y)	69.2
95% CI for the mean (y)	67.9 to 70.6
Median (y)	70
95% CI for the median (y)	68.1 to 71

It is not noted that the stages of the cohort of subjects with single point samples are different when compared with the cohort of subjects using the initial samples from serially monitored subjects. The distribution of tumor stages (in the TNM staging system) is as follows:

	Single point samples	Earliest serial sample from serially monitored subjects	total
stage T1	25	8	33
stage T2	41	20	61
stage T3	6	25	31
stage T4	0	0	0
stage TX	1	19	20
no info	10	2	12
All stages	83	74	

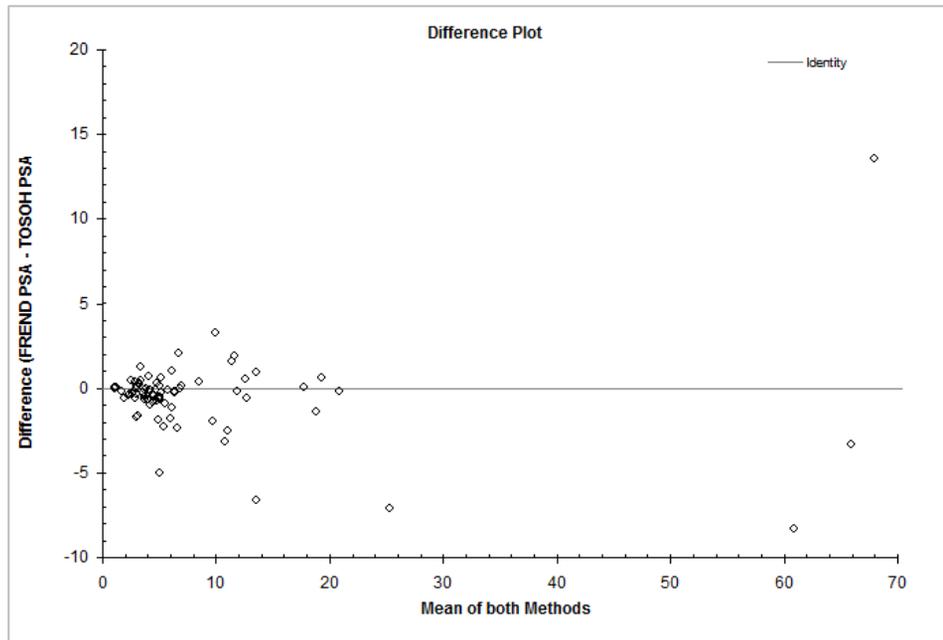
As noted in the table, single point samples are largely stage T2 (41/83 = 49%) while the stage of patients providing the initial serum sample when serially monitored are largely stage T3 (25/74 = 34%) with a lower percentage of stage T2 subjects (20/74 = 27%). This indicates that the subjects providing a single serum sample are not as equally representative of all stages as the earliest serum samples from serially monitored subjects. Single point samples are from subjects at prostate cancer diagnosis and so are more typically tumor stage T1 and T2 rather than higher stages. Therefore, a separate analysis will be performed of all serum samples from subjects undergoing serial surveillance monitoring comparing predicate and proposed PSA test results. This second analysis will provide comparison test results on patients whom are the usual target population of the proposed PSA assay.

For 85 subjects providing single serum samples, the distribution of PSA test results for the proposed and predicate assays are as follows:

Percentiles	FREND™ PSA Plus			TOSOH AIA-PACK PSA		
	PSA value	count per interval	Frequency (%)	PSA value	count per interval	Frequency (%)
2.5	1.23	3	4%	1.21	3	4%
5	1.70	2	2%	2.22	2	2%
10	2.34	4	5%	2.69	4	5%
20	3.06	8	9%	3.14	9	11%
25	3.42	5	6%	3.75	4	5%
40	3.97	12	14%	4.62	12	14%
60	5.08	17	20%	5.74	17	20%
75	8.65	13	15%	8.30	13	15%
80	10.50	4	5%	10.91	4	5%
90	17.96	8	9%	18.46	8	9%
95	49.70	4	5%	54.73	4	5%
99	1014.12	4	5%	1074.32	4	5%
	>1100	1			1	
total number		85			85	

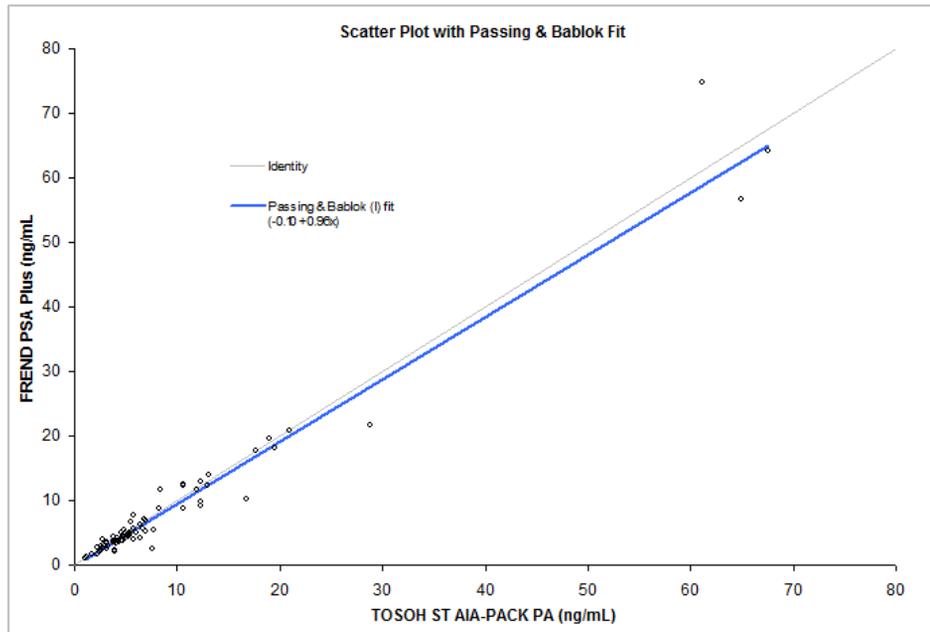
As noted in this table, the distribution of PSA test results in this cohort of subjects diagnosed with prostate cancer (at or near initial diagnosis) is very similar.

A Bland-Altman plot of the difference between the proposed and predicate assay (vs. the mean of both assays) is as follows:



The median difference of samples in the central 95% percentile region was -0.3 ng/mL (95% confidence interval of the median -0.48 to -0.12 ng/mL). Though the 95% confidence interval does not include 0, the difference is believed to be not clinically significant.

A scatter plot of 83 of 85 samples (2 samples not utilized since the differences were 2 extreme values in the distribution of differences) is as follows:



The slope of the best fit line from a Passing-Bablok regression analysis was 0.963 (95% confidence interval 0.905 to 1.029). The intercept of the best fit regression line was -0.10 ng/mL (95% confidence interval -0.45 to 0.12 ng/mL). Both the slope and intercept of the best fit line are equivalent with 1.0 and 0 respectively. This indicates that there is minimal difference in PSA test result in this cohort of subjects.

A comparison of tPSA results from the immediately preceding tPSA value for subjects undergoing serial surveillance monitoring will be described below in the clinical study.

b. Matrix comparison:

Thirty six (36) matched serum and plasma specimens (anti-coagulated with heparin, EDTA, and citrate) were collected over a period of six months, aliquoted, and frozen upon collection until use. Specimens were collected under an Institutional Review Board (IRB)-approved protocol with informed consent at a site outside the U.S. Sample testing was performed on 1 lot of cartridges using 1 FRENDA system. The samples were tested in 2 replicates and the mean of the 2 replicates was used for the data analyses. Six to nine (6~9) pairs of specimens were tested in duplicate per day.

To determine the statistical relationship between the PSA concentrations of the plasma specimens and the serum specimen, linear regression and Passing-Bablok regression analyses were performed. Ninety-five percent (95%) confidence intervals on the regression parameters were calculated. Bias plots were generated to assess the

agreement between the two matrices. Equivalence of repeatability in duplicate runs for all matrices and equivalence of variance between serum and each of three different plasma matrices in three partitions were also evaluated. The acceptance criteria for serum vs. plasma samples with PSA Plus assay value are as follows:

- 1) Passing-Bablok slope: $1.00 \pm 10\%$
- 2) Spearman Rank Correlation Coefficient ≥ 0.90

An assessment of the repeatability of variance for each matrix type was performed to ensure that variation in serum samples was equivalent to variation in each plasma type. The assay range was partitioned into 3 parts ($<1.32\text{ng/mL}$, 1.32 to 9.085ng/mL , $\geq 9.085\text{ng/mL}$ PSA). Assessment of equivalence of variation was estimated using the ratio of variances for the degrees of freedom used. The standard deviations of repeatability for serum and for each plasma type in each assay range (partition), the ratio with 95% confidence intervals based on F distribution, degrees of freedom (sample size), and the p-value for hypothesis that the ratio equals 1 were calculated. The hypothesis of equivalence of variance (null hypothesis) cannot be rejected for all matrices in all partitioned assay ranges.

	Partition I ($<1.32\text{ng/mL}$)				Partition II				Partition III (9.085ng/mL)			
	Serum	Heparin	EDTA	Citrate	Serum	Heparin	EDTA	Citrate	Serum	Heparin	EDTA	Citrate
Sample size	12	12	12	12	12	12	12	12	12	12	12	12
95% CI of mean	0.51 to 0.88	0.46 to 0.84	0.50 to 0.89	0.39 to 0.69	2.54 to 5.61	2.55 to 6.18	2.33 to 5.47	1.99 to 4.47	13.0 to 18.5	13.8 to 20.0	13.0 to 18.5	10.8 to 15.4
Variance	0.088	0.093	0.093	0.055	5.85	8.14	6.10	3.82	18.55	23.43	19.00	13.19
STDEV	0.2976	0.3054	0.306	0.236	2.4198	2.8548	2.47	1.9561	4.3076	4.8407	4.3591	3.6326
Variance ratio		1.0536	1.0573	1.5894		1.3918	1.0419	1.5304		1.2628	1.0241	1.4062
Significance level		P = 0.933	P = 0.928	P = 0.455		P = 0.593	P = 0.947	P = 0.492		P = 0.706	P = 0.969	P = 0.581

The following table summarizes the comparison results of heparin, EDTA and citrate plasmas to sera by Passing-Bablok analyses.

	N	tPSA (ng/mL)	Slope (95% CI)	Intercept (ng/mL) (95% CI)	Correlation coefficient
Serum vs. heparin plasma	36	0-25	1.07 (1.04 to 1.12)	-0.09 (-0.21 to -0.02)	0.996 (P<0.0001)
Serum vs. EDTA plasma	36		1.004 (0.94 to 1.06)	0.006 (-0.13 to -0.05)	0.991 (P<0.0001)
Serum vs. citrate plasma	36		0.814 (0.79 to 0.83)	0.077 (-0.12 to 0.01)	0.994 (P<0.0001)

For the matrix analysis, Serum, heparin-plasma, and EDTA-plasma can be used for the FRENDS™ PSA Plus assay. Citrate-plasma is not recommended to be used for the FRENDS™ PSA Plus assay. It is recommended to use the same specimen matrix when following patients because the results may not be interchangeable.

3. Clinical studies:

a. *Clinical Sensitivity:*

The percent change in tPSA from the immediately preceding value was calculated for each serum sample from 75 subjects undergoing serial surveillance monitoring for cancer progression. The percent change in total PSA ($\geq 20\%$ compared with $< 20\%$) was correlated with the clinical outcome of progression or non-progression at each serum sampling interval. The following 2 x 2 contingency table indicates the subject count for each of 4 categories.

Proposed device	progressive disease	Not progressive disease	total
Test Positive ($\geq 20\%$ change)	84	46	130
Test Negative ($< 20\%$ change)	24	82	106
total	108	128	236

	Value	\pm standard error	lower 95% conf. limit	upper 95% conf. limit
clinical sensitivity	77.8%	$\pm 4.0\%$	69.1%	84.6%
clinical specificity	64.1%	$\pm 4.2\%$	55.5%	71.9%
PPV	64.6%	$\pm 4.2\%$	58.7%	70.2%
NPV	77.4%	$\pm 4.1\%$	70.1%	83.3%
likelihood ratio positive	2.164	0.129	from 1.682	to 2.785
likelihood ratio negative	0.347	0.192	from 0.238	to 0.505

There is a significant association between %change in PSA value from the preceding value and the clinical outcomes of progression or non-progression. Additionally, receiver-operator curve (ROC) analysis of the %change $> 20\%$ with progressive disease outcome found that the area under the ROC curve for the proposed assay was 0.759 (95% confidence interval from 0.697 to 0.822; $p < 0.001$). This analysis indicates that the assay using a 20% change in PSA value from the immediately

preceding PSA value discriminates between subjects with progressive disease compared with non-progressive disease.

b. Clinical specificity:

See table above in the clinical sensitivity section.

c. Other clinical supportive data (when a. and b. are not applicable):

Clinical Study – Brief introduction and study design

Seventy-five (75) serial serum sample sets with a minimum of three sequential blood draw dates were obtained from a retrospective sample bank containing specimens from patients with confirmed prostate cancer undergoing serial surveillance monitoring and auditable medical records. From the available catalog of samples, serial sets were chosen for this study to represent a variety of stages at diagnosis, samples with Gleason Scores from 5 – 9, and a variety of treatments including prostatectomy, radioactive seeds, external beam radiation, chemotherapy and hormone therapy alone or in combination.

The serial monitoring cohort consisted of 311 serum samples with a minimum of three longitudinal samples per serial set and a maximum of nine samples per serial set – mean sample number across all serial sets was 4.2 samples. The table below defines the distribution of the samples within the serial cohort. The “comparison” column refers to the number of changes of status (changes from one visit to the next in clinical status or PSA result) that can be computed in a single serial set.

Serum sample sets collected from patients with confirmed prostate cancer who have been followed during the course of their disease were analyzed for values of PSA by the test device and the predicate device in single replicates according to the manufacturer’s instructions.

Distribution of the Serial Sample Sets				
Number of Samples	Number of Comparisons	Frequency	Total Sample	Point to Point
3	2	15	45	30
4	3	42	168	126
5	4	13	65	52
6	5	4	24	20
9	8	1	9	8
Total		75	311	236

Inclusion Criteria

Inclusion Criteria were set for this study as follows:

- 1) Serial sets must include a minimum of three (3) draws with an average of four (4) draws or more per subject if available. Samples are to be from blood draws performed at or after clinical visits throughout the clinical course of the disease.
- 2) Samples must have been in storage at minus seventy degrees (-70°C) with complete freeze-thaw records and storage records

- 3) Institutional Review Board approval for use of the samples and Informed Consent must be available for each sample set if required.
- 4) Sufficient serum must be available per sample to allow testing on both the predicate and the experimental device.
- 5) Clinical information detailing 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.
- 6) Clinical information concerning the subject at the time of initial diagnosis including the following is requested:
 - a) Patient age at diagnosis, race and gender
 - b) Initial Diagnosis with tests used to confirm diagnosis
 - c) Stage and grade at diagnosis (if available)

Exclusion Criteria

- 1) Subjects failing to meet any of the above inclusion criteria.
- 2) Patients were excluded from this study if they had been previously diagnosed with a malignancy (non-melanoma skin cancer excluded) other than prostate cancer within the five years prior to the blood draw unless this malignancy was organ-confined and no evidence of this disease existed at the time of initial draw.

Statistical analysis plan

The outcome measure for this analysis was the determination of progression of disease from time point i (clinical visit i , $i=1$ to $n-1$) to a succeeding time point j (clinical visit j , $j= i+1$ to n). In this analysis n is the number of clinical visits made by a patient after diagnosis of prostate cancer and prior to death, loss to follow up, or remission of disease.

Disease progression (w) from visit i to visit j was determined by the subject's physician based on any or a composite of all of the following and confirmed by the Principal Investigator at the bank site at the time the CRFs are completed and signed. Current status of disease at the time of a specific blood draw will be determined by:

1. Examination of the patient for clinical signs and symptoms, including the results of laboratory tests that are current standard of care for the assessment of prostate cancer disease status.
2. Comments and conclusions drawn by the physicians involved in the patient care and recorded on history and physical reports, notes of visits, status notes – all confirmed in the medical records for the subject.
3. Examination of radiographic findings (imaging) ordered as standard of care that can be used for the assessment of prostate cancer disease status. Radiographic findings include results from CAT scans, PET scans, MRI and x-Ray images.
4. Interviews with the subject as to how he is feeling and any symptoms he may be experiencing, how the subject feels compared to previous time intervals, etc.

Definition of %change in tPSA value (v) during Monitoring based on Values of the Test Device:

Let d equal 2.5 times the coefficient of variation determined in the complex precision study at approximately 4 ng/mL of the test device. This definition was chosen under the rationale that the change in the test device from one visit to the next could not be attributed solely to assay variation and could be statistically significant. For the use of the %CV of the assay multiplied by 2.5 to define the PSA %change that is considered significant, the following facts were used. TOSOH ST AIA-PACK PA %CV according to their package insert is 3.4%. The significant PSA change for the ST AIA-PACK PA is calculated to be 8.5%. For the FREND™ PSA Plus, the overall %CV determined in the precision study was 8% which translates to a significant PSA change of 20%.

With w_{ij} and v_{ij} defined above, a 2x2 contingency table is constructed for the analysis of these data. This table has the format of the table below.

	$w_{ij}=1$ Disease Progression	$w_{ij}=0$ No Progression	Total
$v_{ij}=1$ Significant Increase with test device	A	b	(a+b) = p_2N
$v_{ij}=0$ No Significant Increase with test device	C	d	(c+d) = (1- p_2)N
Total	(a+c) = p_1N	(b+d) = (1- p_1)N	N

For fixed marginal total (p_1N , p_2N) concordance, C, can be redefined as

$$C = \frac{a}{N}$$

Where

P_1 = the proportion of v-w pairs that show a progression in disease

P_2 = the proportion of v-w pairs that show an increase in PSA

Justification of Sample Size

With concordance defined in terms of a, p_1 , p_2 and N the following assumptions are made:

- The value of p_1 is no less than 0.8.
- The value of p_2 is no less than 0.7

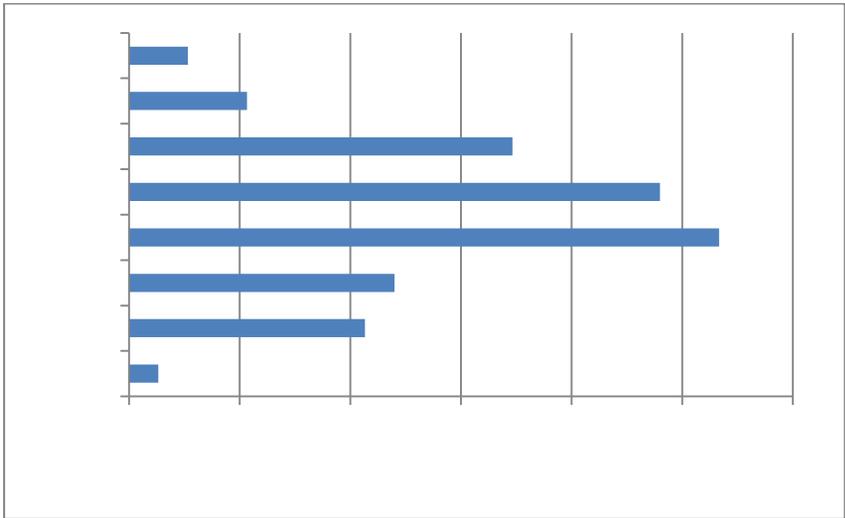
- There is a mean of 3 v-w pairs in the patient cohort.

A bootstrap sampling distribution for the concordance (C) was constructed. The estimate of the standard error for this distribution is 0.03 which translates into a 95% confidence interval width of ± 0.06 . This confidence interval width was believed sufficient to show the efficacy of the test device.

Given the above assumptions and calculations, the minimum sample size for this study was proposed to be 50 patients. Seventy-five (75) serial sets were actually used for this monitoring study.

Analysis and results

The distribution of age among the 75 men undergoing surveillance monitoring is the following:



Age by half-decade	Count	Frequency (%)
50	1	1%
55	8	11%
60	9	12%
65	20	27%
70	18	24%
75	13	17%
80	4	5%
85	2	3%
>85	0	
min age	50	
max age	85	

Note from the distribution that men aged 60 to 75 represent 80% of the men in the study.

The race of men undergoing monitoring in this study was the following:

Race	Count	Frequency (%)
African-American	11	15%
Asian	0	0%
Caucasian	61	81%
Hispanic	3	4%

Based on the distribution, 80% of subjects were Caucasian.

Seventy-two percent (72%) of men in the study had no family history of prostate cancer in first degree relatives.

In the serial cohort represented in this study, subjects were staged at the time of diagnosis as shown in the following table. Subjects followed longitudinally with changes in clinical status tend to be subjects with later stage cancer. The study surveillance cohort was not enrolled with respect to initial stage at diagnosis but randomly chosen from available subjects who met the criteria for enrollment.

Stage of Disease at Diagnosis for Serial Cohort

Stage Unknown	2
Stage I	2
Stage II	26
Stage III	25
Stage IV	20

Gleason Score at Diagnosis for Serial Cohort

Per literature on the subject of Gleason scores and the use of Gleason scores in defining a patient's prostate cancer aggressiveness, almost all patients today present with a Gleason score of 6, 7, or 8. The study cohort shows this trend as well.

Gleason Score	Number of Subjects
3	1
4	4
5	8
6	14
7	22
8	15
9	7
10	1
Information not available	3

Note from the distribution that 51 of the 75 subjects (68%) undergoing monitoring in the study were Gleason grade 6 to 8. It is unusual from a clinical viewpoint that subjects with

Gleason grade below 6 were undergoing surveillance monitoring (13 of 75 subjects were Gleason 3 to 5).

Treatment:

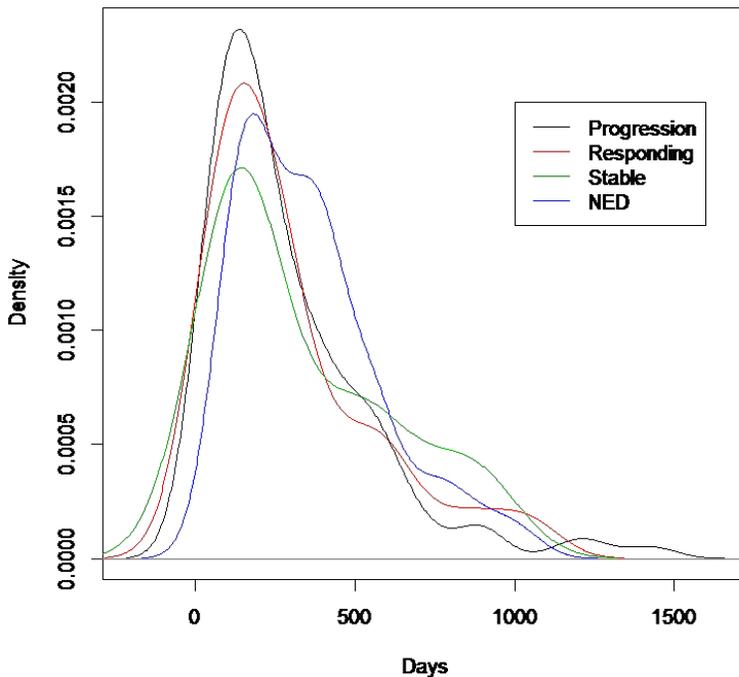
Treatment	count	frequency (%)
Chemotherapy	2	3%
Hormone Treatment	17	23%
Radiation Treatment	24	32%
Surgery Treatment	27	36%
No treatment	5	7%

As a general comment, the distribution of treatment is typical.

To summarize the time interval between serum sampling dates and infer the median interval between patient follow-up, the following table was generated.

time interval (months) by Disease Status	n	Min	1st Quartile	Median	95% CI		3rd Quartile	Max	InterQuartile Range
Progression	108	0.4	3.3	6.8	5.6	8.4	12.9	47.1	9.5
Responding	23	0.9	3.0	7.2	3.1	11.3	13.4	34.0	10.4
Stable	21	1.4	3.9	6.7	3.9	16.1	17.3	30.1	13.4
NED	84	2.2	5.8	10.9	8.3	12.5	15.4	33.8	9.5

Distribution of Time Intervals between Two Visits



The time intervals have similar distributions across disease status. Note from the table that the median interval between serum sampling and thus the median interval of follow-up is 6.7 to 7.2 months for active diseases states (progression, responding disease, and stable disease). However, the range of time intervals is wide within these groups. The range was 3-4 months (first quartile) to 13-17 months (third quartile) for subjects with active disease. The median time interval for subjects with no evidence of disease (NED) is 11 months, a slightly longer period. Except for subjects with no evidence of disease, the interval between serum sampling, and hence clinical follow-up intervals, covers a period from ~3 months to approximately 15 months; a range that is not significantly different by disease status. The median interval between serum sampling for subjects with no evidence of disease is somewhat longer and ranges from 6 months (25th quartile) to approximately 15 months (75th quartile). This longer period of follow-up is typical of subjects without evidence of disease and differs from subjects with active disease only at the first quartile (~6 months vs. ~ 3 months). This difference is not likely to be significant for subjects with no evidence of disease.

The change in clinical status for each of the measurement intervals was evaluated for “Progression” or “No Progression”. The clinical status of the patient at that visit was taken from medical records and verified by the Principal Investigator at the site. The percentage change in PSA (either $\geq 20\%$ or $< 20\%$) was correlated with the clinical status at each follow-up time period. The four choices of “Disease Status at the Time of the Visit” are: Stable, Progressing, Responding, and NED (No Evidence of Disease).

Comparison: Change in FRENDS™ PSA Plus vs. Progression/No Progression			
% Change in PSA Value	Progression	No Progression	Total
Change $\geq 20.0\%$	84	46	130
Change $< 20\%$	24	82	106
Total	108	128	236

Comparison: Change in ST AIA-PACK PA vs. Progression/No Progression			
% Change in PSA Value	Progression	No Progression	Total
Change $\geq 8.5\%$	88	46	134
Change $< 8.5\%$	20	82	102
Total	108	128	236

Table of Concordances				
Concordance	FREND™ PSA Plus	95% CI*	TOSOH ST AIA-PACK PA	95% CI*
Positive	77.06%	69.02% to 84.85%	80.73%	73.19% to 88.03%
Negative	63.78%	55.30% to 71.97%	64.57%	56.25% to 75.59%
Total	69.92%	63.98% to 75.85%	72.03%	66.10% to 77.54%

*Confidence Intervals are based on 10,000 resamples of the patient data

Based on the 95% confidence intervals there is no differences between the concordances for the FREND™ PSA assay and TOSOH AIA-PACK PSA assay.

Subject counts comparing % change categories of the proposed and predicate PSA assays were prepared for comparison in subjects with positive concordance and negative concordance. The tables are as follows:

Samples with no Progression (Negative Concordance)

		Tosoh		
		≥8.5%	<8.5%	Total
FREND	>20%	31	15	46
	<20%	15	67	82
	Total	46	82	128

Of 82 subjects absent progressive disease with a non-significant change in PSA from the preceding PSA value using the predicate PSA assay (< 8.5%), 67 subjects were categorized as having a %change in PSA from the preceding value less than 20% (82%). In both the proposed and predicate assay, 82 of 128 subjects absent progressive disease were categorized as less than the specific %change in the respective PSA assay.

Samples with Progression (Positive Concordance)

		Tosoh		
		≥8.5%	<8.5%	Total
FREND	≥20%	83	1	84
	<20%	5	19	24
	Total	88	20	108

Of 88 subjects having progressive disease with a significant change in PSA from the preceding PSA value using the predicate PSA assay ($\geq 8.5\%$), 83 subjects were categorized as having a %change in PSA from the preceding value $\geq 20\%$ (94%).

A McNemar test of the paired data in the above table yields a p value of 0.218. The associated 95% confidence interval for the true difference is -0.0157 to 0.0551. Both estimators agree: There are no statistically significant differences in the Positive Concordances (PC) between the two assays.

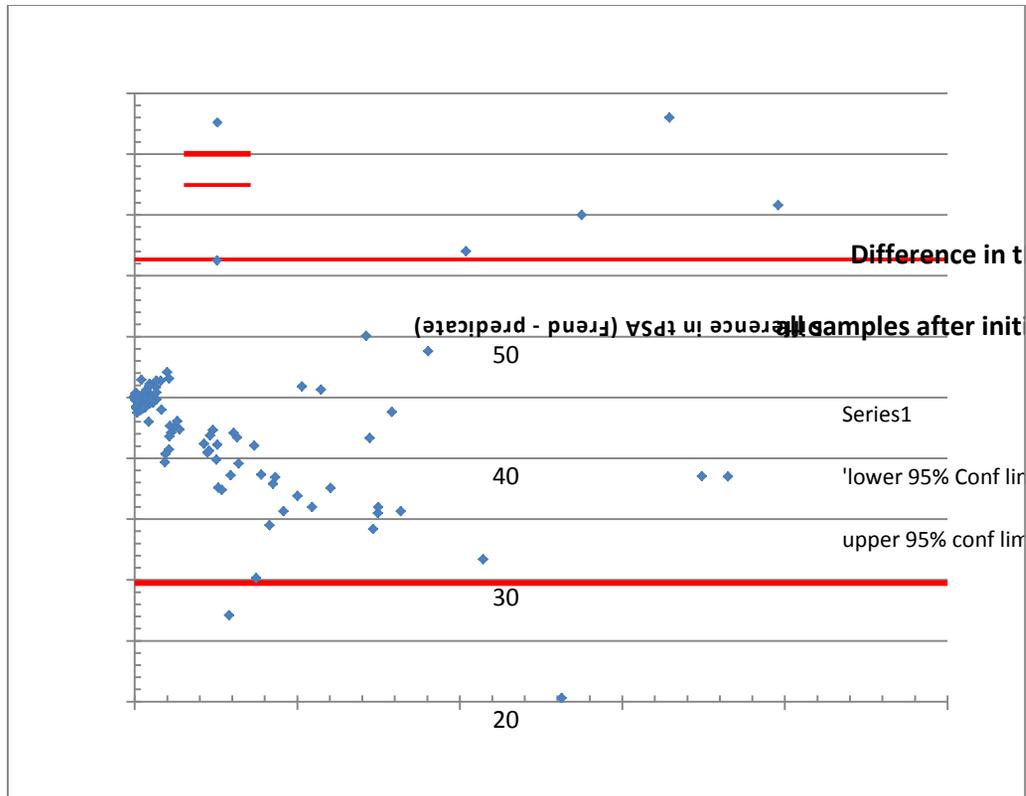
In order to use a different approach to evaluate the differences in the positive concordances, the sponsor statistician states that a resampling algorithm was also run where 10,000 differences in the positive concordances were constructed for the two assays. The middle 95% of the distribution of these differences constitutes an empirical sampling distribution for the difference. The middle 95% interval for these differences was [0.000, 0.083]. These results are yet another indication that there is no significant difference in the positive concordances between the two assays, test and predicate.

Comparison of tPSA values in tPSA values for subjects undergoing surveillance monitoring

As part of a substantial equivalence determination, it is important to evaluate the total PSA values and the %change in tPSA from the immediately preceding PSA value for actual differences between proposed and predicate assays for all subjects undergoing clinical surveillance monitoring. Such subjects undergoing monitoring are the target population for device use. In the clinical study, there were 311 such PSA values for the proposed and predicate assay containing PSA values at various points during the follow-up from each patient. Some patients had at least 2 such follow-up PSA values during monitoring. Some patients had more serial samples (as many as 8 serial samples; average of 3.1 samples per subject).

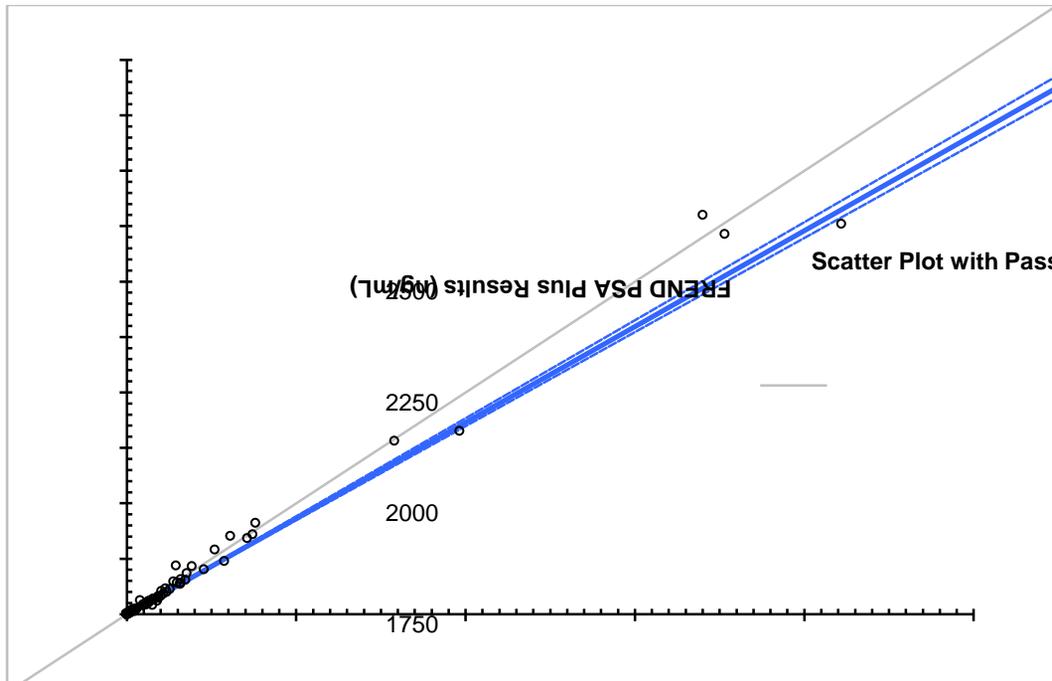
Of these 311, 75 were the initial PSA value from both assays and 236 values from both assays in subjects undergoing monitoring. The minimum detectable PSA concentration in the predicate assay is 0.05 ng/mL. For this comparison, tPSA values in either assay were utilized as stated, even if below the minimum detectable concentration. In this analysis, 236 paired serum samples from subjects undergoing serial surveillance monitoring were chosen. Among 236 paired serum samples where PSA results were from the predicate PSA assay, 13% of results were less than 0.05 ng/ml, the minimum detectable concentration. Among 236 paired serum samples where PSA results were from the proposed PSA assay, 22% were greater than 25 ng/mL, the upper limit of the assay.

The difference in tPSA value for the two assays ranged from -1558 ng/mL to 100 ng/mL. A plot of the differences in tPSA value vs. the mean of the two PSA methods (on the x-axis) is as follows for differences between -50 ng/mL to differences of 50 ng/mL.



The differences between tPSA values were not normally distributed. The median difference in total PSA was -0.16 ng/mL. Ninety-five percent of the differences were from -30.5 ng/mL to 22.7 ng/mL. For total PSA concentrations below approximately 20 ng/mL, the differences appear to be constant. For total PSA concentrations above 100 ng/mL, the differences appear to be proportional to the PSA concentration.

Regression analysis of the PSA concentrations in each assay for the 236 subjects providing serum samples indicates that there is bias in the total PSA concentrations from each assay. The slope of the best fit line from Passing-Bablok regression analysis was 0.865 (95% confidence interval 0.849 to 0.884, a slope not equivalent with 1.0). The intercept of the best fit line was 0.038 (95% confidence interval 0.025 to 0.051, an intercept not equivalent with 0).



The differences in total PSA value between the proposed and predicate PSA assays is related to the disease states. The following summary indicates the mean PSA difference (with 95% confidence interval) by disease state:

Disease status	Number samples	mean difference	lower CI	upper CI
progression	105	-22	-52	8
responding	23	-1	-4	1
stable	21	-0.15	-0.27	-0.03
NED	84	-0.07	-0.18	0.04
not progression	128	-0.29	-1	0.17

For subjects with progressive disease, the difference in PSA value is largest. For subjects with other disease states (or pooled together in the not progression category), the mean difference in PSA value is smaller and possibly not significant. It is likely that the wide range of 95% of the differences (from -30.5 ng/mL to 22.7 ng/mL) is found among subjects with progressive disease.

4. Clinical cut-off:

Definition of %change in tPSA value during Monitoring based on Values of the Test Device:

Let d equal 2.5 times the coefficient of variation determined in the complex precision study at approximately 4 ng/mL of the test device. Let x_i be the value of the test device obtained from the assay of a blood sample drawn from the patient at visit i and x_j be the

value of the test device obtained from the assay of a blood sample drawn from the patient at visit j . Define v_{ij} as:

$$v_{ij} = 1 \text{ if } (x_j - x_i) \geq d \cdot x_i$$

$$v_{ij} = 0 \text{ otherwise}$$

This definition was chosen by the sponsor under the rationale that the change in the test device ($x_j - x_i$) from one visit to the next could not be attributed solely to assay variation and could be statistically significant. The use of the %CV of the assay multiplied by 2.5 to define the PSA %change that is considered significant, the following facts were used. TOSOH ST AIA-PACK PA %CV according to their package insert is 3.4%. The significant PSA change for the ST AIA-PACK PA is calculated to be 8.5%. For the FRENDS™ PSA Plus, the overall %CV determined in the precision study was 8% which translates to a significant PSA change of 20%.

5. Expected values/Reference range:

Distribution of PSA Values in Normal Individuals

Testing of ambulatory male subjects fifty years old and older who reported themselves as healthy without any known illnesses, diseases or conditions was performed using both the FRENDS™ PSA Plus on the FRENDS System and legally marketed PSA method – TOSOH ST AIA-PACK PA on the Tosoh AIA-600II.

The sponsor recommends that a reference interval corresponding to the characteristics of the population being tested should be determined by each laboratory. Results from the FRENDS™ PSA Plus on the FRENDS System should be interpreted in the light of other clinical findings and diagnostic procedures such as DRE, various imaging studies, etc. since certain treatments can cause PSA values to decrease by virtue of the treatment while the cancer is still progressing.

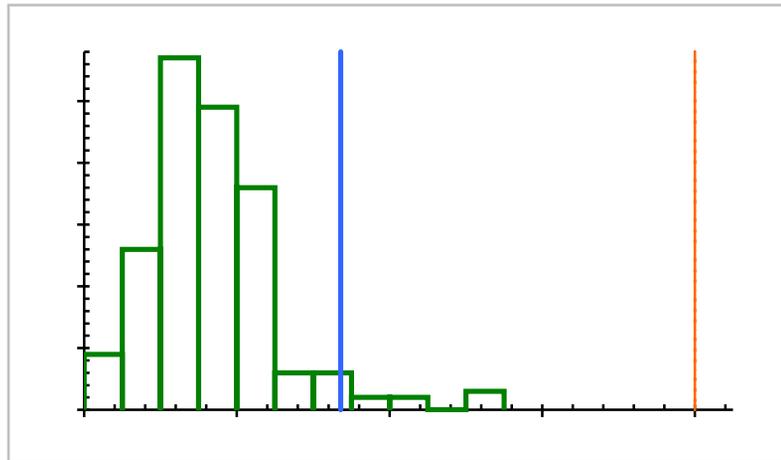
In normal men greater than 50 years of age, greater than 99% of the subjects had serum PSA concentrations less than or equal to 4.0 ng/mL by FRENDS™ PSA Plus and the predicate assay.

A tabulation of the percentile distribution for the proposed and predicate tPSA assays, as performed by the sponsor, are as follows:

Percentiles	AIA-Pack PA PSA	95% Confidence Interval	FRENDS™ PSA Plus	95% Confidence Interval
0.5	0.0000		0.0248	
10	0.3200	0.1412 to 0.3400	0.411	0.3685 to 0.4600
20	0.4100	0.3407 to 0.4594	0.53	0.4603 to 0.5600
25	0.4500	0.4000 to 0.4860	0.56	0.5200 to 0.6300

Percentiles	AIA-Pack PA PSA	95% Confidence Interval	FREND™ PSA Plus	95% Confidence Interval
40	0.5700	0.5157 to 0.6442	0.679	0.6457 to 0.7442
60	0.7900	0.7200 to 0.8400	0.841	0.8000 to 0.9700
75	0.9400	0.8540 to 1.0100	1.04	0.9800 to 1.1400
80	0.9930	0.9306 to 1.0800	1.133	1.0306 to 1.1700
90	1.1300	1.0800 to 1.2743	1.219	1.1700 to 1.5601
95	1.4590	1.1500 to 1.7379	1.677	1.2734 to 2.0379

Further information for the proposed tPSA assay in normal men age 50 years or older is shown in the following graph of the distribution:



The upper 95th percentile PSA value using the proposed assay calculated from 196 normal men age 50 years or older was 1.68 ng/mL (90% confidence interval 1.28 to 2.03 ng/mL). The upper 95th percentile value for the same cohort of men using the predicate assay was 1.52 ng/mL (90% confidence interval 1.15 to 1.73 ng/mL).

Distribution of PSA Values in Patients with Benign Diseases

The cohorts shown in the following table were assembled to determine the distribution of values in benign diseases that may be co-existent in patients with confirmed prostate cancer. Prospectively collected stored samples were utilized for this study.

Cohort	Number
Benign prostate disease (BPH, prostatitis, etc.)	104
Benign Diseases of the GI tract	107
Subjects with Diabetes of any type	97
Cardiovascular Disease/Hypertension	102
Total	410

To determine an approximate distribution of PSA values in these cohorts, the indicated number of samples for the benign cohorts was enrolled. Values were determined in single replicate for the proposed assay and in single replicate for the predicate device. A cumulative distribution was created with the results from both assays. Descriptive statistics were constructed. Distribution tables for all the single point samples were constructed. Once the sampling distributions for each order statistic are constructed, approximate 95% confidence intervals were developed. This was accomplished by the identification of the value of tPSA at the 2.5th and 97.5th percentiles of the sampling distribution for each of the assays. These values will be compared to those found in the normal population. Results from the test assay and the predicate device will be compared.

Descriptive statistics for the benign sample set are displayed in the following table.

Cohort	Percentiles	FREND™ PSA Plus	ST AIA-PACK PA
Benign GI Tract	25	0.04	0.00
	50	0.17	0.00
	75	1.11	0.93
	Percentile for tPSA = 4.0 or lower	0.941	0.941
Benign Prostate Disease	25	1.95	1.85
	50	3.46	3.69
	75	5.79	6.33
	Percentile for tPSA = 4.0 or lower	0.565	0.551
Diabetes, any type	25	0.05	0.00
	50	0.18	0.00
	75	1.15	0.79
	Percentile for tPSA = 4.0 or lower	0.950	0.949
Heart Disease/Hypertension	25	0.06	0.00
	50	0.28	0.12
	75	0.93	0.81
	Percentile for tPSA = 4.0 or lower	0.949	0.951

Distribution of PSA Values in Patients with malignant Diseases

The patient cohorts (shown in the table below) were assembled to determine the distribution of PSA values in patients with known malignancies (a mixture of treated and untreated are represented).

<i>Cohort</i>	<i>Number</i>
Lung/Liver Cancer	52
Gall Bladder/Gastric/Pancreatic Cancer	31
Prostate Cancer (Divided by Gleason Score)	85
Colorectal Cancer	89
Other Cancers	45
Total	302

Descriptive statistics for the benign sample set are displayed in the following table.

Cohort	Percentiles	FREND™ PSA Plus	ST AIA-PACK PA
Lung/Liver Cancers	25	0.11	0.00
	50	0.22	0.11
	75	0.42	0.51
	Percentile for tPSA = 4.0 ng/mL or lower	0.982	0.983
Gall Bladder /Gastric/ Pancreatic Cancers	25	0.10	0.00
	50	0.32	0.26
	75	0.99	0.74
	Percentile for tPSA = 4.0 ng/mL or lower	1.0	0.934
Colorectal Cancers	25	0.07	0.00
	50	0.30	0.00
	75	0.87	0.91
	Percentile for tPSA = 4.0 ng/mL or lower	0.942	0.932
Other Cancers	25	0.31	0.25
	50	0.57	0.40
	75	0.84	0.73
	Percentile for tPSA = 4.0 ng/mL or lower	0.958	0.956

Prostate cancer

The following table represents the nonparametric distribution for 85 prostate cancer subjects (single serum samples) not undergoing serial surveillance monitoring.

Percentiles	FREND™ PSA Plus assay		Predicate PSA assay	
	PSA value	Frequency (%)	PSA value	Frequency (%)
2.5	1.23	4%	1.21	4%
5	1.70	2%	2.22	2%
10	2.34	5%	2.69	5%
20	3.06	9%	3.14	11%
25	3.42	6%	3.75	5%
40	3.97	14%	4.62	14%
60	5.08	20%	5.74	20%
75	8.65	15%	8.30	15%
80	10.50	5%	10.91	5%
90	17.96	9%	18.46	9%
95	49.70	5%	54.73	5%
99	1014.12	5%	1074.32	5%
median	4.438		4.96	
lower 95% CI	4.041		4.652	
upper 95% CI	5.061		5.612	
percentile for 4.0 or lower	0.406		0.316	
Total number	85			

These subjects had varying pathological stages, but 77% had stage T1 or T2 (29% T1, 48% T2, 8% T3, 0% T4, 12% no stage information). The age of these men ranged from 50 years of age to greater than 75 years of age (median age 68, 95% confidence interval 64.4 to 70.6 years). The distribution of ages was:

Age range	Frequency (%)
age <50	0%
age 50-55	11%
age 55-60	14%
age 60-65	17%
age 65-70	17%
age 70-75	14%
age > 75	27%

The table below indicates the distribution by different PSA concentration categories and Gleason grade of 85 prostate cancer subjects for the proposed and predicate PSA assays.

Proposed PSA method	N	0 – 4.0 ng/mL	4.1 – 10.0 ng/mL	10.1 – 20 ng/mL	20.1 – 40 ng/mL	>40 ng/mL
Prostate Cancer**	85	31.76%	44.71%	15.29%	2.35%	5.88%
Gleason Score 5-6	43	41.86%	51.16%	4.65%	2.38%	0%
Gleason Score 7	31	29.03%	41.94%	22.58%	0%	6.45%
Gleason Score 8-9	11	0%	27.27%	36.36%	9.09%	27.27%
Predicate PSA assay						
Prostate Cancer**	85	40.00%	38.82%	12.95%	2.35%	5.88%
Gleason Score 5-6	43	51.16%	44.19%	2.38%	2.38%	0%
Gleason Score 7	31	35.48%	38.72%	19.35%	0%	6.45%
Gleason Score 8-9	11	9.09%	18.18%	36.36%	9.09%	27.27%

**Serial samples are not included in this cohort.

N. Instrument Name:

FREND System

O. System Descriptions:

FREND™ System is a bench top fluorescence reader containing a touch-screen user interface. The FREND™ System includes a simple computerized user interface to order tests, display results and operate the mechanical functions of the instrument. All reactions occur in a self-contained plastic cartridge and the reading is done in the cartridge. Cartridges are loaded manually by the operator. The System 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. A high-level schematic and process diagram of the FREND™ system are included in the User Manual.

The FREND™ System is a closed instrument system. The user does not have access to configuration parameters that could affect the assay process, test analysis or result calculation, or any other parameter that could affect test result outcomes.

The FREND™ System software consists of a graphical user interface and hardware control elements. The software controls the graphical user interface, communication with hardware, database management and data analysis. The software also controls access and functions of mechanical components such as motor, laser and printer control and acquisition of data from the sensor.

1. Modes of Operation:

The instrument is a system allowing single use only with the appropriate test cartridge.

2. Software:

Yes or No

3. Specimen Identification:

The analyzer does not perform these functions, with the exception that the instrument user can manually add specimen identification.

4. Specimen Sampling and Handling:

The analyzer does not perform these functions.

5. Calibration:

Patient samples are introduced into the device, hydrate fluorescent labeled anti-PSA antibody in buffer, and flow by capillary action into the test cartridge. At the test region of the cartridge, the PSA antibody complex interacts with surface bound anti-PSA antibody. After continued flow into the reference zone un-captured PSA antibody complexes are captured by surface bound PSA. Any secondary antibodies that do not bind to captured PSA antigen in the test zone will flow through and bind to the PSA that is fixed on the surface in the reference zone. A signal in the reference zone indicates that the microfluidics mechanism is working. Microfluidic flow can vary because of differences in blood viscosity. Therefore, the reference signal can be used to “normalize” the result.

The fluorescence is measured in both the test zone and reference zone by the FRENDS system analyzer and a ratio of signal in test zone to reference zone is calculated. The ratio is compared with a calibration curve (lot specific) which relates the ratio signal to known amounts of PSA. The concentration is calculated by the FRENDS system analyzer and the quantitative amount of PSA is reported.

6. Quality Control:

All software and control parameters are verified by the system’s redundancy check which is established at the time of software release. In the event the protected configuration is modified, the application will be prevented from running.

P. ~~Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:~~

None

Q. Proposed Labeling:

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

R. Conclusion:

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