VIDAS® TPSA (TPSA)

IVD

VIDAS® TPSA is an automated quantitative test for use on the VIDAS family instruments, for the quantitative measurement of prostate specific antigen (PSA) levels in human serum or plasma (lithium heparin or EDTA), using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

Prostate-specific antigen (PSA) is a glycoprotein which belongs to the kallikrein family (1, 2, 3). PSA has a molecular weight of 30,000 daltons (3, 4, 5).
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 (2, 3, 6).
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 (5, 6). 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.
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 (4, 5). The VIDAS TPSA assay is an equimolar test (7): the molar ratio (concentration of solution containing 100% Free PSA over concentration of solution containing 100% PSA-ACT) is between 105 and 125%.
The third form of PSA, bound to alpha-2-macroglobulin, cannot be detected by immunoassays.
The VIDAS TPSA assay is used in the diagnosis of prostate disorders, including cancer of the prostate, and for the prognosis and monitoring of patients with diagnosed malignant tumors.

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. 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 reaction medium is cycled in and out of the SPR several times.
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, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS:

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. After reconstitution, stable 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 + preservatives. MLE data indicate the confidence interval in ng/mL ("Control C1 Dose Value Range").
TPSA calibrator 2 x 2 ml (lyophilized)	S1	Reconstitute with 2 ml of distilled water. Let stand for 30 minutes, then mix. After reconstitution, stable 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 + preservatives. MLE data indicate the concentration in ng/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range").
TPSA sample diluent 2 x 4 ml (liquid)	R1	Ready-to-use. Calf serum + 0.9 g/l sodium azide.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar codes printed on the box label.		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* 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.

The SPR

The SPR is coated during production with monoclonal anti-PSA antibodies (mouse). Each SPR is identified by the code TPSA. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, 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 wells in the center section of the strip contain the various reagents required for the assay.

Description of the TPSA 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: 4-Methyl-umbelliferyl-phosphate (0.6 mmol/l) + diethanolamine* (DEA) (0.62 mol/l or 6.6 %, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 2 ml and 200 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **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 - WHO - Geneva - latest Edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not 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 (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless gloves**, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- 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.
- The substrate in well 10 contains an irritant agent (6.6 % diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- 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 User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- 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. Refer to the kit composition table for special storage conditions.

SPECIMENS

Speciment type and collection

Human serum or plasma (lithium heparin or EDTA).
As some collection tubes contain substances which interfere with test results, it is recommended that each laboratory checks the compatibility of collection tubes used.

Samples containing impurities must be centrifuged before analysis.
None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin: 0 to 300 µmol/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 10 mg/ml equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 500 µmol/l).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric, and if possible to collect a new sample.

Specimen stability

Samples can be stored at 2-8°C in stoppered tubes for a maximum of 24 hours; if longer storage is required, freeze the sera or plasma at -25 ± 6°C. A study performed on frozen samples over a period of 2 months showed that the quality of results is not affected. Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User'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 have been entered. Calibration 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 User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

VIDAS TPSA is calibrated against the reference standard: 1st WHO international standard 96/670 (8). Depending on the dilution mode and the type of diluent used with the international standard, a bias of up to 15% may be observed.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "TPSA" strip and one "TPSA" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "TPSA" code on the instrument. The calibrator, identified by "S1" should be tested **in duplicate**. If the control needs to be tested, it should be identified by C1.
4. Mix the calibrator, control and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. **For this test, the calibrator, control, and sample test portion is 200 µl.**
6. Insert the "TPSA" SPRs and "TPSA" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to the required temperature after pipetting.
9. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

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 SPR is introduced into the substrate. 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 instrument (4-parameter logistic model); the concentrations are expressed in ng/ml.

Samples with TPSA test value concentrations > 100 ng/ml should be reassayed after diluting with TPSA sample diluent (R1).
If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result by the dilution factor to obtain the sample concentration.
Interpretation of test results should be made taking into consideration the patient history, and the results of any other tests performed.

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 calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1. Results cannot be validated if the control value deviates from the expected values.

Note
It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted as part of a complete clinical profile.
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.

TPSA assays should not be performed on patients who have received a contrast medium within the previous 24 hours (9).

RANGE OF EXPECTED VALUES

Expected values were determined using 1041 samples collected from healthy subjects. PSA concentrations per age group are as follows:

	PSA concentrations (ng/ml)	
Age (years)	Low limit*	High limit*
< 40	0.21	1.72
40 - 49	0.27	2.19
50 - 59	0.27	3.42
60 - 69	0.22	6.16
> 69	0.21	6.77

* including 95% of the population.

For a healthy male population aged 50 years or more, the distribution of values is as follows:

PSA (ng/ml)	0 - 2	2 - 4	4 - 6	> 6
Frequency	83.5%	13.5%	1.3%	1.7%

For a population of 54 patients with benign prostate hypertrophy, the distribution of values is as follows:

	No. of subjects	Distribution in percentage (%) in relation to the zone of values in ng/ml		
		< 4 ng/ml	4-10 ng/ml	> 10 ng/ml
Mean = 3.08 ng/ml Standard deviation = 2.3 ng/ml	54	74 (n=40)	24 (n=13)	2 (n=1)

These results are given as a guide; it is recommended that each laboratory establish its own reference values from a rigorously selected population.

PERFORMANCE

Measurement range

The measurement values of the VIDAS TPSA kit range from 0.07 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.**

Hook effect

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

Precision

The results presented in the following tables are given for information purposes only.

Within-run reproducibility (intra-assay)

Five samples were tested 30 times in the same run.

Sample	1	2	3	4	5
Mean (ng/ml)	0.55	3.0	7.6	17.4	31.6
CV %	6.5	3.9	3.5	3.4	3.9

Between-run reproducibility (inter-assay)

Five samples were tested in 29 different runs on the same VIDAS instrument.

Sample	1	2	3	4	5
Mean (ng/ml)	0.52	2.8	7.5	16.9	32.1
CV %	6.8	3.9	3.9	4.3	5.5

Accuracy

Dilution testa

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 1/2 1/4 1/8 1/16	92.9 46.4 23.2 11.6 5.8	92.9 41.9 19.8 9.9 5.0	100 90 85 85 86
2	1/1 1/2 1/4 1/8 1/16	52.8 26.4 13.2 6.6 3.3	52.8 26.1 12.5 6.4 3.2	100 99 95 97 97
3	1/1 1/2 1/4 1/8 1/16	18.2 9.1 4.6 2.3 1.1	18.2 8.1 4.2 2.2 1.1	100 89 92 96 96

Diagnostic sensitivity and specificity

109 samples from patients with benign prostatic hyperplasia and 205 from patients with prostate cancer were tested using VIDAS® TPSA.

A ROC curve was established using the 314 samples. 2 cut-off values were identified:

- 3.03 ng/ml: sensitivity = 95%
- 6.73 ng/ml: sensitivity= 80%

Cut-off value	3.03 ng/ml		6.73 ng/ml	
	(IC 95%)		(IC 95%)	
Sensitivity	91.16	97.36	73.86	84.99
Specificity	9.88	23.75	46.41	65.09
Positive predictive value	67.94		77.36	
Negative predictive value	60.71		59.22	

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.
Correlation was established between VIDAS® TPSA (Y), and another enzyme immunoassay method (X)
X = 0,99 Y - 0,17 r = 0,994
(n = 126)

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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9. WATANABE N. and al. *In vitro* effect of contrast during immunoradiometric assay for tumour-associated antigens. Nuclear Medicine Communication, 1998, 19, 63-70.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	09296I	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE

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