VIDAS® Total Prostate Specific Antigen (TPSA)

IVD

VIDAS TPSA is intended for use with a VIDAS (VITEK ImmunoDiagnostic Assay System) instrument as an automated enzyme-linked fluorescent immunoassay (ELFA) for the quantitative measurement of total prostate specific antigen in human serum. The VIDAS TPSA assay is indicated as an aid in the management of patients with prostate cancer and as an aid in the detection of prostate cancer in conjunction with digital rectal examination (DRE) in men age 50 years or older. Prostate biopsy is required for diagnosis of prostate cancer.

SUMMARY AND EXPLANATION

Prostate-specific antigen (PSA) is a glycoprotein which belongs to the kallikrein family. PSA has a molecular weight of 30,000 daltons.

PSA is principally produced by the glandular epithelium of the prostate, and is secreted in the seminal fluid. PSA is also present in urine and blood. PSA acts on seminal fluid to fluidify and increase sperm mobility.

PSA levels rise in prostatic pathologies such as benign prostatic hyperplasia (BPH) or prostate cancer. Testing for PSA and its evolution is useful for monitoring and controlling the efficacy of prostatic carcinoma therapy.

PSA is present in blood with three main forms. The most important immunoreactive form is PSA bound to Alpha-1-antichymotrypsin (PSA-ACT). Free PSA is the other immunoreactive form present in serum. Equimolar PSA assays detect the bound form (PSA-ACT) and the free form in the same manner. The VIDAS TPSA assay is an equimolar test.

The third form of PSA, bound to alpha-2-macroglobulin, cannot be detected.

Determination of PSA levels enables the detection of the onset of metastases or the persistence of disease following prostate cancer therapy. An elevated PSA level after therapy or a persistently high level during therapy indicates residual or recurrent disease.

PRINCIPLE

The assay principle combines a two step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay.

Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The sample is cycled in and out of the SPR several times. This operation enables the antibody fixed onto the interior wall of the SPR to capture the prostate specific antigen present in the sample. Unbound components are eliminated during the washing steps. Alkaline phosphatase labeled antibody is then incubated in the SPR where it binds with the prostate specific antigen. Unbound conjugate is then eliminated during the washing steps.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR.

The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of prostate specific antigen present in the sample.

At the end of the assay, results are automatically calculated by VIDAS in relation to the calibration curve stored in memory, and then printed out.

KIT COMPOSITION (60 TESTS) :

60 TPSA strips	STR	Ready-to-use.
60 TPSA SPRs 2 x 30	SPR	Ready-to-use. Interior of SPR coated with monoclonal anti-PSA immunoglobulins (mouse).
TPSA Control 1 x 2 ml (lyophilized)	C1	Reconstitute with 2 ml of distilled water. Let stand for 30 minutes, then mix. Stable after reconstitution for 24 hours at 2-8°C or until the expiration date on the kit at -25 ± 6°C. 5 freeze/thaw cycles are possible. Human serum* + human PSA + 0.1 g/l sodium merthiolate. The range in ng/ml is indicated on the vial label.
TPSA Calibrator 2 x 2 ml (lyophilized)	S1	Reconstitute with 2 ml of distilled water. Let stand for 30 minutes, then mix. Stable after reconstitution for 24 hours at 2-8°C or until the expiration date on the kit at -25 ± 6°C. 5 freeze/thaw cycles are possible. Human serum* + human PSA + 0.1 g/l sodium merthiolate. The titer in ng/ml is indicated on the vial label.
TPSA diluent 1 x 8 ml (liquid)	R1	Ready-to-use. Calf serum + 0.9 g/l sodium azide.
MLE card		Specifications sheet containing the factory master calibration data required to calibrate the test.
1 Package Insert		

* This product has been tested and shown to be negative for HBs antigen, and antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

Description

The SPR

The interior of the SPR is coated during production with monoclonal anti-PSA antibodies (mouse). Each SPR is identified. Only remove the required number of SPRs from the pouch. Make sure the pouch is well closed after opening.

The Reagent Strip

The polypropylene strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which indicates the type of test carried out, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The four wells in the center section of the strip contain the various reagents required for the assay.

Description of the TPSA Reagent Strip :

Wells	Reagents
1	Sample well.
2 - 3 - 4 - 9	Empty wells.
5	Conjugate : Alkaline phosphatase labeled monoclonal anti-PSA immunoglobulins (mouse) + 0.9 g/l sodium azide (400 µl).
6 - 7	Wash buffer : Tris (0.05 mol/l, pH 7.4) + NaCl (0.4 mol/l) + Tween (0.05 %) + 0.9 g/l sodium azide (600 µl).
8	Diluent : Tris (0.1 mol/l) + NaCl (0.1 mol/l) + calf serum (5 %) + 0.9 g/l sodium azide (400 µl).
10	Reading cuvette with substrate : Diethanolamine DEA* (0.62 mol/l or 6.6%, pH 9.2) + 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + 1 g/l sodium azide (300 µl).

*** IRRITANT reagent :**

- **R 36** : Irritating to eyes.
- **S 26** : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip calibrated to dispense 200 µl.
- Powderless, disposable gloves.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.

1. **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety manual – 1993, 2 nd edition WHO Geneva).**
2. **This kit contains products of animal origin. It is therefore recommended that they be treated as potentially infectious and handled observing the usual safety precautions.**
3. Do not use reagents after the expiration date indicated on the label.
4. Do not mix reagents (or disposables) from different lots.
5. Do not use the SPRs if the pouch is pierced.
6. Do not use visibly deteriorated SPRs (damaged foil or plastic).
7. When the analysis is completed, remove the used SPRs and strips, and dispose of them appropriately (i.e autoclaving). All other contaminated material such as disposable gloves and pipette tips should be disposed of in a similar manner.
8. Use **powderless gloves**, as powder has been reported to cause false results for certain enzyme immunoassay tests.
9. Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
10. The Optical Cuvette with Substrate (well 10) contains an irritant agent (diethanolamine). Refer to the risk sentence "R" and the precautions "S" above.
11. Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5 % sodium hypochlorite. See the VIDAS Operator's Manual for cleaning spills on or in the VIDAS instrument. Do not autoclave solutions containing bleach.
12. The VIDAS and mini-VIDAS instruments should be regularly cleaned and decontaminated (see the VIDAS Operator's Manual).

STORAGE

- Store the VIDAS TPSA kit at 2-8°C.
- **Do not freeze SPRS and strips.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all the components are stable until the expiration date indicated on the label.

SAMPLE COLLECTION

Human serum. It is recommended to validate collection tubes before use as some contain substances which interfere with test results. Samples can be stored at 2-8°C for a maximum of 24 hours; if longer storage is required, freeze at -25 ± 6°C. Avoid successive freezing and thawing. Samples containing impurities must be centrifuged before analysis.
None of the following factors have been found to significantly influence this assay: hemolysis (5 mg/mL), lipemia (5 mg/mL), or bilirubinemia (0.6 mg/mL). However, it is recommended not to use clearly hemolyzed, lipemic or icteric samples, and if possible to collect a new sample.

INSTRUCTIONS FOR USE

MASTER LOT DATA ENTRY

Before each new lot of reagents is used, specifications (or factory master calibration curve data) must be entered into the instrument (VIDAS or mini-VIDAS) using the master lot entry (MLE) card (specifications sheet) included in each kit. If this operation is not performed **before initiating the tests**, the protocol will not run. The master lot data need only be entered once for each lot. It is possible to enter data automatically using the MLE card or manually.
For complete instructions, see the VIDAS or mini-VIDAS Operator's Manual.

CALIBRATION

Calibration, using the calibrator provided in the kit, must be performed upon receipt of a new lot of reagents after the master lot data has been entered. Recalibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested in **duplicate** (see VIDAS Operator's Manual). The calibration value must be within the set RFV (Relative Fluorescence Value) range or the mean will not be stored in memory, if this is not the case, recalibrate.
VIDAS TPSA has been calibrated using the Stanford reference standard. This standard contains 90% of PSA-ACT and 10% of free PSA.

PROCEDURE

1. **Remove the kit from the refrigerator and allow it to come to room temperature for at least 30 minutes.**
2. Remove one TPSA strip and one TPSA SPR from the kit for each sample, control or calibrator to be tested. **Make sure the storage pouch has been resealed after the required SPRs have been removed.**
3. Place the TPSA strip and SPR on the VIDAS Preparation/Loading tray.
4. Enter the appropriate assay and patient data using the keyboard to create a Work List (code TPSA). Type "TPSA" to enter the code and indicate the number of tests to be run. If the calibrator should be tested, enter "S1" for the sample identification. The calibrator should be tested **in duplicate** if it is to be stored in memory (see Operator's Manual). If the control needs to be tested, it should be identified by C1.
5. Mix the samples, the calibrator and/or the control with a vortex.
6. Pipette **200 µl** of sample, calibrator, or control into the sample well (Note: the samples and the control are tested singly).
7. Insert the VIDAS SPRs and strips into the positions indicated on the screen. Check to make sure the color labels with the three letter assay code on the SPRs and the reagent strips match.
8. Initiate the assay processing as directed in the VIDAS Operator's Manual. All the assay steps are performed automatically by the instrument. Results are obtained within approximately 60 minutes.
9. After the assay is completed, dispose of the used SPRs and strips into an appropriate recipient.

QUALITY CONTROL

A control is included in each VIDAS TPSA kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each recalibration must also be checked using this control. The instrument will only be able to check the control value, if the control is identified by C1 and the calibrator by S1.
Results cannot be validated if the control value deviates from the expected values.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the cuvette and substrate before the substrate is introduced into the SPR. The second reading is taken after the substrate in the SPR has been incubated. The RFV is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
The results are automatically calculated by the instrument using calibration curves which are stored by the VIDAS (4-parameter logistic model); the concentrations are expressed in ng /mL. Samples with TPSA test value concentration > 100 ng/mL should be reassayed after diluting with TPSA diluent. If the dilution factor has not been entered when the Work List was created (see Operator's Manual), multiply the result by the dilution factor to obtain the sample concentration.

TPSA test value ≥ 4.00 ng/mL is suspicious for prostate cancer. See ASSAY LIMITATIONS.

ASSAY LIMITATIONS

Samples collected from patients receiving mouse monoclonal antibody preparations for diagnostic or therapeutic purposes may contain human anti-mouse antibodies (HAMA). These samples may give falsely high or low concentrations when tested with kits containing mouse antibodies.
The serum PSA value in an isolated specimen can only be used in conjunction with clinical data and information available from other diagnostic procedures. An abnormal PSA level does not necessarily signify a malignant disorder.

EXPECTED VALUES

Expected values were determined using samples from 400 healthy and 700 symptomatic men in the United States.

Studies were conducted to support the detection of prostate cancer in men aged 50 years or older with the following objectives:

- Assess clinical validity by the use of clinical sensitivity and specificity as measured by the device alone and in conjunction with DRE results. The added value of total PSA over DRE alone is assessed.
- Assess clinical reliability using positive and negative predictive values as measured by the device alone and in conjunction with DRE result.
- Determine the 95th order statistic of total PSA (tPSA) in a cohort of apparently healthy men aged 50 years of age or older.
- Determine the distribution of results in a patient and apparently healthy cohort to support a threshold of 4.0 ng/ml.

The patient cohort was composed of 700 retrospectively obtained serum samples from men aged 50 years of age or older, regardless of race, presenting to a practicing urologist with symptoms that would lead to an evaluation for prostate cancer, including a trans-rectal prostate biopsy who had no history of prostate disease for 6 months or had no history of an evaluation for prostate cancer. These samples were collected from 34 clinical sites in the United States under an IRB approved protocol with patient informed consent. The average age of the men in this cohort was 66.6 years (95% CI: 66.0 years to 67.2 years), with a range of 50.8 years to 88.5 years; the median age was 66.6 years. The racial composition of this cohort was 81.9% Caucasian, 13.3% African American (non- Hispanic); the remainder (4.8%) from other racial groups.

The apparently healthy men cohort consisted of 400 men 50 years of age or older who had met the criteria required for blood donation to the American Red Cross and who had no known prostate disease or no history of prostate disease. These samples were collected under an IRB approved protocol with informed consent. The average age of the apparently healthy cohort was 63.7 years (95% CI: 62.8 to 64.6 years); the median age was 62 years. Of these 400, 97.3% were Caucasian and 1.9% were Black, non- Hispanic males. The remaining 0.8% of the cohort was of the other racial groups.

The observed overall cancer rate was 33.6%. Of 234 cancer subjects with Gleason grading results available, 13% had Gleason grading of 4 or 5, 76% of subjects had Gleason grade 6 or 7, and 11% had Gleason grade 8 or 9 cancers. Of 700 subjects, 207 subjects (29.6%) had a digital rectal examination a physician characterized as abnormal suspicious for cancer or as other. While 493 subjects (70.4%) had a digital rectal examination a physician characterized as abnormal not suspicious for cancer or normal. Of 700 subjects, 80% had PSA values greater than or equal to 4.0 ng/ml. Of 235 cancer subjects, 89.4% had PSA values greater than or equal to 4.0 ng/ml while 75% of 465 non-cancer subjects had PSA values greater than or equal to 4.0 ng/ml. The median PSA value of cancer subjects (8.0 ng/ml) was significantly different from the median PSA value of non-cancer subjects (5.6 ng/ml; $p < 0.001$ by Wilcoxon test).

The estimates of the probability (risk) of positive biopsy results are presented by the following table:

	Probability (Risk) of Positive Biopsy	**95% CI**
Pre-test	33.6% (235/700)	
DRE+	41.1% (85/207)	34.3% to 48.1%
DRE–	30.4% (150/493)	26.4% to 34.7%
PSA ≥ 4.0	37.6% (210/559)	33.5% to 41.7%
PSA < 4.0	17.7% (25/141)	11.8% to 25.1%
PSA ≥ 4.0 and DRE+	49.0% (75/153)	40.9% to 57.2%
PSA ≥ 4.0 and DRE –	33.3% (135/406)	28.7% to 38.1%
PSA < 4.0 and DRE+	18.5% (10/54)	9.3% to 31.4%
PSA < 4.0 and DRE –	17.2% (15/87)	10.0% to 26.8%
PSA ≥ 4.0 DRE+ or PSA ≥ 4 DRE– or PSA < 4.0 DRE+	35.9% (220/613)	32.1% to 39.8%

The probability (risk) of positive biopsy represents the proportion of cancer subjects having the designated diagnostic test result.

Expected values were determined using samples from 400 healthy men in the United States. Using a cutoff of 4.0 ng/ml, 93% of apparently healthy men aged 50 to 60 years of age had PSA values less than 4.0 ng/ml. Similarly, 88.5% of apparently healthy men aged 60 to 70 years of age had PSA values less than 4.0 ng/ml and 80% of apparently healthy men aged 70+ years had PSA values less than 4.0 ng/ml.

Distribution of tPSA in Healthy Men:

Age (years)	N	Order Statistic (ng/mL)	95% Confidence Interval
50 – 59	155	4.0	2.5 to 4.8
60 – 69	135	5.4	4.1 to 6.6
> 69	110	7.2	6.0 to 9.4

Ninety-five percent confidence intervals determined by re-sampling the PSA distribution 10,000 times and determining the 95th order statistic.

Total PSA Statistics by Device and Biopsy Result:

Disease State	N	Mean (ng/mL)	Median (ng/mL)	95% Confidence Interval
Prostate Cancer	235	23.2	8.0	12.1–34.2
Benign Prostate Disease	465	6.9	5.6	6.3–7.5

It is recommended that each laboratory establishes its own reference values using a rigorously selected population.

PERFORMANCE

The figures presented in the following tables are taken from tests which have been performed as indicated in this package insert, and are given for information purposes only.

Hook effect

No hook effect was found up to prostate specific antigen concentrations of 100 000 ng/mL.

Interfering Substances

None of the following substances interfered with the assay result: albumin (3 mg/mL), IgG (3 mg/mL).

Measurement range

The measurement range of the VIDAS TPSA kit goes up to 100 ng/ml.

Detection limit

Defined as the smallest concentration of prostate specific antigen which is significantly different from the zero concentration with a probability of 95 % : **0.07 ng/ml.**

Precision:

Three samples were evaluated with three lots of kits at 3 U.S. sites in accordance with NCCLS procedures. The coefficient of variation for the averaged results between runs and within a run are found in the following table:

%CV for Total PSA

Site		Sample 1 85 ng/mL	Sample 2 4.0 ng/mL	Sample 3 0.6 ng/mL
1	Between Runs	0.0	0.0	2.4
	Within Run	2.9	4.1	8.6
2	Between Runs	0.7	0.7	1.8
	Within Run	3.4	3.6	7.2

3	Between Runs	0.0	0.3	0.9
	Within Run	2.9	2.3	7.6

Accuracy - Dilution test

The serum matrix of the sample can influence the results of the dilution test. When printing out results, it is recommended to indicate the level of dilution used. Three samples were diluted in the TPSA diluent and tested singly in 3 runs. The measured mean concentration compared to the expected mean concentration is expressed as a mean recovery percentage.

Sample	Dilution factor	Expected mean concentration (ng/ml)	Measured mean concentration (ng/ml)	Mean recovery percentage (%)
1	1/1	92.9	92.9	100
	1/2	46.4	41.9	90
	1/4	23.2	19.8	85
	1/8	11.6	9.9	85
	1/16	5.8	5.0	86
2	1/1	52.8	52.8	100
	1/2	26.4	26.1	99
	1/4	13.2	12.5	95
	1/8	6.6	6.4	97
	1/16	3.3	3.2	97
3	1/1	18.2	18.2	100
	1/2	9.1	8.1	89
	1/4	4.6	4.2	92
	1/8	2.3	2.2	96
	1/16	1.1	1.1	96

COMPARISON WITH OTHER TEST METHODS

The concentration of prostate specific antigen may vary in a sample determined using kits from different manufacturers, depending on the test methods used. If the test method is changed, and in the case of patient monitoring, laboratories
should confirm the concentrations previously found.

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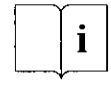
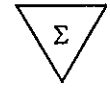
INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	In vitro diagnostic medical device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult instructions for use
	Contains sufficient for "n" tests

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