FREND™ PSA Plus

Prostate Specific Antigen

Caution:

The sale and distribution of this device is restricted by United States federal law to, by, or on the order of a physician. In addition, the use of this device is restricted to, by, or on the order of a physician. Because of differences in reagent specificity and assay methods, the concentration of PSA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for PSA is changed during the course of monitoring patients with serial PSA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and 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 K_3-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.

Summary and Explanation of Test

Prostate-specific antigen (PSA) is a single-chain glycoprotein with molecular weight of 34 kilodaltons.[1, 2, 3] As a serine protease with chymotrypsin-like activity, PSA belongs to the kallikrein family. In blood, PSA exists as a free or complex form with protease inhibitors such as α-1-antichymotrypsin (ACT). Total PSA represents the sum of both free and complex forms.[4] PSA is uniquely associated with prostate tissues from normal, inflamed, or cancerous stages. Elevated PSA in serum or plasma is found in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory tissues. Studies on a variety of PSA methods have shown that PSA can be useful as an indicator for the diagnosis and management of prostate cancer.[5]

PSA has been found in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid as well as in seminal fluid.[6] PSA is not found in any other tissue in men, and it is not produced by cancers originating in the lung, colon, rectum, stomach, pancreas or thyroid.[7] Though increased concentrations of PSA are found in the serum of patients with benign prostate hyperplasia (BPH), prostatitis and prostate infections and inflammation, they are also found in patients with cancer of the prostate.[8, 9] PSA measurement is an essential tool in assessing the status of disease in patients with prostate cancer when serial samples are measured over time.[10] The clinical value realized by monitoring tPSA concentrations in patients with prostate cancer regardless of the treatment regimen is well known.[11] Since the mid-1980s, there has been a growing body of literature concerning the utility of Prostate Specific Antigen (PSA) for both the monitoring and detection of prostate cancer (CaP).

Principle of the Assay

The FREND™ PSA Plus is a rapid quantitative “sandwich” immunoassay using fluorescent nanoparticles which measures the concentration of total PSA. 35µL of patient serum or plasma (sodium heparin or K_3-EDTA only) is manually presented to the inlet on the individual single-unit test cartridge where it is mixed with fluorescent nano-particles conjugated with PSA antibodies. PSA molecules in the specimen bind to conjugated antibodies to form immune complexes which then move by capillary action through the reagent cartridge channel to the detection area. When the specimen reaches the test zone, it hydrates dried solid-phase anti-PSA antibodies.

PSA-fluorescent particle-immune complexes in the specimen are grabbed by the capture antibodies to form sandwich immune-complexes.
The residual PSA-unbound fluorescent nano-particles conjugated with PSA-antibodies pass through the test zone and bind to PSA antigens in the reference zone. As the sample moves forward to the waste reservoir, non-specific binding components are washed away. The intensity of fluorescence measured by a light source (laser) is proportional to the amount of total PSA in the original sample. The result is calculated using information stored on the lot-specific FREND™ PSA Plus Code Chip and then is displayed on the FREND™ System screen. A hard copy printout can be obtained if desired. A ratio calculated between the Reference zone and the Test zone corrects for test-to-test variations.

Total PSA concentration in a sample analyzed with the FREND™ PSA Plus on the FREND™ System correlates directly with the fluorescence intensity - the higher the tPSA concentration, the greater the fluorescence. The FREND™ PSA Plus has a measuring range determined as 0.08 ng/mL to 25.0 ng/mL.

The FREND™ PSA Plus uses single-use transparent plastic cartridges in which all required reagents are stored within the cartridge itself. All that is added by the user is a 35 µL test sample. The cartridge is inserted into the FREND™ System in a prescribed fashion indicated with a black arrow on the cartridge. The reaction is read multiple times as the sample moves via capillary action through the cartridge. This type of assay system is sometimes referred to as one which incorporates laminar flow.

Material Provided

*Catalog Number : FRPS 025

FREND™ PSA Plus Cartridges	25
Disposable pipette tips	30
FREND™ PSA Plus Code chip	1
FREND™ PSA Plus Package Insert	1

One Cartridge contains:

Monoclonal anti-PSA1 ---------- 48 ± 9.6 ng
Monoclonal anti-PSA2 ---------- 144 ± 28.8 ng
Fluorescent particle ---------- 2.4 ± 0.48 µg

Materials Required But Not Provided

The following materials are not provided with the reagent but are required to perform Prostate Specific Antigen analysis using the FREND™ PSA Plus on the FREND™ System.
They are available separately from NanoEntek.

Materials	Cat. No.
FREND™ System	F10

Warnings and Precautions

- The FREND™ PSA Plus cartridges are intended for *in vitro* diagnostic use only.
- PSA Plus cartridges are only to be used on the NanoEntek FREND™ System.
- Allow cartridges to come to room temperature for 15 – 30 minutes prior to use.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.
- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Cartridges should not be frozen.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab maybe derived from human materials, please use standard laboratory safety procedures when handling all specimens and controls.
- Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package Insert and User Manual.
- PSA Plus cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Keep the cartridge sealed in the pouch until just ready for use.
- Use the cartridge immediately after opening its pouch.
- Wear disposable gloves when handling the cartridges and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- For professional use only.

PSA Plus has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. Samples with PSA concentrations between 25 and 1,200 ng/mL will read > 25ng/mL. The “hook effect” phenomenon may occur only at PSA concentrations > 1,200 ng/mL.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twenty four months from the date of manufacture.

The expiration date is clearly indicated on the product box and the cartridges.

Materials	Cat. No.
Refrigerator Temperature (2~ 8°C) :	FRPS 025
PSA Plus cartridges	
Room Temperature :	None
Pipette tips	

Specimen Collection and Handling

Serum or plasma (heparinized or K_3-EDTA only) is required for the assay. Citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. To use serum, a blood sample is collected aseptically without additives by venous puncture. After allowing the sample to clot for 30 minutes at room temperature, the collection tube should be centrifuged for 10 minutes at 3000 rpm.

For heparinized or K_3-EDTA plasma, a venous blood sample is collected aseptically with the designated additive. The plasma should be separated from the packed cells as soon as possible.

Prostatic manipulation has been shown to affect the PSA results so samples should be drawn before any prostatic procedures such as DRE, prostatic massage and TRUS are performed.

Samples may be stored at 2°-8°C for up to 6 hours prior to analysis.
If the analysis is scheduled to be done at some later time, the sample should be stored frozen at -20°C or below for future use.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter such as fibrin clots or strands should be centrifuged before being tested. Prior to assay, slowly bring frozen samples to room temperature (18° C - 25° C) and mix gently but thoroughly before testing.

Procedure

Reagent Preparation

- **Cartridges**

There is no reagent preparation required to measurer PSA using the FREND™ PSA Plus cartridge on the FREND™ System. However, the cartridges needed for a particular run should be removed from the refrigerator and allowed to reach room temperature for 15 ~ 30 minutes before they are used.

- **Calibration**

The calibrators used during the reagent manufacture process to create the information placed electronically on the FREND™ PSA Plus Code chip are prepared gravimetrically and are compared to international reference standards (WHO International Prostate Specific Antigen (90:10) NIBSC code: 96/670). However, for the end- user, there is no need for calibration as is generally performed on other automated laboratory equipment. All calibration statistics and information have been electronically stored on the FREND™ PSA Plus Code chip included in each box of FREND™ PSA Plus reagent. The FREND™ PSA Plus Code chip is specific for that manufactured lot of FREND™ PSA Plus.

The appropriateness of the calibration information should always be checked by running sufficient external quality control materials as samples to verify that the results obtained for tPSA on the FREND™ System using the FREND™ PSA Plus cartridges of a particular lot meet the laboratory criterion for acceptability.

- **PSA Plus Code chip Installation**

Please refer to the FREND™ System User Manual for more detailed instructions relative to the Code Chip installation. Abbreviated instructions follow here:

(1) Insert the FREND™ system electrical cord into an appropriate outlet.
(2) Insert the Code chip into the Code chip slot at the rear of the FREND™ Systemfollowing the arrows.
(3) Press the ‘Setup’ button on the ‘Main’ screen.

(4) Press the 'Code chip' button on the 'Setup' screen.
(5) The information embedded on the FREND™ PSA Plus Code chip is automatically saved on the FREND™ System.
(6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
(7) Press the 'Item' button on the 'Setup' screen.
(8) Check the FREND™ PSA Plus cartridge lot number and the installation date of the Code chip.
(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

Quality Control

• FREND System QC Cartridge

The FREND™ QC cartridge contains multiple controls to check the optics of the system. By testing with the QC cartridge, the analytical components of the system; (1) laser power (2) alignment, and (3) mechanical integrity are confirmed. For each day of patient testing, perform QC Cartridge testing. Refer to the Quality Control Procedures section in the User Manual of FREND™ System. In brief, perform QC Cartridge testing for the following conditions:

(1) Upon initial setup of the system
(2) Each day of patient testing.
(3) When the system has been transported or moved.
(4) Whenever there is uncertainty about the performance of the system.
(5) Whenever required by your laboratory's quality control requirements.

• Internal procedural controls

The FREND™ PSA Plus test cartridge contains built in control features. Fluorescence signal in the Reference Zone of each cartridge shows: (1) that enough sample volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this Reference Zone signal is missing or lower than the threshold, the FREND™ System considers it as an incorrect or failed test, and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors, in part, for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

- **External quality control testing**

Commercially available controls that contain total PSA as a measured analyte are available from a variety of manufacturers. It is recommended that a minimum of two (2) levels of controls be run at least once per month or once for each new lot, whichever comes earlier. However, Controls should be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should establish its own criteria based on the following parameters.

(1) Each new lot
(2) Each new shipment (even if from the same lot previously received)
(3) Each new operator (an individual who has not run the tests for at least two weeks)
(4) Monthly, as a continued check on storage condition
(5) Whenever problems (storage, operator, or other) are identified
(6) Or other times as required by your laboratory's standard QC procedures

Individual laboratory policy will dictate exactly which control materials and lot numbers should be run, the frequency with which controls are to be tested, criteria for acceptance of the results and required corrective action to be taken if results do not meet laboratory criteria. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument or software malfunction. Do not assay patient samples on the FREND™ System using the FREND™ PSA Plus if quality control results do not give expected values. Refer to your laboratory policies on how to determine acceptability of external control material results.

Each laboratory operates under a different set of regulations.
Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

Specimen Processing

• Preparation

Remove from the refrigerator sufficient cartridges of FREND™ PSA Plus to test the number of patient samples and required external quality control materials. Allow the cartridges to come to room temperature for 15 – 30 minutes prior to the start of the testing sequence.

If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing.

There are no other reagents or sample preparations necessary.

• Assay Procedure

(1) Prepare the FREND™ PSA Plus and specimen.
(2) Record the Sample ID on the cartridge in the designated area.
(3) Drop the sample (35 μL) into the sample inlet on the cartridge using a calibrated micro-pipette with a fresh pipette tip.
(4) Press the 'Test' button on the 'Main' screen of the FREND™ System.
(5) The system moves to the Patient ID screen automatically.
(6) Type the Patient ID and press the 'Enter' button to begin the test.
(7) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.

⚠ ***Caution:*** *Please check the direction of the cartridge before insertion and assure the insertion is complete.*

(8) When the reaction in the cartridge is complete within 4 minutes, the FREND™ System will automatically begin the reading process.
(9) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠ ***Caution:*** *Do not remove power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.*

(10) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.
(11) For more detailed instructions, please refer to the 'FREND™ System User Manual.'

Procedural Notes

If a specimen Prostate Specific Antigen concentration is found to be greater than the linearity limit of the assay of 25 ng/mL and a definitive result is required, the specimen should be diluted with sera that has been previously measured on the FREND™ PSA Plus and found to contain < 0.08 ng/mL tPSA and then re-assayed according to the Assay Procedure. The exact dilution will depend upon the original value but we suggest that one begin with a 1:5 and a 1:10 dilution. If the result of the 1:10 dilution is still outside the linear limits, a 1:20 and 1:50 dilution should be made and these dilutions re-assayed. It is desirable to dilute the sample so that the diluted sample reads between 1 and 20 ng/mL. Dilutions must be made manually and the final result on the diluted sample calculated manually by multiplying the result obtained on the diluted sample by the dilution factor.

Below is an example using a final diluted sample result of 12.3 ng/mL, a 1:10 dilution and a dilution sample concentration of 0.1 ng/mL where "X" equals the original concentration of the unknown sample and * indicates multiplication.

$$123 \text{ ng/mL} = \frac{(1*X \text{ ng/mL}) + (9 * 0.1 \text{ ng/mL})}{10}$$

$$123 \text{ ng/mL} = \frac{X \text{ ng/mL} + 0.9 \text{ ng/mL}}{10}$$

$$(10) * (123) \text{ ng/mL} = X \text{ ng/mL} + 0.9 \text{ ng/mL}$$
$$123 \text{ ng/mL} = X \text{ ng/mL} + 0.9 \text{ ng/mL}$$
$$123 \text{ ng/mL} - 0.9 \text{ ng/mL} = X \text{ ng/mL}$$
$$122.1 \text{ ng/mL} = X \text{ ng/mL}$$

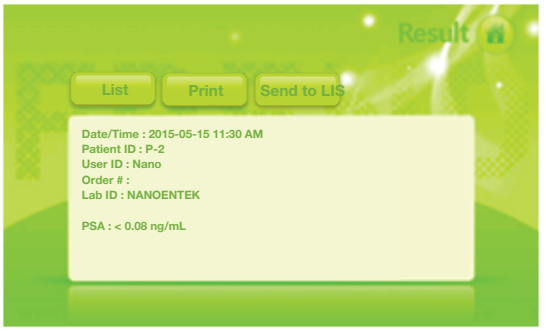
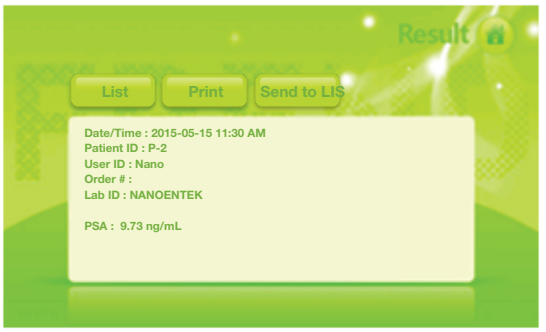
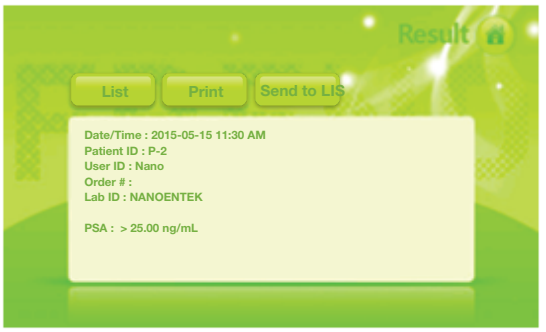
※Original unknown concentration of PSA equals 122.1 ng/mL

To use this formula to calculate the original concentration of any unknown sample PSA which exceeds the assay linearity and is diluted with another sample previously measured with the FREND™ PSA Plus, just substitute the appropriate measured result on the diluted sample, the dilution factor and the diluent PSA concentration for those used above in the example. To do a quick check on your result, the original concentration for the unknown should always be just slightly less than the diluted result multiplied by the dilution factor without taking into consideration the PSA value of the sample used as the diluent.

Calculation of Results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been manually added to the sample well in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank readings are subtracted after which the net rate is automatically converted to total Prostate Specific Antigen concentration in ng/mL based upon information stored on the PSA code chip. This result is then output on the screen and to the optional printer. It is also stored in memory on the FREND™ System.

Screen Displays for Various Concentration Scenarios

Displayed result	Description
	PSA concentration Less than 0.08 ng/mL
	PSA concentration Not less than 0.08 ng/mL and not higher than 25.00 ng/mL
	PSA concentration Higher than 25.00 ng/mL

Limitations of the Procedure

When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, medical history, therapy, etc.).

The FREND™ System, paired with a FREND™ PSA Plus cartridge, is programmed to report 25 ng/mL as the highest concentration of PSA measurable without dilution. The lowest measurable concentration is 0.08 ng/mL – the assay sensitivity limit.

Heterophilic antibodies in a sample have the potential to cause interference in immunoassay systems.[12,13] Infrequently, PSA levels may appear elevated due to heterophilic antibodies present in the patient's serum or plasma or to nonspecific protein binding. If the PSA level is inconsistent with clinical evidence, additional PSA testing is suggested to confirm the results.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated or decreased PSA values.

Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.[14]

The concentration of tPSA in a given sample determined with assays from different manufacturers can vary due to differences in assay methods, calibration, and reagent specificity.[15]

Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Clinical results must be interpreted with regard to medications administered to the patient.[14]

The ability of the assay to detect both free and complexed forms of total PSA (free PSA complexed with alpha-1-antichymotrypsin) on an equal molar basis (equimolarity) has not been established.

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Because of differences in reagent specificity and assay methods, the concentration of PSA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for PSA is changed during the course of monitoring patients with serial PSA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Expected Values

As with every clinical diagnostic test, a reference interval corresponding to the characteristics of the population being tested should be determined by each laboratory. The FREND™ PSA Plus on the FREND™ System is to be used on serial blood samples to manage patients with prostate cancer.

Testing of 121 ambulatory male subjects fifty years old and older who reported themselves as healthy without any known illnesses, diseases or conditions was performed using the FREND™ PSA Plus on the FREND™ System according to the CLSI guideline C28-A3.

As is true for all PSA methods, not PSA results can be interpreted as being definitive for the presence or absence of prostate cancer. Patients with levels of PSA within the reference interval found in apparently healthy subjects may have prostate cancer; patients with levels exceeding those in the reference interval maybe prostate cancer free. Results from the FREND™ PSA Plus on the FREND™ 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.

Reference Ranges

The interval given here was determined in serum samples from 121 apparently healthy male subjects from the age of 50 – 88 years.

Category	Men
Number of Samples (n)	121
Reference Interval (95th percentile)	2.92 ng/mL
Reference Interval (99th percentile)	4.59 ng/mL

Expected Values for Management of Patients with Prostate Cancer

Distribution of serum FREND™ PSA Plus Concentrations Healthy, Benign and Various Malignant Disease States

	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
Healthy Subjects	121					
Men ≥ 50 yrs.	121	98.35%	1.65%	0%	0%	0%
Benign Disease/Cond.*	410					
Benign Prostate	104	56.73%	25.96%	11.54%	3.85%	1.92%
Diabetes	97	95.88%	3.09%	1.03%	0.00%	0.00%
HTN/Heart Disease	102	95.10%	4.90%	0.00%	0.00%	0.00%
Benign GI	107	94.4%	4.67%	0.00%	0.93%	0.00%
Malignant Diseases*	302					
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%
Lung/Liver Cancer	52	98.08%	0%	1.92%	0%	0%
GB,Gastric,Pancreatic	31	100%	0%	0%	0%	0%
Colorectal Cancer	89	94.38%	4.49%	1.13%	0%	0%
Other Cancers	45	97.78%	2.22%	0%	0%	0%
TOTAL Subjects	833					

* Treated and untreated subjects
** Serial samples are not included in this cohort.

Performance Characteristics

Performance characteristics were evaluated for the FREND™ PSA Plus as follows:

Precision

Imprecision study was performed at the NanoEntek laboratory as described in the CLSI guideline EP5-A3. Four serum pools were assayed for 20 days, 2 runs per day in duplicate using three lots of PSA Plus reagent cartridge. The results are summarized below:

	Mean (ng/mL)	Repeatability		Between Run		Between Day		Within Lot		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low1	0.08	0.011	14.1%	0.002	2.4%	0.011	14.6%	0.000	0.0%	0.011	14.6%
Low2	0.10	0.012	12.3%	0.002	2.0%	0.012	12.5%	0.000	0.0%	0.012	12.5%
Medium	4.00	0.290	7.2%	0.000	0.0%	0.297	7.4%	0.000	0.0%	0.297	7.4%
High	21.40	1.196	5.6%	0.000	0.0%	1.301	6.1%	0.086	0.4%	1.304	6.1%

A multi-site imprecision study was also performed at three sites. Three serum samples were assayed for 5 days, 5 replications per day using single lot of PSA Plus reagent cartridge. The results are summarized below:

	Mean (ng/mL)		Repeatability		Between Day		Within Lot		Between Site / Operator / Instrument		Reproducibility (TOTAL)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
C	75	0.258	0.026	10.1%	0.011	4.4%	0.028	11.0%	0.000	0.0%	0.028	11.0%
D	75	2.874	0.218	7.6%	0.034	1.2%	0.221	7.7%	0.053	1.8%	0.227	7.9%
E	75	11.485	1.137	9.9%	0.485	4.2%	1.236	10.8%	0.219	1.9%	1.255	10.9%

Linearity

A study was done to demonstrate the linearity of the assay according to the dilution protocol outlined in CLSI guideline EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. At each dilution level, the samples were tested in quadruplicate or quintuplicate to determine the measured value of tPSA. Linearity was demonstrated from 0.08 to 25. The measuring range for the FREND™ PSA Plus is 0.08 ~ 25.00 ng/mL. Results of unweighted least squares regression analysis are shown below:

Parameter	Estimate	95%CI
Intercept	0.000	-0.101 to 0.101
Slope	1.001	0.9926 to 1.010

Correlation with the expected linearity showed $R^2 = 0.996$.

Method comparison

Comparison studies were performed in a CLIA-certified laboratory using de-identified frozen specimens. The reference method was the Abbott ARCHITECT total PSA assay run on the Abbott ARCHITECT i System. All samples (207) analyzed in the clinical testing were split and tested by both the ARCHITECT total PSA assay and the FREND™ PSA Plus assay.

Results generated using the FREND™ PSA Plus on the FREND™ System (y) were compared to those obtained using a previously FDA cleared ARCHITECT total PSA assay (x) by Passing-Bablok regression analysis. Results of this study are shown below:

Slope: 0.975 (95% CI 0.936 to 1.014)	**y-Intercept: -0.047 (95% CI -0.101 to - 0.0004)**
Number of samples: 207	Range Tested: 0.08 to 23.56 ng/mL
r: 0.976	

Comparability using CLSI guideline EP9-A3 shows that the two methods compare favorably.

Matrix comparison

The matrix comparison study was performed according to CLSI guideline EP14-A3. PSA concentration s in 40 paired serum, lithium heparin, and K_3-EDTA samples were measured using the FREND™ PSA Plus. The range tested is 0.08 - 23.59 ng/mL with serum samples. Passing-Bablok regression analysis of serum results (x) compared to lithium heparin plasma results (y_1) and K_3-EDTA plasma results (y_2), yielded acceptable regressions shown below:

	Lithium heparin plasma (y1)	K_3-EDTA plasma (y2)
Slope (95% CI)	0.961 (0.894 to 1.031)	1.030 (0.985 to 1.090)
y-Intercept (95% CI)	0.026 (-0.152 to 0.242)	-0.077 (-0.370 to 0.078)
Number of samples	40	40
r	0.985	0.988

FREND™ PSA Plus can be measured equally well in serum, lithium heparin plasma and K_3-EDTA plasma.

Sensitivity

The Limit of Detection (LoD) for the FREND™ PSA Plus was established according to the CLSI guideline EP17-A2 and was determined to be 0.03 ng/mL. The Limit of Quantitation (LoQ) was determined to 0.08 ng/mL.

Specificity

Interference was defined as recovery values outside of 15% of the know specimen mean concentration. Recovery within 85% to 115% of the expected total PSA was considered as lack of interference. The interference studies were performed as recommended in the CLSI guideline EP07-A2 protocol using two concentrations of total PSA. Results are summarized in the table below:

Interference Study Results for FREND™ PSA Plus on the FREND™ System.

	Interferent	Conc. Tested	Average %Recovery PSA (1 ng/mL)	Average %Recovery PSA (4 ng/mL)
Endogenous	Hemoglobin	500 mg/dL	91.2	104.8
	Bilirubin (unconj.)	20 mg/dL	95.2	98.5
	Triglycerides	3 g/dL	107.1	101.2
	Total protein	50 mg/mL	97.4	107.8
Pharmace-uticals	Acetaminophen	250 ng/mL	101.4	101.1
	Aspirin	600 mg/mL	100	100.4
	ibuprofen	500 mg/mL	102.7	104.1
	Flutamide	10 mg/mL	94.0	95.9
	Diethylstilbestrol(DES)	5 mg/mL	91.8	104.1
	Goserelin	40 ng/mL	103.3	100.7
	Leuprolide	275 ng/mL	95.7	94.3
	Finasteride	250 ng/mL	105.5	105.7
	Tamsulosin	100 ng/mL	96.8	107.1
	Docetaxel	10 mg/mL	94.3	103.6
Potential cross-reactant	Prostatic acid phosphatase	10 ng/mL	105	95.3
Heterophilic Antibodies	RF	1,075 IU/mL	108.9	106.3
	HAMA	70 ng/mL	92.3	93.1

There was no significant interference from the tested substances that would affect the interpretation of a tPSA result as assayed on the FREND™ PSA Plus.

Serial measurements and concordance with medical status

Since the FREND™ PSA Plus Indication for Use is that the assay results will be used as a tool in managing the care for patients with prostate cancer, it is imperative that the changes in the marker are compared to clinical status changes to determine the efficacy of the test. Therefore, as an important part of the clinical studies performed to characterize the FREND™ PSA Plus, serial samples collected longitudinally from patients previously diagnosed with prostate cancer and treated in a variety of ways over the clinical course of their disease (including prostatectomy, cryoablation, lymph node resection, TURP, chemotherapy, orchiectomy and so on) were assayed for tPSA with the modified FREND™ PSA Plus assay as well as the previously cleared FREND™ PSA Plus assay.

For each point to point in a sample serial set, the change in the tPSA concentration was compared to the change in the clinical status of the patients as measured by other laboratory tests, patient interviews, physical examinations, and imaging studies of a variety of types and recorded on a Clinical Report Form.

For both predicate and test devices, the significance of PSA increase was defined as the change over 0.2 ng/mL at low concentration (≤1.0 ng/mL), and the percent increase over 20% at the higher concentration. Any significant increase in value from one time period to the next that did not exceed the criteria was logged as ≤0.2 ng/mL or ≤ 20% Change.

Disease status for the patients falls into one of four different categories by the physician - NED (no evidence of disease), Responding, Stable and Progression. The table below shows each disease status as determined for the FREND™ PSA Plus results for all subjects compared to the Clinical Status changes.

		NED	Responding	Stable	Progression	Total
FREND™ PSA Plus	**Positive**	16	1	6	46	69
	Negative	80	9	9	27	125
	Total	96	10	15	73	194

The table below is stratifying the results based on the cancer stage at the time of diagnosis. As per the guidelines from American Cancer Society, the TNM category information is combined (along with the Gleason score and PSA level) to get the overall stage of the cancer.

Change	Clinical status				
	NED	Responding	Stable	Progression	Total
Stage I					
Positive	0	0	0	2	2
Negative	4	1	0	1	6
Total	4	1	0	3	8
Stage II					
Positive	5	0	2	22	29
Negative	39	4	2	11	56
Total	44	4	4	33	85
Stage III					
Positive	7	0	2	15	24
Negative	21	4	3	8	36
Total	28	4	5	23	60
Stage IV					
Positive	3	1	2	7	13
Negative	13	0	4	7	24
Total	16	1	6	14	37
Stage Unknown					
Positive	1	0	0	0	1
Negative	3	0	0	0	3
Total	4	0	0	0	4

The table below shows the summary of diagnostic performance of FREND™ PSA Plus.

	FREND™ PSA Plus	
	Proportion	**95% CI**
Sensitivity	0.630	0.515 to 0.732
Specificity	0.810	0.731 to 0.870
Accuracy (Overall Concordance)	0.742	0.675 to 0.802
Positive Likelihood ratio	3.315	2.227 to 5.008
Negative Likelihood ratio	0.457	0.328 to 0.609
Positive Predictive value	0.667	0.571 to 0.750
Negative Predictive value	0.784	0.727 to 0.832

Results of subgroup analysis (surgery group, non-surgery group, and prostatectomy group) are shown below:

Surgery Group (N = 119)	**Clinical status**				
	NED	**Responding**	**Stable**	**Progression**	**Total**
Positive	11	1	1	27	40
Negative	54	5	3	17	79
Total	65	6	4	44	119

Non-surgery Group (N = 75)	**Clinical status**				
	NED	**Responding**	**Stable**	**Progression**	**Total**
Positive	5	0	5	19	29
Negative	26	4	6	10	46
Total	31	4	11	29	75

Prostatectomy Group (N = 53)	**Clinical status**				
	NED	**Responding**	**Stable**	**Progression**	**Total**
Positive	4	0	0	10	14
Negative	32	2	0	5	39
Total	36	2	0	15	53

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Glossary of Symbols

	Caution, warning, Consult accompanying documents
	Catalogue number/Reference number
	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
	Authorized representative in the European Community
	CE marking
	*In vitro* diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged
	For prescription use only
	Irritant

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