

Summary of Safety and Effectiveness

I. General Information

Device Generic name(s): Immunoassay for the *in vitro* quantitative determination of free prostate specific antigen (PSA) in human serum and plasma

Device Trade name(s): IMMULITE®/IMMULITE® 1000 Free PSA Immunoassay
IMMULITE® 2000 Free PSA Immunoassay

Applicant's name and address Siemens Medical Solutions Diagnostics
5210 Pacific Concourse Drive
Los Angeles, CA 90045-6900
United States

PMA number: P060005

Date of Panel recommendation: None

Date of notice of approval to the applicant May 11, 2007

II. Indications for Use

The IMMULITE®/IMMULITE® 1000 Free PSA assay is intended for use as follows:

For *in vitro* diagnostic use with the IMMULITE®/IMMULITE® 1000 Analyzer for the quantitative measurement of free prostate-specific antigen (PSA) not bound to α 1-antichymotrypsin or other binding proteins (uncomplexed) in human serum (including serum collected in serum glass, serum plastic and serum gel separator tubes).

Measurement of Free PSA is used in conjunction with IMMULITE®/IMMULITE® 1000 Total PSA to determine a ratio of Free PSA to Total PSA (percent Free PSA). The percent Free PSA is used as an aid in discriminating prostate cancer from benign disease in men 50 years or older with IMMULITE®/IMMULITE® 1000 Total PSA values between 4 and 10

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ng/mL and digital rectal exam (DRE) findings not suspicious of cancer. Prostate biopsy is required for the diagnosis of prostate cancer.

The IMMULITE[®] 2000 Free PSA assay is intended for use as follows:

For *in vitro* diagnostic use with the IMMULITE[®] 2000 Analyzer for the quantitative measurement of free prostate-specific antigen (PSA) not bound to α 1-antichymotrypsin or other binding proteins (uncomplexed) in human serum (including serum collected in serum glass, serum plastic and serum gel separator tubes). Measurement of Free PSA is used in conjunction with IMMULITE[®] 2000 Total PSA to determine a ratio of Free PSA to Total PSA (percent Free PSA). The percent Free PSA is used as an aid in discriminating prostate cancer from benign disease in men 50 years or older with IMMULITE[®] 2000 total PSA values between 4 and 10 ng/mL and digital rectal exam (DRE) findings not suspicious of cancer. Prostate biopsy is required for the diagnosis of prostate cancer.

III. Contraindications

There are no known contraindications for the IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 free PSA Immunoassay.

IV. Warnings and Precautions

Warnings and precautions are stated in the product labeling.

V. Device Description

Test Principle

The IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA assays are automated, solid-phase sequential chemiluminescent immunometric assays. The solid phase, a polystyrene bead, is coated with a monoclonal antibody specific for epitopes on PSA that would be rendered inaccessible by complex formation between PSA and α 1-antichymotrypsin (ACT).

The patient sample and a buffer/serum matrix are simultaneously introduced into the test unit/reaction tube, and incubated for approximately 30 minutes at 37°C with intermittent agitation. During this time, free PSA (fPSA) in the sample binds to the anti-fPSA monoclonal antibody-coated bead. Unbound serum is then removed by a centrifugal wash. An alkaline phosphatase-labeled polyclonal goat anti-total PSA (tPSA) antibody is introduced and the test unit/reaction tube is incubated for another 30-minute cycle. The unbound enzyme conjugate is removed by a centrifugal wash. Substrate is then added, and the test unit/reaction tube is incubated for a further 12 minutes on the IMMULITE[®]/IMMULITE[®] 1000 or 5 minutes on the IMMULITE[®] 2000.

The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light. The bound complex, and thus also the photon output as measured by the luminometer, is proportional to the concentration of fPSA in the sample. A quantitative result is then obtained by comparing the patient result to a stored Master Curve.

Description of the Device

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Kit Components:

The IMMULITE®/IMMULITE® 1000 assay kit contains matched set of the following components (the barcode labels are needed for the assay):

1. Free PSA Test Units (LPF1 100 units; KPF5 500 units)
Each barcode-labeled unit contains one bead coated with monoclonal antibody specific for the fPSA. Stable at 2-8°C until expiration date.
2. Free PSA Reagent Wedges (LPFA: 1 set. , LPFB: 5 sets)
With barcodes, LPFA: 7.5 mL of a protein buffer matrix, with preservative.
LPFB: 7.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-PSA antibody specific for PSA in a buffer containing human serum, with preservative. Stored capped, stable at 2-8°C until expiration date.
3. Free PSA adjustors (LPFL: 1 set. LPFH: 2 sets)
Two vials (Low and High) of lyophilized fPSA in a buffer solution, with preservative. Reconstitute each vial with 3.0 mL distilled or deionized water.
4. Other kit components supplied separately
 - a) Free PSA Sample Diluent (LPFZ)
 - b) Chemiluminescent Substrate (LSUBX)
 - c) Probe Wash Module (LPWS2)
 - d) Probe Cleaning Kit (LKPM)
 - e) Sample Cup Holders (barcoded) (LCHx-y)
 - f) Sample Cups (disposable) (LSCP)
 - g) Sample Cup Caps (optional) (LSCC)
 - h) Tumor Marker Controls (Tri-level, multi-constituent, human serum-based control) (TMCO)
 - i) Sample transfer pipets, distilled or deionized water, controls.

The IMMULITE® 2000 assay kit contains components are matched set of the following components (the barcode labels are needed for the assay):

1. Free PSA Bead Pack (L2PF12 1 pack)
Each barcode. 200 beads, coated with monoclonal murine anti-PSA antibody specific for the fPSA. Stable at 2-8°C until expiration date.
2. Free PSA Reagent Wedges (L2PFA2: 1 wedge) stable at 2-8°C until expiration date.
With barcodes, 11.5 mL of a protein buffer matrix, with preservative; 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-PSA antibody specific for PSA in a buffer containing human serum, with preservative.
3. Free PSA adjustors (L2KPF2)
Two vials (Low and High) of lyophilized fPSA in a buffer solution, with preservative. Reconstitute each vial with 3.0 mL distilled or deionized water. Stable at 2-8 °C for 30 days after reconstitution, or for 6months (aliquotted) at -20 °C.
4. Other kit components supplied separately
 - Multi-Diluent 2 (L2M2Z: 25 mL, L2M2Z4: 55 mL) with barcode labels.
 - Chemiluminescent Substrate (L2SUMB)
 - Probe Wash (L2PWSM)
 - Probe Cleaning Kit (L2KPM)
 - Reaction Tubes (disposable) (LRXT)
 - 250 Sample Diluent Test Tubes (16 x 100 mm) (L2ZT)

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- 250 Sample Diluent Tube Caps (L2ZC)
- Tri-level, multi-constituent control) (TMCO)
- Sample transfer pipets, distilled or deionized water, controls.

VI. Alternate Practices and Procedures

Alternative practices and procedures for diagnosis of prostate cancer include measurement of tPSA or complexed PSA (cPSA), digital rectal exam (DRE), ultrasonography, and biopsy of the prostate gland.

VII. Marketing History

The IMMULITE® Free PSA kit was released internationally on February 6, 1995 and the IMMULITE® 2000 Free PSA kit was released internationally on April 29, 1998.

The IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA kits have been marketed in the following countries since their respective international release dates: Argentina, Australia, Austria, Bangladesh, Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, Cyprus, Dominican Republic, Ecuador, Egypt, Finland, France, Germany, Greece, Hong Kong, Hungary, India, Israel, Italy, Japan, Jordan, Korea, Kuwait, Lebanon, Malaysia, Malta, Mexico, Netherlands, Pakistan, Peru, Philippines, Portugal, Puerto Rico, Republic of Panama, Russia, Saudi Arabia, Singapore, South Africa, Spain, Sri Lanka, Sweden, Switzerland, Syria, Taiwan, Thailand, Turkey, United Arab Emirates (UAE), United Kingdom, Uruguay and Venezuela.

These products have not been withdrawn from any of these markets for any reason.

VIII. Potential Adverse Effects of the Device on Health

The IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assay kits are for *in vitro* diagnostic use, there should be no direct adverse effect on the health of a patient. However, failure of the product to perform as intended or errors in the use of the product could lead to a false result.

Routine clinical practice for diagnosis of prostate cancer includes DRE, tPSA measurement and assessment of clinical signs, symptoms and risk factors. Thus, a false measurement of fPSA alone is less likely to have an adverse effect on health as other diagnostic tools are used in conjunction with fPSA. It is possible that a confirming biopsy of the prostate gland would or would not be ordered in error. If a biopsy is ordered in error, the health danger involved is minimal, similar to that of other minor surgeries. If the biopsy is not ordered, subsequent and regular patient evaluations with tPSA, fPSA and DRE are routine practice.

Since low ratios of free to total PSA occur in patients with benign prostatic disorders and elevated ratios are not always associated with absence of disease, assessment of patient status must not be based entirely on fPSA results. Potential adverse effects are:

- a. A falsely low ratio could lead to a medical decision causing unnecessary biopsy.
- b. A falsely elevated ratio could lead to a medical decision depriving the patient of potentially diagnostic biopsy results and subsequent treatment.

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The concentration of fPSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methodology and reagent specificity. The results reported by the laboratory to the physician must include the identity of the free and total PSA assay used for %fPSA ratio calculation. The IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 fPSA values must be used with tPSA values obtained on the same IMMULITE[®] instruments to calculate the % fPSA to tPSA ratio. The tPSA values obtained from total PSA assays on other SIEMENS MEDICAL SOLUTIONS DIAGNOSTICS instruments should not be used interchangeably with tPSA values derived from the IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 analyzer for %fPSA ratio calculation. Use of another manufacturer's total PSA assay may lead to significantly biased or misleading results, cutoffs, and cancer probabilities than represented in the expected values.

Free PSA devices are not indicated as the sole diagnostic tool to confirm the presence or absence of malignant prostate disease. Patients with confirmed prostate cancer may have serum tPSA serum levels within the normal range. Some patients with non-malignant diseases of the prostate, including benign prostatic hyperplasia (BPH), may have elevated serum tPSA levels. Therefore, serum PSA values should be used in conjunction with the information from a complete clinical evaluation including DRE or other diagnostic tests. Confirmation of prostate cancer can only be determined by prostatic biopsy.

Manipulations of the prostate including DRE, needle biopsy and transurethral resection can cause transient and often large increases in serum free and total PSA levels. Therefore, blood samples for PSA measurement should be taken before performing these procedures. Additional blood testing should be delayed at least 2 weeks to allow serum PSA to return to original levels.

IX. Summary of Pre-clinical Studies

Calibration Range and Standardization

The IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA assays are standardized to the WHO NIBSC 1st IS 96/668. The reportable range is 0.07 ng/mL to 25.0 ng/mL.

There are 11 calibrators used to establish the Master Curve for the IMMULITE family Free PSA assay at the site of manufacture. The assigned values for these calibrators are shown below.

Calibrator	Assigned Dose (ng/mL)
A	0.00
B	0.05
C	0.10
D	0.20
E	0.41
F	0.81
G	1.60
H	3.20

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I	6.60
J	13.80
K	29.00

The Master Curve and the reference counts per second (cps) for the adjustors are incorporated into the kit bar code. They have concentrations assigned by Siemens Medical Solutions Diagnostics, and are not provided to the customers. The calibration of a specific kit lot is completed at the Siemens Medical Solutions Diagnostics manufacturing site.

There are two adjustor (Low and High Adjustor) in the assay kit with target value 0.35 and 10 ng/mL. The adjustors are used to correlate the counts per second (CPS) of the IMMULITE® systems in the user's lab to those of the Master Curve and to account for the changes in reagent enzyme activities and/or operating conditions.

Performance at Low Levels

The Limit of Blank defined as the highest value that is likely to be observed (with 95.0% probability) for a sample with no analyte, was calculated as the concentration associated with two standard deviations above the mean counts per second for 20 replicates of the zero calibrator A. The Limit of Detection defined as the lowest amount of analyte in a sample that can be detected with 95% probability, was determined in accordance with CLSI EP17-A. The Limit of Quantitation (functional sensitivity) defined as the lowest concentration with 20% coefficient of variation, was determined in accordance with CLSI EP17-A. The table below summarizes results of these three parameters for the two IMMULITE® platforms.

Instrument	Limit of Blank	Limit of Detection	Limit of Quantitation
IMMULITE®/IMMULITE® 1000	0.02 ng/mL	0.07 ng/mL	0.07 ng/mL
IMMULITE® 2000	0.02 ng/mL	0.07 ng/mL	0.07 ng/mL

High dose Hook Effect

Serial dilutions of a stock solution containing the highest available analyte concentration were made to bring the analyte concentration into the assay range. Data were reviewed to determine if any of the diluted stock solution samples with concentrations higher than the highest calibrator yielded results within the assay range.

IMMULITE®/IMMULITE® 1000

The high dose hook study was conducted using 1 kit lot. No high dose hook effect was observed up to 14,555 ng/mL.

IMMULITE® 2000

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The high dose hook study was conducted. Results were similar across lots. No high dose hook effect was observed up to 15,118 ng/mL.

Interfering substances

Potential interfering substances were spiked into aliquots of patient samples. The unspiked aliquot was the control sample. Samples were chosen such that the levels of fPSA span as much of the range of the assay as possible. The IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assays had low to no detectable interference with endogenous or naturally occurring compounds tested that might be present in patient samples including bilirubin and triglycerides (Intralipids). The maximum concentrations of bilirubin and Intralipids that did not interfere with the assays were 200 mg/L and 3,000 mg/dL respectively. Hemolysis may cause significant positive bias of the free PSA results (maximum interference 26-55%) for hemoglobin concentrations of 156 mg/dL.

Cross-Reactivity

Cross Reactivity to complexed PSA

1. Analysis of PSA antibody by blotting methods (SDS-PAGE/Western Blot)

The SDS-PAGE/Western Blot analyses were performed with purified fPSA, PSA/ACT Complex, ACT, undiluted normal female serum, undiluted prostate cancer patient serum, and undiluted BPH serum. The blotted proteins and sera were probed with biotinylated capture antibody-fPSA Mab (1A5) and biotinylated PSA tracer polyclonal antibody followed by detection with Streptavidin-Alkaline phosphatase. The data indicated that:

- a) Both antibodies showed reactivity with purified fPSA and PSA/ACT complex. No apparent reactivity was seen with purified ACT. Blots of normal female serum and BPH serum did not show any obvious staining. A faint staining of what appears to be PSA/ACT complex was observed with prostate cancer serum probed with PSA tracer polyclonal antibody. There was no reactivity of the blotted proteins and sera when probed with the negative control biotinylated Vancomycin Mab.
- b) Literature review indicated in an independent study by Becker et al. (Tumor Biology 20 [Suppl. 1]:13-17, 1999), 53 anti-PSA monoclonal antibodies were evaluated by SDS-PAGE and Western Blotting for staining of purified PSA and PSA/ACT complex. Of these 53 antibodies, 9 were classified as being specific for fPSA (Stenman et al. Tumor Biology 20 [Suppl. 1]:1-12, 1999) and were designated with ISOBM codes 25, 26, 33, 68, 73, 77, 78, 80, and 85. The 1A5 monoclonal antibody used in the SIEMENS MEDICAL SOLUTIONS DIAGNOSTICS Free PSA assays were designated as ISOBM 54 which can react with both fPSA and cPSA in 50:50 ratio.
- c) It was demonstrated that all of the fPSA antibodies tested under non-reducing conditions reacted with both purified fPSA and PSA/ACT complex. The problem with SDS-PAGE and Western Blotting method was that the PSA/ACT complex was denatured during the process and thus revealed fPSA binding epitope(s) that reacted with the fPSA antibody, making the procedure ineffective for determination of possible crossreactivity of fPSA antibodies to PSA/ACT. Agarose gel should be

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used as indicated in the study by Becker et al. (Tumor Biology 20 [Suppl. 1]:13-17, 1999).

- d) On the SDS-PAGE and Western Blotting of PSA or PSA/ACT complex in patient sera, little to no staining of fPSA and cPSA was found, even with the loading of an undiluted prostate cancer patient sample with very high tPSA level (> 6000 ng/mL).

2. Liquid-phase Cross Reactivity and Interference Study #1

Additional liquid phase cross-reactivity/interference experiments were performed using a single concentration of "added" cPSA, but making 5-10 replicate determinations of the fPSA concentration and also replicate determinations in the tPSA assays on this single solution of cPSA on both IMMULITE[®] instrument platforms. Calculations for percent crossreactivity are made using the observed PSA concentrations from both the free and total PSA assays.

Complexed PSA a purified preparation of bound PSA and α -1-chymotrypsin (ACT), was obtained from Scripps Laboratories (San Diego, CA). Sizing chromatography was performed to separate any dissociated fPSA or free ACT from the cPSA preparation that is known to occur during storage. The peak cPSA fraction was then assigned a value after serial dilution into the working range of the current IMMULITE[®] Total PSA assays. Free PSA from the WHO was then mixed with cPSA in the Free PSA assay's "zero" calibrator using the following mixtures:

- 9 ng/mL cPSA
- 19 ng/mL cPSA
- 99 ng/mL cPSA
- 1 ng/mL fPSA
- 1 ng/mL fPSA : 9 ng/mL cPSA
- 1 ng/mL fPSA : 19 ng/mL cPSA
- 1 ng/mL fPSA : 99 ng/mL cPSA

Each sample was then tested in the IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA assays. Each of these samples was also tested (in duplicate with the mean taken as the final result) for tPSA. The study indicated:

- a) The observed Free/Total PSA ratio for the sample prepared with 9 parts cPSA and 1 part fPSA was 9.0% (1.06/11.72); the observed Free/Total PSA ratio for the sample prepared with 19 parts cPSA and 1 part fPSA was 5.2% (1.18/22.48) the observed Free/Total ratio for the sample prepared with 99 parts cPSA and 1 part fPSA was 1.6% (1.59/96.88). These represent deviations of 1%, 0.2% and 0.6% when the expected %fPSA was at 10%, 5% and 1% respectively.
- b) The percents recovery of fPSA from added cPSA were at 106%, 118% and 159% when the added cPSA is at 9, 19 and 99 ng/mL, respectively. However, the change in %fPSA is likely negligible for tPSA result between 4-10 ng/mL.

3. Liquid-phase Cross Reactivity and Interference Study #2

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For this study, purified PSA-ACT was obtained from Scripps Laboratories (San Diego, CA). Sizing chromatography was used to separate dissociated fPSA and free ACT from the PSA-ACT complex. The separated material was then assigned a dose value by diluting serially into the working range of the currently marketed IMMULITE[®] family of instruments total PSA assay. A constant concentration of 1 ng/mL fPSA (determined using the WHO standard) was spiked into the Free PSA assay's "zero" calibrator and mixed with either 9 ng/mL separated PSA-ACT or a comparable volume of diluent. Each sample was tested in replicates of 10 in the IMMULITE[®] 2000 Free PSA assay and the IMMULITE[®] 2000 Total PSA assay. The study showed:

- a) The observed interference from PSA-ACT was 7.6% ($1.135-1.059/1.059*100$) when 9 ng/mL PSA-ACT was added to 1 ng/mL fPSA.
 - b) The observed Free/Total PSA ratio for the sample prepared with 9 parts cPSA and 1 part fPSA is 10.3% ($1.135/10.979*100$) which represents 0.3% deviation of the fPSA if there is no crossreactivity of the anti-fPSA antibody.
 - c) As demonstrated by the spiking recovery experiment with 1 ng/mL of fPSA into zero calibrator, the free and total PSA assays have significant analytical biases of 5.9% and 31.9% respectively.
4. FDA recalculated the performance data for IMMULITE[®] 2000 fPSA concentration and %fPSA assuming that the fPSA concentration listed in the patient database as the result of approximately 10% crossreactivity was 90% of what was listed and using the proposed 20% fPSA cutoff. For fPSA, sensitivity of %fPSA was 98% and specificity was 15% while for tPSA at a cutoff of 4.02 ng/mL, sensitivity was 98.5% and specificity was 2.4%. The 95% confidence interval for specificity of %fPSA was 9.6 to 23.1%. The 95% confidence interval for the specificity of tPSA was 0.5% to 7.0%. The confidence intervals of specificity for tPSA and %fPSA did not overlap indicating that the incremental increase in specificity for %fPSA compared to specificity of tPSA alone (15.0% vs. 2.4%) was statistically significant.
5. Conclusion of the cross-reactivity studies
- a) Study #1 suggested cPSA exhibited less than 1.0% variation in the final %fPSA calculation when the amount of cPSA added varied from 9 to 99 ng/mL, fPSA fixed at 1 ng/mL and %fPSA changed from 10% to 1%.
 - b) Study #2 demonstrated that the observed recovery of fPSA in zero calibrator was 1.059 ng/mL as compared to 1.135 ng/mL when in the presence of a 9-fold excess of cPSA. This difference in recovery (7.2%) was due to crossreactivity of the anti-fPSA antibody with cPSA and resulted in a 0.3% increase in the expected %fPSA value of 10% ($1.135/10.979*100 = 10.3%$).
 - c) From the blotting experiment and published literature presented, it can be concluded that the 1A5 monoclonal antibody is not specific to fPSA. It can crossreact with cPSA in a ratio of 50:50 of free to cPSA (Becker C et al Tumor Biol 1999;20(suppl 1):13-17).

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- d) The total PSA assay has not been shown to be equimolar in reactivity to cPSA and fPSA. The crossreactivity study data indicated the total PSA assay and the free PSA assay has 31.9% and 5.9% bias respectively toward fPSA when 1 ng/mL of fPSA was spiked into the “zero” calibrator. It means that the total PSA assay would likely inflate the fPSA value than that of 1A5 monoclonal antibody used in the free PSA assay.
- e) Since the total PSA is the denominator and the fPSA is the numerator in the calculation of %fPSA, it is expected that falsely low %fPSA could arise due to greater proportion of false elevation of the total PSA measured by the total PSA assay comparing to minor degree of false elevation of fPSA derived from the free PSA assay with increase in tPSA concentration in serum. It could be expected that the observed %fPSA would be relatively stable in the assay range of tPSA of 4.0 to 10.0 ng/mL as demonstrated in Cross-reactivity Study #2 when 1 ng/mL fPSA was spiked into 9, 19, 99 ng/mL of cPSA with changes in %fPSA ratio merely at an average of 0.6%.

In summary, because of the crossreactivity of the fPSA monoclonal antibody to cPSA, %fPSA results are valid only when the tPSA is in the range of 4 to 10 ng/mL. This limitation is clearly stated in the package insert.

Reactivity to Other Cross Reactants

The IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assays were tested for cross reaction to other naturally occurring compounds that might be present in patient samples including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), ferritin, human chorionic gonadotropin (HCG), prostatic acid phosphatase (PAP), and prolactin.

Maximum concentrations of potential cross-reactants were tested (see table below) and found to have no effect on the recovery of the on the IMMULITE® 2000 Free PSA calibrator A indicating that the Free PSA assays are not affected by these compounds.

IMMULITE®/IMMULITE® 1000 and IMMULITE®2000 Cross Reactivity

Material	Maximum Concentration Not Interfering
AFP	10,000 ng/mL
CEA	100 ng/mL
Ferritin	10,000 ng/dL
HCG	100,000 mIU/mL
PAP	1.000 mg/L
Prolactin	200 ng/mL

Linearity

Assay linearity was evaluated by measuring dilutions created from two calibrators and six patient samples (fPSA concentration ranged from 0.07 to 29.0 ng/mL). For each dilution, percent of recovery was calculated. The results of the linearity study were evaluated using CLSI EP6-A in which linear and nonlinear (quadratic and cubic) models of the data were considered and the magnitudes of nonlinearity at every level for each sample were

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estimated. The IMMULITE® 1000 has been demonstrated to be linear from 0.07 ng/mL to 28.95 ng/mL with deviation from linearity not more than $\pm 11.0\%$ within this interval. The IMMULITE® 2000 has been demonstrated to be linear from 0.07 ng/mL to 29.92 ng/mL with deviation from linearity not more than $\pm 7.2\%$ within this interval.

Precision

A Reproducibility Study was performed on the IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 instruments using Siemens Medical Solutions Diagnostics's tri-level multi-constituent Tumor Marker Control (TMCO) and 5 proficiency samples. The proficiency samples contained different levels of spiked fPSA. These materials were assayed in quadruplicate (in study sites) or duplicate (in house) with each of the three lots of fPSA assay in 1 run (in study sites) or 2 runs (in house) per day for 20 days. Analysis of variance was used to estimate the within-run precision and total variance.

IMMULITE®/IMMULITE® 1000

For IMMULITE®/IMMULITE® 1000, average statistics across 3 lots and 3 sites indicated that within-run precision and total precision CV% for three levels of controls and 5 levels of human serum proficiency samples were not greater than 4.8% and 11.1%, respectively.

IMMULITE® 2000

For IMMULITE® 2000, average statistics across 3 lots and 3 sites indicated that within- and between-assay CV% for three levels of controls and 5 levels of human serum proficiency samples were not greater than 4.6% and 8.2%, respectively.

Spiked Recovery

Spiked recovery experiments test the ability of an assay to quantitatively recover added analyte. Spiked recovery experiments are not applicable to the Free PSA assay as some or all of the spiked fPSA would bind to serum proteases forming cPSA which is unrecognizable in the Free PSA assay.

Stability Testing

Sample Stability

Routine serum preparation and refrigerated (2-8°C) storage of samples for 24 hours or frozen storage at -70°C is acceptable for measurement of Free PSA.^{1,2,3} Results of testing at various storage conditions and durations are listed in the table below.

Storage Condition	Duration
+ 20°C	No Data
+ 2-8°C	2 days
- 70 °C	2 Years
Freeze-thaw	No Data

Sample shipping stability from referred subjects has not been tested for samples stored at -70°C upon receipt and then assayed later.

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Reagent and Component Stability

1. Long term shelf, stress, and accelerated stress stability

Long term shelf, stress, and accelerated stress stability studies were conducted to evaluate the effects of long-term storage and handling and temperature extremes on the IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 instruments Free PSA assay kit reagents and components. Materials used included 3 levels of the Tumor Marker Controls and 6 pools spanning the range of the assay. Results of these real time stability studies support a 360 day shelf life claim for the IMMULITE® 1000 and 2000 instruments Free PSA assay kit reagents/components stored in 2-8°C.

2. Reagent stability on analyzer

On-board reagent stability was not tested on the IMMULITE/IMMULITE® 1000 analyzer. Reagents are not refrigerated on this analyzer, user takes the reagent wedge off the instrument and returns it to refrigerated storage after use.

Reagent stability determined on the IMMULITE® 2000 Immunoassay Analyzer with two reagent kits stored continuously on board for a three month period. Three additional kits from the same lot were store at 4°C and run as controls. Both on-board kits and a control kit were adjusted with freshly reconstituted adjustors and assayed in triplicate using fresh vials of control on each test day as per IMMULITE®2000 SOP and CLSI protocol. The results supported recommendation of 90 day on-board stability.

Calibration Stability

Calibration stability for a single reagent pack was evaluated on both the IMMULITE® 1000/2000 Immunoassay Analyzers by testing human serum pools and controls covering the assay range for a 20 day period in the imprecision study. Results supported 14 days of calibration stability for a single reagent pack stored on instrument.

Upper Limit of Reference Interval

The upper limit of reference interval of the Free/Total PSA ratio was determined in 119 healthy, asymptomatic males, ≥ 50 years with no underlying diseases and absence of infection/fever for the last 2-4 weeks from one site based upon the CLSI document C28-A. The nonparametric 95th, 97.5th and 99th percentiles are shown below.

Normal/Healthy Population (N=119)	95 th Percentile	97.5 th Percentile	99 th Percentile
IMMULITE®/IMMULITE® 1000 Free PSA (ng/mL)	0.62	0.74	0.90
IMMULITE® 2000 Free PSA (ng/mL)	0.70	0.75	0.92
IMMULITE®/IMMULITE® 1000 Total PSA (ng/mL)	3.60	4.20	5.40
IMMULITE® 2000 Total PSA (ng/mL)	3.83	4.77	5.77

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Normal/Healthy Population (N=119)	95 th Percentile	97.5 th Percentile	99 th Percentile
IMMULITE [®] /IMMULITE [®] 1000 %Free PSA	37%	41%	42%
IMMULITE [®] 2000 %Free PSA	39%	47%	51%

The mean values of %fPSA by age groups are presented below:

	50-59 years (n=58)	60-69 years (n=50)	70-79 years (n=11)
IMMULITE [®] 1000 %Free PSA	19.79%	19.94%	20.82%
IMMULITE [®] 2000 %Free PSA	19.83%	20.52%	20.27%

The mean values of Free/Total PSA Ratio by ethnicity are presented below:

	Caucasian (n=82)	African- American (n=5)	Hispanic (n=2)	Asian (n=2)	Unknown (n=11)
IMMULITE [®] 1000 %Free PSA	19.99%	21.67%	20.50%	20.50%	18.45%
IMMULITE [®] 2000 Free PSA%	20.22%	22.17%	20.50%	21.00%	18.27%

Comparison of IMMULITE[®] 1000 and 2000 Analyzer Performance

Comparison of total PSA with historical (institutional) total PSA

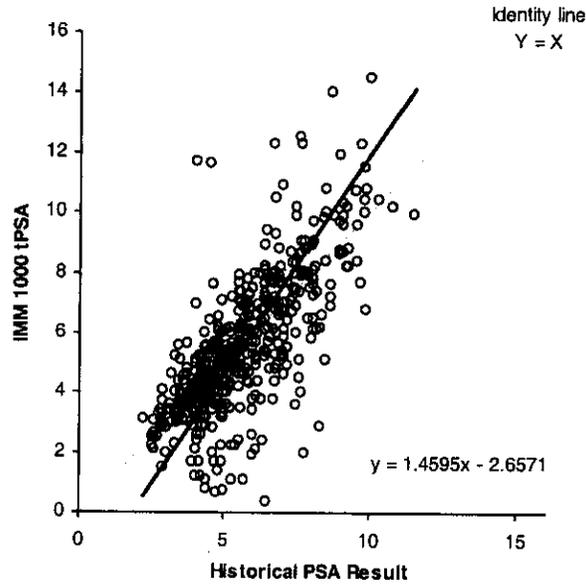
An analysis was performed to determine whether there was a systematic bias between IMMULITE[®] 1000 total PSA vs. the historical total PSA and IMMULITE[®] 2000 total PSA vs. historical total PSA used for enrolled patients of the clinical study described in Section X.

IMMULITE[®] 1000 tPSA vs. Historical PSA

N=503

Deming regression: slope = 1.46 (95% CI: 1.34 to 1.58); intercept = -2.7 (95% CI: -3.3 to -2.0)

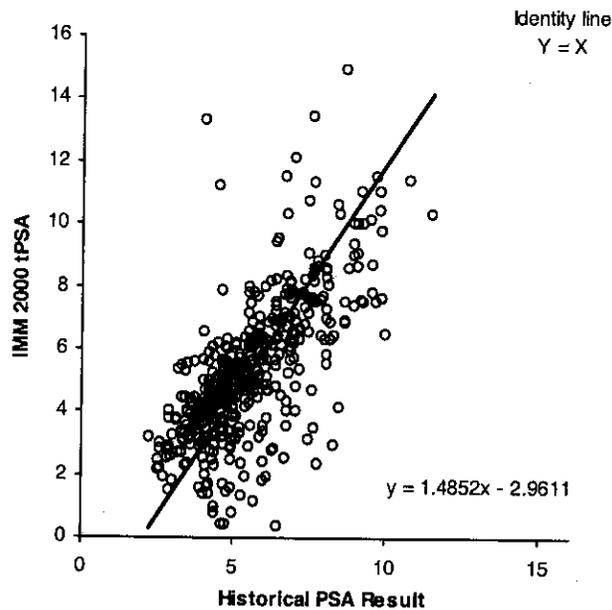
SUMMARY OF SAFETY AND EFFECTIVENESS



IMMULITE® 2000 tPSA vs. Historical tPSA

N=472

Deming regression: slope = 1.49 (95% CI: 1.35 to 1.62); intercept = -3.0 (95% CI: -3.7 to -2.2)



These data indicated that there were significant systemic biases for the IMMULITE® 1000 and 2000 platforms when compared to the historical (institutional) total PSA results.

Comparison of Free PSA and %fPSA at different sites

For comparability among IMMULITE® instruments, 680 serum samples (1 outlier excluded) from DRE positive, DRE negative results and Apparently Healthy subjects

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collected from 4 clinical sites were tested on IMMULITE® 1000 and IMMULITE® 2000.. The fPSA concentrations ranged from 0.5 to 2.8 ng/mL.

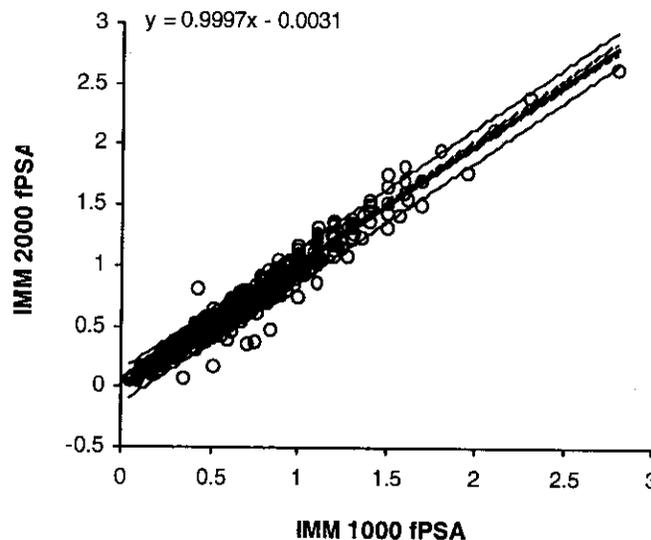
- a. Free PSA and %fPSA values with IMMULITE® 2000 vs. IMMULITE® 1000 for each site separately

Results of the Deming regression analysis (slope and intercept with 95% CI) at each site are presented in the following table:

	SITE	Free PSA	% Free PSA
IMMULITE® 2000 vs. IMMULITE® 1000	1 (University of Washington) N=194	Slope = 1.08 (1.06 to 1.09) Intercept = 0.00 (-0.00 to 0.01) (1 outlier removed)	Slope = 1.08 (1.04 to 1.11) Intercept = -0.01 (-0.02 to -0.00) (1 outlier removed)
	2 (Johns Hopkins) N=211	Slope = 0.98 (0.96 to 1.00) Intercept = -0.01 (-0.02 to 0.00)	Slope = 0.99 (0.96 to 1.02) Intercept = -0.00 (-0.00 to 0.00)
	3 (University of Virginia) N= 187	Slope = 1.05 (1.01 to 1.08) Intercept = -0.05 (-0.08 to -0.03)	Slope = 1.09 (1.06 to 1.12) Intercept = -0.00 (-0.01 to 0.00)
	4 (Michigan Institute of Urology) N=89	Slope = 1.04 (0.99 to 1.09) Intercept = -0.03 (-0.07 to 0.01)	Slope = 0.83 (0.76 to 0.90) Intercept = 0.01 (0.00 to 0.02)

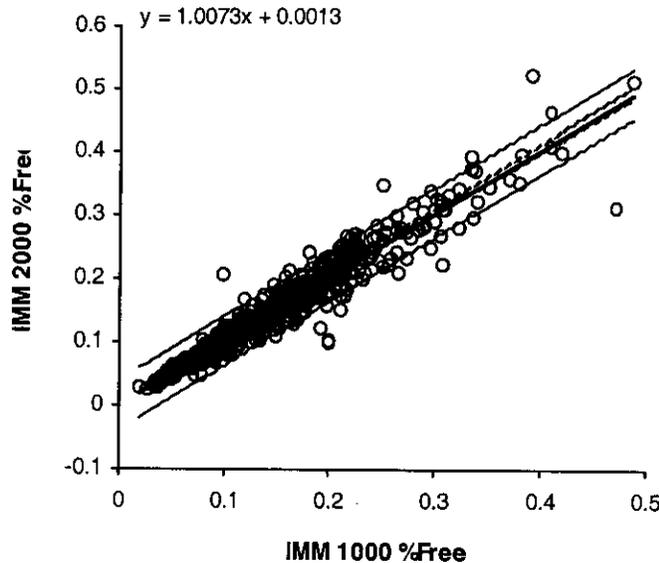
- b. Free PSA and %fPSA values with IMMULITE® 2000 vs. IMMULITE® 1000 for all sites combined

Results of the linear regression analysis of fPSA are: slope = 0.9997 with 95%CI: 0.985 to 1.01 and intercept = -0.0031 with 95%CI: -0.013 to 0.007.



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Results of the linear regression analysis of %fPSA are: slope = 1.007 (95%CI: 0.987 to 1.03) and intercept = 0.0013 (95%CI: -0.002 to 0.005).



X. Summary of Clinical Investigations

Clinical Study

Study Objective

A clinical study was undertaken to assess the clinical utility of the IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA assays for differentiating between benign prostatic conditions and prostate cancer using the ratio of fPSA to tPSA in patients with tPSA levels in the range of 4 to 10 ng/mL, and with DRE results that are not suspicious of cancer. An ultrasound-guided six-sector (or greater) needle biopsy of the prostate was required to confirm a diagnosis of cancer.

Patient Inclusion and Exclusion Criteria

Inclusion Criteria:

- Males ≥ 50 years.
- Historical/routine institutional PSA assay value between 4.0 and 10 ng/mL.
- Medical history information available to confirm and document clinical status at the time of the enrolled specimen draw.
- No evidence of acute prostatitis or urinary tract infection at the time of specimen draw.
- No personal history of a previous occurrence and treatment of prostate cancer prior to specimen draw.
- Adequate documentation of DRE no more than 6 months before specimen draw and no more than one month after. If DRE was performed before the blood draw, the blood draw must have been at least 1 week after the DRE.
- Ultrasound guided needle biopsy results of six sectors or more of the prostate gland. The biopsy must have been performed within 6 months (± 2 weeks tolerance) of the

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enrolled blood specimen. If the biopsy results were positive, the Gleason's score was recorded on the Patient History Form.

- Retrospective specimens included in the study should be ideally no more than approximately 2 years old and be stored at least at -60°C . A tolerance of 2.25 years was allowed.

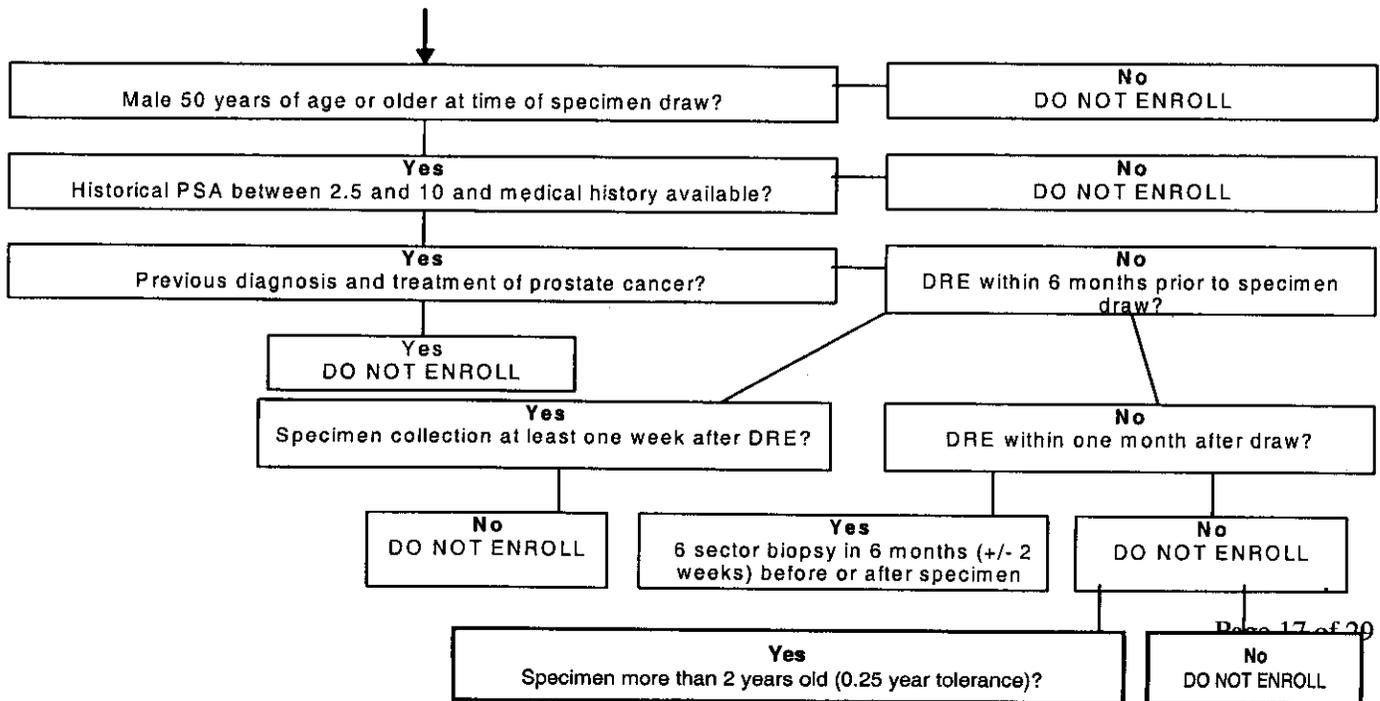
Exclusion Criteria:

- Prior transurethral resection of the prostate.
- Men on medications that might alter serum PSA concentrations.
- Men that have had prostatic needle biopsy within the previous 6 weeks of blood draw.
- Men who underwent prostatic massage or ultrasonography within the previous 3 weeks of blood draw.
- Treatment of BPH symptoms within the previous 4 weeks of blood draw.
- Inadequate documentation of TRUS guided biopsy results.

Study Design

The study was reviewed and approved by the Institutional Review Board at each participating site. Retrospective or prospectively collected serum specimens were used in this investigation. Informed consent was received for each patient specimen used in the study. Specimens included in the study were no more than approximately 2 years old and were stored at -60°C or colder. Control materials recovering in range indicated acceptance of the patient run. All patient samples were assayed in singlicate, following instructions in the respective IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA package inserts. Specimens included in the study were no more than approximately 2 years old and were stored at -60°C or colder.

Free PSA Study Inclusion Requirements



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Study Results

Patient Enrollment

The number of patients enrolled in each of the five clinical sites is shown in the following table. Of the 760 patients, 171 were excluded from the study for protocol nonconformance. Of the remaining 593 patients, 503 were DRE negative and 90 were DRE positive.

Site	# Patients DRE negative	# Patients DRE positive	Excluded
1	164	47	64
2	31	0	72
3	88	1	19
4	162	25	7
5	58	17	9
TOTAL	503	90	171

Of the 503 subjects, every subject had total and free PSA results by IMMULITE[®]/IMMULITE[®] 1000. Four hundred seventy-two (472) of these subjects had total and free PSA results by IMMULITE[®] 2000 and 31 subjects had the total and free PSA results by IMMULITE[®] 2500. Since the Free PSA assay for the IMMULITE[®] 2500 was

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not being considered for approval, samples assayed on this instrument were excluded from analyses.

The range of tPSA values among 503 subjects assayed by the IMMULITE®/IMMULITE® 1000 Total PSA was 0.34 ng/mL to 14.5 ng/mL. One hundred twenty nine (129) subjects had tPSA values less than 4.0 ng/mL and 21 subjects had tPSA values greater than 10.0 ng/mL. For the evaluation of the effectiveness of the IMMULITE®/IMMULITE® 1000 Free PSA assay, 354 subjects were available from the intended use population, i.e. tPSA values by IMMULITE®/IMMULITE® 1000 Total PSA were from 4.0 ng/mL to 10.0 ng/mL.

The range of tPSA values among 472 subjects assayed by the IMMULITE® 2000 Total PSA was 0.35 ng/mL to 14.9 ng/mL. One hundred thirty two (132) subjects had tPSA values less than 4.0 ng/mL and 19 subjects had tPSA values greater than 10.0 ng/mL. For the evaluation of the effectiveness of the IMMULITE® 2000 Free PSA assay, 321 subjects were available, i.e. tPSA values by IMMULITE® 2000 Total PSA were from 4.0 ng/mL to 10.0 ng/mL.

Patient Specimens Measured at Each Site

Patient samples were assayed using each of the IMMULITE® immunoassay analyzers for total and free PSA. The table below summarizes the distribution of the patient samples from the 5 sites tested by the different analyzers.

SITE	IMMULITE®/IMMULITE® 1000	IMMULITE® 2000
1	124	128
2	24	0
3	67	66
4	102	88
5	37	39
TOTAL	354	321

Patients being evaluated for prostate disease

The clinical study patient population included males ≥50 years with historical/routine institutional PSA value between 4 and 10 ng/mL, with (1) no evidence of acute prostatitis or urinary tract infection at the time of specimen draw; (2) no personal history of a previous occurrence and treatment of prostate cancer; (3) no prior transurethral resection of the prostate; (4) no medications that might alter serum PSA concentrations; (5) no prostatic massage or ultrasonography within the previous 3 weeks before specimen draw; (6) adequate documentation of DRE exam no more than 6 months before (but at least one week before) specimen draw and no more than one month after; (7) ultrasound guided needle biopsy results of six sectors or more of the prostate gland performed within 6 months of the enrolled blood specimen with accompanying Gleason’s score if positive.

Distribution of Age and Free PSA Results

Univariate analysis including comparison of means, medians was performed to determine if the distribution of age and Free PSA assay results differed among the populations enrolled at individual clinical sites. The table below presents results among the different site populations. The data were pooled for overall analysis.

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Site	Age (years)			Biopsy Negative Free PSA ng/mL			Biopsy Positive Free PSA ng/mL				
	Mean (95%CI)	Median (95%CI)	Range	N	Mean (95% CI)	Median (95% CI)	Range	N	Mean (95% CI)	Median (95% CI)	Range
Site 1 N=124	60.64 (59.71-61.57)	60 (59-62)	50-79	12	0.80 (0.56-1.03)	0.78 (0.49-1.11)	0.30-1.37	112	0.62 (0.57-0.68)	0.59 (0.50-0.69)	0.11-1.62
Site 2 N=24	64.25 (61.46-67.04)	63.5 (63-67)	51-79	11	0.95 (0.68-1.22)	0.99 (0.50-1.40)	0.30-1.60	13	0.82 (0.56-1.08)	0.66 (0.45-1.20)	0.42-1.80
Site 3 N=67	65.49 (63.56-67.43)	64 (62-68)	52-81	36	0.83 (0.69-0.96)	0.77 (0.61-1.00)	0.21-1.70	31	0.74 (0.60-0.87)	0.77 (0.51-0.87)	0.24-2.10
Site 4 N=102	64.84 (63.34-66.35)	65 (63-67)	50-80	70	0.84 (0.75-0.93)	0.84 (0.72-0.92)	0.18-2.80	32	0.70 (0.59-0.81)	0.67 (0.54-0.86)	0.21-1.50
Site 5 N=37	62.24 (60.12-64.37)	63 (62-67)	52-75	17	0.78 (0.59-0.96)	0.77 (0.48-1.00)	0.11-1.60	20	0.76 (0.63-0.90)	0.73 (0.58-1.00)	0.25-1.40

Comparison of Patient Age and IMMULITE®/IMMULITE® 1000 Free PSA Values among Sites

Comparison of Patient Age and IMMULITE® 2000/IMMULITE® 2500 Free PSA Values among Sites

Site	Age (years)			Biopsy Negative Free PSA ng/mL			Biopsy Positive Free PSA ng/mL				
	Mean (95%CI)	Median (95% CI)	Range	N	Mean (95% CI)	Median (95% CI)	Range	N	Mean (95% CI)	Median (95% CI)	Range
Site 1 N=128	60.55 59.64-61.47)	60.0 (59-62)	50-79	12	0.78 (0.55-1.01)	0.77 (0.45-1.05)	0.31-1.41	116	0.61 (0.55-0.66)	0.57 (0.48-0.65)	0.13-1.56
Site 2 N=26*	64.73 (62.15-67.31)	64.5 (62-67)	51-79	13	0.99 (0.70-1.27)	0.95 (0.44-1.40)	0.28-1.85	13	0.86 (0.55-1.18)	0.69 (0.41-1.30)	0.40-1.99
Site 3 N=66	65.30 (63.41-67.20)	63.5 (62-68)	52-79	35	0.84 (0.70-0.99)	0.76 (0.58-1.11)	0.21-1.83	31	0.70 (0.57-0.83)	0.70 (0.50-0.76)	0.26-2.13
Site 4 N=88	65.98 (64.32-67.63)	65 (63-68)	50-85	57	0.88 (0.77-0.99)	0.86 (0.75-1.01)	0.17-2.63	31	0.70 (0.58-0.81)	0.66 (0.51-0.84)	0.23-1.44
Site 5 N=39	62.00 (60.04-63.96)	63 (59-65)	52-75	19	0.87 (0.70-1.04)	0.91 (0.59-1.10)	0.18-1.61	20	0.77 (0.64-0.91)	0.73 (0.55-0.99)	0.27-1.37

* The study data were measured with IMMULITE® 2500 analyzer and excluded from final analysis.

Summary of Clinical Performance

Receiver Operating Curve (ROC) Analysis

Using the biopsy confirmed clinical diagnosis of prostate cancer vs. no prostate cancer as the reference standard, the receiver operator characteristic (ROC) analysis was used to evaluate the clinical performance of the %FPSA.

For demonstration of effectiveness of %FPSA,

- a. It should be demonstrated that %FPSA is at least informative, i.e. better than a random test. It should be demonstrated that the true positive rate (TPR) of %FPSA with the

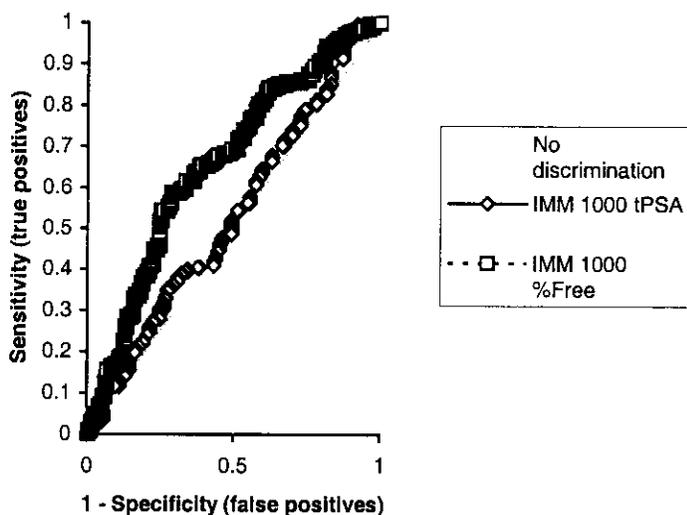
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appropriate cutoff is statistically higher the false positive rate (FPR) (the same as $TPR > FPR$, $Se > 1 - Sp$ or $Se + Sp > 1$)

- b. Note that if the biopsy procedure will be performed on every subject from the population of subjects with total PSA value of 4.0 -10.0 ng/mL and DRE negative results, then sensitivity will be 100% and specificity will be 0%. Every subject from this population has quantitative value of total PSA and the quantitative total PSA test values with cutoff higher than 4.0 will improve specificity (more than 0%) and decrease sensitivity (less than 100%). Therefore in addition, it should be demonstrated an improvement in free/total PSA (%fPSA) specificity over the specificity of the total PSA test when the sensitivities of %fPSA and the total PSA are the same.

IMMULITE®/IMMULITE® 1000 %fPSA

In the dataset, there were 354 subjects with IMMULITE®/IMMULITE® 1000 total PSA values of 4.0 -10.0 ng/ml and negative DRE. Among them, 208 subjects had positive biopsy results and 146 subjects had negative biopsy results. The ROC curves for total PSA and %fPSA are presented below.



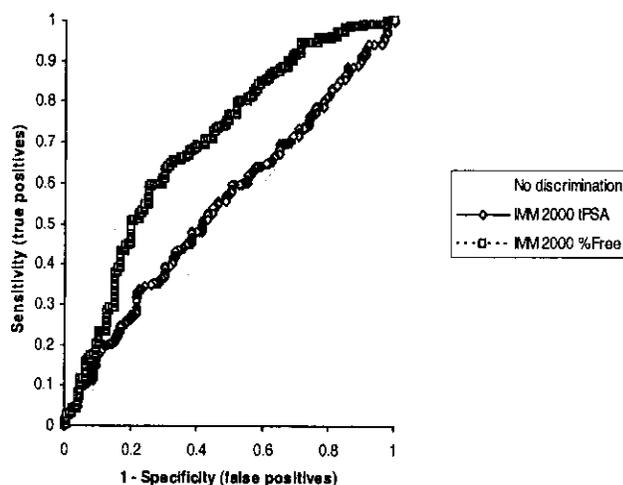
- a. Whether %fPSA measured by IMMULITE®/IMMULITE® 1000 was informative.
- The area under ROC curve for %fPSA was 0.656 with 95% CI: 0.598 to 0.714.
 - In the particular region of interest (sensitivity=95%), the %fPSA was informative. For the cutoff of 20%, sensitivity was 95.2% (198/208) and specificity was 15.8% (23/146). The difference between $TPR=95.2\%$ and $FPR=84.2\%$ was statistically significant. Difference between TPR and FPR was +11.0% with 95% CI: 4.7% to 18.3%.
- b. Whether %fPSA specificity is higher than tPSA specificity when sensitivities are the same.
- Area under ROC curves for %fPSA and tPSA were 0.656 and 0.533 respectively. The increase in areas under ROC curves was statistically significant.

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- For the level of sensitivity of 95%, specificity of %fPSA was 15.8% (23/146) with 95% CI: 10.3% to 22.7% and specificity of tPSA was 11.0% (16/146) with 95% CI: 6.4% to 17.2%. The improvement in specificities (15.8% vs 11.0%) was not statistically significant (calculated by bootstrap).
- For the level of sensitivity of 85%, specificity of %fPSA was 36.3% (53/146) with 95% CI: 28.5% to 44.7% and specificity of tPSA was 17.1% (25/146) with 95% CI: 11.4% to 24.2%. The improvement in specificities (36.3% vs 17.1%) was statistically significant.

IMMULITE[®] 2000 %fPSA

In the dataset, there were 321 subjects with IMMULITE[®] 2000 total PSA values of 4.0 - 10.0 ng/ml and negative DRE. Among them, 198 subjects had positive biopsy results and 123 subjects had negative biopsy results. The ROC curves for total PSA and %fPSA are presented below.



- Whether %fPSA measured by IMMULITE[®] 2000 was informative.
 - The area under ROC curve for %fPSA was 0.696 with 95% CI: 0.636 to 0.756.
 - In the particular region of interest (sensitivity=95%), the %fPSA was informative. For the cutoff of 20%, sensitivity was 95.5% (189/198) and specificity was 23.6% (29/123). The difference between TPR=95.5% and FPR=76.4% was statistically significant. Difference between TPR and FPR was +19.1% with 95% CI: 11.4% to 27.8%.
- Whether %fPSA specificity is higher than tPSA specificity when sensitivities are the same.
 - Area under ROC curves for %fPSA and tPSA were 0.696 and 0.547 respectively. The increase in areas under ROC curves was statistically significant.
 - For the level of sensitivity of 95%, specificity of %fPSA was 23.6% (29/123) with 95% CI: 16.4% to 32.1% and specificity of tPSA was 3.3% (4/123) with 95% CI: 0.9% to 8.1%. The improvement in specificities (23.6% vs 3.3%) was statistically significant.

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- For the level of sensitivity of 85%, specificity of %fPSA was 39.8% (49/123) with 95% CI: 31.1% to 49.1% and specificity of tPSA was 17.1 % (21/123) with 95% CI: 10.9% to 24.9%. The improvement in specificities (39.8% vs 17.1%) was statistically significant.

Summary of Clinical Performance of Free/Total PSA Ratio with cutoff of 20%

	IMMULITE[®]/IMMULITE[®] 1000	IMMULITE[®] 2000
N	354	321
Sensitivity (Se)	95.2% (198/208), 95% CI: 91.3% to 97.7%	95.5% (189/198), 95% CI: 91.6% to 97.9%
Specificity (Sp)	15.8% (23/146), 95% CI: 10.3% to 22.7%	23.6% (29/123), 95% CI: 16.4% to 32.1%
Se +Sp	111.0% lower limit of CI is 104.7% p-value = 0.0004	119.1% Lower limit of CI: 111.4% p-value=0.0000
Prevalence	58.8% (208/354)	61.7% (198/321)
NPV	69.7% (23/33)	76.3% (29/38)
PPV	61.7% (198/321)	66.8% (189/283)
AUC of tPSA	0.533	0.547
AUC of %fPSA	0.656	0.696
Difference in AUC	Statistically significant	Statistically significant

The percentage of biopsy reduction using the IMMULITE[®] systems is comparable to published results (20%)¹.

Results of these studies support measurement of fPSA in patients with tPSA values between 4 and 10 ng/mL and non-suspicious DRE to determine the %fPSA to be used as an aid in distinguishing prostate cancer from benign disease.

Performance of %fPSA (sensitivity and specificity and Negative and Positive Predictive Values) with the cutoff of 20% at each site

IMMULITE[®]/IMMULITE[®] 1000

Site	Prevalence of positive biopsy	Sensitivity (Se) of %fPSA	Specificity (Sp) of %fPSA	Sum of Se and Sp
1 N=124	90.3% (112/124)	98.2% (110/112)	8.3% (1/12)	106.5%
2 N=24	54.2% (13/24)	92.3% (12/13)	0% (0/11)	92.3%
3 N=67	46.3% (31/67)	93.5% (29/31)	16.7% (6/36)	110.2%
4 N=102	31.4% (32/102)	87.5% (28/32)	18.6% (13/70)	106.1%
5 N=37	54.1% (20/37)	100% (20/20)	11.8% (2/17)	111.8%
Total N=354	58.8% (208/354)	95.7% (199/208)	15.1% (22/146)	110.8% 90% CI: 105.6% to 116.7%

The data indicated Site #2 with 24 subjects showed lower performance than other sites.

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Site	Prevalence of positive biopsy	NPV of %fPSA	PPV of %fPSA	% subjects positive by %fPSA
1 N=124	90.3% (112/124)	33.3% (1/3)	90.9% (110/121)	97.6% (121/124)
2 N=24	54.2% (13/24)	0% (0/1)	52.2% (12/23)	95.8% (23/24)
3 N=67	46.3% (31/67)	75.0% (6/8)	49.2% (29/59)	88.1% (59/67)
4 N=102	31.4% (32/102)	76.5% (13/17)	32.9% (28/85)	83.3% (85/102)
5 N=37	54.1% (20/37)	100% (2/2)	57.1% (20/35)	94.6% (35/37)
Total N=354	58.8% (208/354)	71.0% (22/31)	61.6% (199/323)	91.2% (323/354)

In this clinical study, clinical site#1 had a prevalence of prostate cancer of 90.3%. Since NPV depends on the prevalence of disease in the intended use population, NPV for sites with such a high prevalence is relatively low. The usefulness of the %fPSA for the populations with high prevalence of prostate cancer is of concern because a) almost all subjects are positive by %fPSA (only around 3% of subjects are negative by %fPSA); and b) a risk to be free of prostate cancer for these 3% of subjects with negative %fPSA (NPV) is very low (only 33% at this site).

IMMULITE® 2000

Performance of IMMULITE® 2000 %fPSA with cutoff =20% (sensitivity, specificity, positive and negative predictive values) at each sites was similar to the performance of IMMULITE®/IMMULITE® 1000 %fPSA.

Sensitivity and specificity of the %fPSA for with the cutoff of 20.0% stratified for different ranges of tPSA

IMMULITE®/IMMULITE® 1000

	Ranges of IMMULITE®/IMMULITE® 1000 Total PSA values (n=354)				
	4.0-5.0	5.01-6.0	6.01-8.0	8.01-10.0	Total
%positive biopsies	55.1% (59/107)	63.8% (60/94)	56.4% (62/110)	62.8% (27/43)	58.8% (208/354)
Sensitivity of %FPSA	94.9% (56/59)	93.3% (56/60)	98.4% (61/62)	96.3% (26/27)	95.7% (199/208)
Specificity of %FPSA	22.9% (11/48)	20.6% (7/34)	8.3% (4/48)	0.0% (0/16)	15.1% (22/146)

IMMULITE® 2000

	Ranges of IMMULITE® 2000 Total PSA values (n=321)				
	4.0-5.0	5.01-6.0	6.01-8.0	8.01-10.0	Total
%positive biopsies	59.5% (66/111)	56.9% (41/72)	65.1% (69/106)	73.0% (27/37)	61.7% (198/321)
Sensitivity of	93.9%	95.1%	95.7%	96.3%	95.5%

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%fPSA	(62/66)	(39/41)	(66/69)	(26/27)	(189/198)
Specificity of %fPSA	31.1% (14/45)	25.8% (8/31)	16.2% (6/37)	10.0% (1/10)	23.6% (29/123)

Among the four ranges of tPSA values, the highest specificity of %fPSA was observed for the subjects with tPSA values of 4.0 – 5.0 ng/mL (22.9% for IMMULITE®/IMMULITE® 1000 and 31.1% for IMMULITE® 2000) and the lowest specificity was observed for the subjects with tPSA values of 8.0-10.0 ng/mL (0% for IMMULITE®/IMMULITE® 1000 and 10.0% for IMMULITE® 2000)

Performance of %fPSA for different cutoffs

The risk of finding a positive biopsy for the different cutoff values of %fPSA (Positive Predictive value) is presented below.

Patients have %fPSA below	IMMULITE®/IMMULITE® 1000			IMMULITE® 2000		
	Positive Predictive Value	% Cancers Correctly Identified	% Non-Cancers Correctly Identified	Positive Predictive Power	% Cancers Correctly Identified	% Non-Cancers Correctly Identified
10.0	73.7%	48.6%	75.3%	79.3%	48.5%	79.7%
16.0	65.8%	82.2%	39.0%	69.9%	83.3%	42.3%
20.0	61.7%	95.2%	15.8%	66.8%	95.5%	23.6%
24.4	59.4%	99.0%	3.4%	63.4%	99.0%	8.1%
29.6	59.3%	100.0%	2.1%	62.3%	100%	2.4%

Probability of Finding Prostate Cancer on Needle Biopsy for Different Ranges of %fPSA

The table below shows the probability of finding prostate cancer on needle biopsy for the cohort of men 50 years of age or older with a total PSA value between 4.0 – 10.0 ng/mL and rectal examination findings not suspicious for cancer for different ranges of %fPSA.

%fPSA	IMMULITE®/IMMULITE® 1000 % (95% CI)	IMMULITE® 2000 % (95% CI)
< 10.0	74 (65.8-81.0)	80 (71.3-86.3)
10.0-15.99	57 (47.3-65.6)	60 (50.1-68.7)
16.0-19.99	44 (31.9-57.5)	51 (36.1-65.9)
20.0-24.99	28 (12.1-49.4)	27 (11.6-47.8)
> 0.25	33 (4.3-77.7)	17 (2.1-48.8)
All	59.4 (53.4-63.9)	62 (56.1-67.0)

Probability of Finding Prostate cancer for Different Ranges of %fPSA Stratified by Age

Since age is a factor in the development of cancer, the table below is stratified by age decade (50-59, 60-69, >70). Logistic regression analysis did not demonstrate that age was an independent statistically significant predictor of prostate cancer in the data set of this clinical study. The data did not demonstrate that for the same range of %fPSA values, probability of finding positive biopsy is increasing for the older subjects.

fPSA/tPSA Ratio(%)	Age Groups					
	IMMULITE®/IMMULITE® 1000			IMMULITE® 2000		
	50-59	60-69	70+	50-59	60-69	70+
<=10	76%	70%	81%	82%	77%	79%

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10-15.99	58%	58%	52%	59%	63%	52%
16-19.99	67%	43%	23%	64%	58%	25%
20-24.99	0%	43%	14%	50%	25%	0%
>25	100%	0%	25%	50%	0%	14%

XI. Conclusions Drawn from the Studies

Risk Benefit Analysis

Cancer of the prostate is the most common noncutaneous cancer diagnosis among American men and is the second leading cause of their cancer mortality⁴ (Weir et al., 2003). As such, prostate cancer burden poses a significant public health problem in the United States. The cause of prostate cancer is unknown. The benefits of screening procedures for prostate cancer using PSA, free PSA, and digital rectal exam include early diagnosis, decrease in health care costs of advanced stage cancer, and decrease in mortality. Risks of prostate cancer screening include minimal complications that can occur with venous blood draw such as hematoma, and false negative and false positive assay results. Medical decisions are not based on free PSA or the free/total PSA ratio alone. Total PSA, digital rectal exam, ultrasonography and other clinical signs and symptoms are used. A falsely low free PSA result (low %fPSA is associated with cancer) may lead to the clinical decision to perform unneeded prostate biopsy. Prostate biopsy is a relatively minor surgery and has minimal health risks associated with the procedure. A falsely high free PSA result (high %fPSA is associated with benign conditions) could lead to a medical decision to avoid prostate biopsy. Prostate cancer is a slow growing disease. It is routine clinical practice to perform subsequent and regular patient evaluations of clinical signs/symptoms, total PSA, free PSA and DRE. Therefore, the risks associated with this device are:

- The risk associated with venipuncture, and
- The risk that interpretation of a free PSA/total PSA ratio would subject the patient to unnecessary biopsy or deprive the patient of a medical treatment.

There is a substantial risk of an unnecessary biopsy for men without cancer when total PSA is between 4 and 10 ng/mL and DRE results are not suspicious for cancer (percentage of subjects without prostate cancer was approximately 40% in this clinical study).

The benefit of the device is the increased specificity offered by the use of the free PSA/total PSA ratio when used to determine whether patients with total PSA between 4-10 ng/mL and DRE results not suspicious for cancer should receive biopsies. With the free/total PSA ratio cutoff of 20% for IMMULITE[®]/IMMULITE[®] 1000, 15.1% (from 10% up to 23%) of men without prostate cancer could be spared unnecessary biopsy and have 95% of cancers correctly identified. With the free/total PSA ratio cutoff of 20% for IMMULITE[®] 2000, 23.6% (from 16% up to 32%) of men without prostate cancer could be spared unnecessary biopsy and have 95% of cancers correctly identified. As a consequence, fewer men would be given unnecessary biopsies as well as possible medical complications (infection, bleeding, urinary retention, and hospitalization).

Safety and Effectiveness

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Based upon the results of the pre-clinical and clinical studies, the IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assays, when used according to the provided directions and in conjunction with digital rectal exam and measurement of total PSA, is safe and effective for the intended use.

The IMMULITE®/IMMULITE® 1000 Free PSA assay is intended for use as follows: For *in vitro* diagnostic use with the IMMULITE®/IMMULITE® 1000 Analyzer for the quantitative measurement of free prostate-specific antigen (PSA) not bound to α 1-antichymotrypsin or other binding proteins (uncomplexed) in human serum. Measurement of Free PSA is used in conjunction with IMMULITE®/IMMULITE® 1000 Total PSA to determine the percent Free PSA. The percent Free PSA is used as an aid in discriminating prostate cancer from benign disease in men 50 years or older with total PSA values between 4 and 10 ng/mL and digital rectal exam (DRE) findings not suspicious of cancer. Prostate biopsy is required for the diagnosis of prostate cancer.

The IMMULITE® 2000 Free PSA assay is intended for use as follows: For *in vitro* diagnostic use with the IMMULITE® 2000 Analyzer for the quantitative measurement of free prostate-specific antigen (PSA) not bound to α 1-antichymotrypsin or other binding proteins (uncomplexed) in human serum. Measurement of Free PSA is used in conjunction with IMMULITE® 2000 Total PSA to determine the percent Free PSA. The percent Free PSA is used as an aid in discriminating prostate cancer from benign disease in men 50 years or older with total PSA values between 4 and 10 ng/mL and digital rectal exam (DRE).

The Free PSA/Total PSA ratio produced by measuring free PSA and total PSA on the IMMULITE® systems demonstrates clinical utility for use in discriminating between prostate cancer and benign diseases of the prostate for total PSA at 4.0 to 10.0 ng/mL. The evaluation demonstrated reduction in the number of unnecessary biopsies over use of total PSA alone by improving specificity of the total PSA assay.

Limitations of the clinical and analytical studies:

1. The submitted data revealed that 92% of the subjects might have had a biopsy either on the sampling date or within 180 days prior to the serum specimen collection date. The serum sample was collected (to perform the tPSA and fPSA assays) after a decision to go to biopsy had been made. The accrual order may have caused sampling bias in intended use population leading to spectrum bias.
2. Subjects of this clinical study can be different from the intended use population of subjects with total PSA values of 4.0 - 10.0 ng/mL and negative DRE because decision about biopsy was already made based on other test results and risk factors, therefore, the study results may be difficult to generalize.
3. The inclusion criteria had used histological information such as previous biopsy results and institutional total PSA assay in the study sites to determine the eligibility into the study. Heterogeneity in the institutional total PSA in terms of the make, types and timing could lead to uncertainties in interpretation and questions of comparability

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of the % fPSA results when they are calculated from different tPSA values other than that of the tPSA assay from Siemens Medical Solutions Diagnostics.

4. Disease prevalence in the study group is higher than that would be seen in actual clinical settings, therefore, the probability of positive biopsy findings (positive predictive value) for %fPSA ratio may be misleading if the test is used in patient population having lower disease prevalence.
5. The anti-total PSA antibody in the IMMULITE[®] family of instruments total PSA assay kit has not been demonstrated as being in 1:1 ratio (i.e. non-equimolarity) in reactivity to complexed PSA and free PSA. The comparison of the IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Total PSA assays to historical or institutional total PSA assays in this study indicated the total PSA values derived from the IMMULITE[®] assays have noticeable systemic bias. In addition, the capturing monoclonal antibody (1A5) in the IMMULITE[®]/IMMULITE[®] 1000 and IMMULITE[®] 2000 Free PSA assays can cross react with free PSA. Therefore, use of the IMMULITE[®] free PSA values with non-IMMULITE total PSA values to calculate %fPSA ratio may result in false and misleading results, cutoffs, and cancer probabilities than represented in the expected values.

The limitations of the clinical and analytical studies described above were addressed in the package insert with addition of the following:

1. The concentration of free PSA in a given specimen determined with different assays can vary due to the differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Free and total PSA values should be obtained using assays from the same manufacturer.
2. Samples should be obtained before biopsy, prostatectomy or prostatic massage.
3. The results of the IMMULITE[®]/IMMULITE[®] 1000 and 2000 Free PSA assay are only valid when total PSA is in the range of 4 to 10 ng/mL.
4. The assay was studied in a population of 354 patients where 81% were 50–69 years of age and 59% had positive biopsies for prostate cancer. The prevalence of prostate cancer in the patients accrued for the clinical study is higher than that which occurs in the general population. Clinical performance results may not be the same in a population with lower prevalence of prostate cancer.
5. A sample containing approximately 1 ng/mL Free PSA was spiked with approximately 9 ng/mL complexed PSA (PSA complexed to alpha-1 antichymotrypsin). The difference in the IMMULITE family Free PSA assay result before spiking complexed PSA (1.06 ng/mL) and after spiking complexed PSA (1.14 ng/mL) was 7.5%.

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Scientific Evidence

This evaluation was a well-controlled investigation conducted in accordance with the FDA Guidance for Industry E6 Good Clinical Practice Consolidated Guidance; International Conference on Harmonization and FDA 21 CFR 58 Good Laboratory Practice. Results are believed to be valid scientific evidence of the clinical utility of IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assays.

Safety and Effectiveness

Based upon the results of the pre-clinical and clinical studies, the IMMULITE®/IMMULITE® 1000 and IMMULITE® 2000 Free PSA assays, when used according to the provided directions and in conjunction with digital rectal exam and measurement of total PSA, is safe and effective for the stated intended use.

XII. Panel Recommendation - None

XIII. CDRH Decision

FDA issued an approval order on May 11, 2007

The applicant's manufacturing facility was inspected on March 20 to April 4, 2006 and was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

XIV. Approval Specifications

Directions for use: See the labeling.

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the Approval Order.

Postapproval Requirements and Restrictions: See Approval Order.

XV. References Cited

1. Woodrum D, York L. Two-year stability of free and total PSA in frozen serum samples. *Urology*;1998 Aug;52(2):247-251.
2. Jacobs DS, Grady HD, editors. *Laboratory Test Handbook*, 4th ed. Hudson (Cleveland): Lexi-Comp Inc., 1996;193.
3. Woodrum D, French C, Shamel LB. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions. *Urol*;1996 (48 6A Suppl):33-39.
4. Weir HK, Thun MJ, Hankey BF, Ries LA, Howe HL, Wingo PA, Jemal A, Ward E, Anderson RN, Edwards BK. Annual report to the nation on the status of cancer, 1975-2000, featuring the uses of surveillance data for cancer prevention and control. *J Natl Cancer Inst*. 2003 Sep 3;95(17):1276-1299.

¹ Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease. *JAMA* 1998;279(19):1542-1547.