abia PSA total

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IVD

CE 0483

Note: Changes highlighted ★

abia

Intended use

abia PSA total is an enzyme immunoassay for the quantitative determination of total prostate specific antigen (tPSA) concentration in human serum or plasma.

For professional use only.

Clinical value

Human prostate specific antigen (PSA) is a serine protease, a single chain glycoprotein with a molecular weight of approximately 33 kDa containing 7% carbohydrate by weight.

PSA is immunologically specific for prostatic tissue. It is present in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid and seminal plasma. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Besides, it is functionally and immunologically different from prostatic acid phosphatase (PAP). Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues, but not in men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Reports have suggested that serum PSA is one of the most useful tumor markers in oncology. It may serves as an accurate marker for assessing response to treatment in patients with prostatic cancer.

Therefore, measurement of serum PSA concentrations can be an important tool in monitoring patients with prostatic cancer and in determining the potential and actual effectiveness of surgery or other therapies.

Principle of the test

abia PSA total is an one-step immunoassay, based on the principle of the “sandwich” method.

The assay system utilizes high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact tPSA molecule.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the tPSA molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of tPSA in the specimen and can be read at 450 nm.

Kit contents

	S	
PSA Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-PSA Ab
Conjugate	1 × 12 ml	ready to use; HRP-labeled monoclonal anti-PSA; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing tPSA; pale yellow liquid
Calibrator 1	1 × 0.5 ml	protein based buffer containing tPSA in concentration approx. 1.50 ng/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	protein based buffer containing tPSA in concentration approx. 3.75 ng/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	protein based buffer containing tPSA in concentration approx. 7.50 ng/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	protein based buffer containing tPSA in concentration approx. 15.00 ng/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	protein based buffer containing tPSA in concentration approx. 45.00 ng/ml; pale yellow liquid
Control serum	1 × 0.5 ml	protein based control containing tPSA; pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer, colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colorless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 1st IRP 96/670. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and deconta-minate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use heparin/EDTA plasma or serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 48 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- perform this procedure 5 times in total ★
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia PSA total for the quantitative determination of total prostate specific antigen (tPSA) concentration in human serum or plasma

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
2	Analyze each calibrator, control serum, and sample duplicate. Reserve one or two wells for TMB/substrate solution control (blank). Add 25 µl of calibrators 0 - 5 into appropriate wells. Add 25 µl of control serum into appropriate wells. Add 25 µl of samples to be tested in rest of the wells. The total time should not exceed 10 min.
3	Add 100 µl conjugate into each well except blank. Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
4	Incubate for 75 minutes at room temperature 20–25 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
6	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 25 ± 5 minutes at 20–25 °C.
7	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
8	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx. 45 ng/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

1 Calculate the mean optical density of each calibrator duplicate.

2 Calculate the mean optical density of each sample duplicate.

3 Subtract the mean absorbance value of the "blank" from the mean absorbance values of the calibrators, control and serum samples.

4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 45 ng/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.046)	Value, ng/ml
Calibrator 0	0.043	0.049	0.000	0.00
Calibrator 1	0.181	0.181	0.135	1.50
Calibrator 2	0.345	0.358	0.306	3.75
Calibrator 3	0.656	0.662	0.613	7.50
Calibrator 4	1.226	1.234	1.184	15.00
Calibrator 5	2.594	2.624	2.563	45.00
Sample	0.363	0.389	0.330	4.00

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The limit of detection (LoD) and limit of quantification (LoQ) were established according to CLSI EP-17A2 protocol.

★ Limit of detection defined at 0.282 ng/ml. Limit of quantification defined at 0.39 ng/ml.

Specificity	Concentration	Cross reactivity, %
Kallikrein-2 (KLK2)	2 000 ng/ml	0.028
Chorionic gonadotropin (hCG)	20 µg/ml	0.004
Carcinoembryonic antigen (CEA)	20 µg/ml	0.0066
Alpha-1-Fetoprotein (AFP)	20 µg/ml	0.0032

Precision	Mean, ng/ml	SD	CV, %
Intra-assay, sample 1	4.20	0.25	6.00
Intra-assay, sample 2	13.10	0.60	4.60
Intra-assay, sample 3	22.30	1.01	4.50
Inter-assay, sample 1	4.20	0.31	7.50
Inter-assay, sample 2	13.10	0.90	6.90
Inter-assay, sample 3	22.30	1.62	7.20

Linearity

13 serum samples within the range from 1.13 to 39.31 ng/ml were diluted with calibrator 0 and tested in 3 replicates. The measured concentrations were within ± 10% of expected concentrations.

Accuracy

The assay was compared with an commercially available enzyme immunoassay as a reference test. The total number of specimens was 234. The values ranged from 0.28 to 37.07 ng/ml. The least square regression equation and the correlation coefficient were computed for abia PSA total in comparison with the reference method.

The least square regression analysis was y = 1.10(x) + 0.09 with correlation coefficient 0.97.

Expected normal value	Range, ng/ml	
‹ 40 years	0.01	1.51
40 - 49 years	0.00	2.27
50 - 59 years	0.02	2.89
› 60 years	0.29	4.96

It is known the normal tPSA levels increase with the age. The age-specific reference ranges are used in the diagnosis of prostate cancer.

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient.
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.
- no hook effect was observed up to a tPSA concentration of 3 000 ng/ml.
- not intended for newborn screening.

References

1. Aus G., Bergdahl S., Lodding P., et al. Prostate cancer screening decreases the absolute risk of being diagnosed with advanced prostate cancer--results from a prospective, population-based randomized controlled trial. Eur Urol 2007; 51:659-664.
2. Catalona W.J., Partin A.W., Slawin K.M., et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. JAMA 1998; 279:1542-1547.
3. Capitanio U., Perrotte P., Zini L., et al. Population-based analysis of normal Total PSA and percentage of free/Total PSA values: results from screening cohort. Urology 2009; 73:1323-1327.
4. Thompson I.M., Pauler D.K., Goodman P.J., et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. N Engl J Med 2004; 350:2239-2246.
5. Thompson I.M., Ankerst D.P., Chi C., et al. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA 2005; 294:66-70.
6. Van Leeuwen P.J., Roobol M.J., Kranse R., et al. Towards an optimal interval for prostate cancer screening. Eur Urol 2012; 61:171-176.
7. Vickers A.J., Ulmert D., Sjoberg D.D., et al. Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40-55 and long term risk of metastasis: case-control study. BMJ 2013; 346:f2023.
8. Ulmert D., Cronin A.M., Bjork T., et al. Prostate-specific antigen at or before age 50 as a predictor of advanced prostate cancer diagnosed up to 25 years later: a case-control study. BMC Med 2008; 6:6.
9. Schaeffer E.M., Carter H.B., Kettermann A., et al. Prostate specific antigen testing among the elderly--when to stop? J Urol 2009; 181:1606-1614.
10. NCCN Clinical Practice Guidelines in Oncology “Prostate Cancer Early Detection” Version 2.2016.

Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components ★

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P 352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate, calibrators 0 - 5, control serum

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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