ms_04491734190V5.0

total PSA

cobas®

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170
04491734 190	200	**cobas e** 601
		cobas e 602

English

Please note

The measured tPSA value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the tPSA assay method used. tPSA values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the tPSA assay procedure used while monitoring therapy, then the tPSA values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

This assay, a quantitative in vitro diagnostic test for total (free + complexed) prostate-specific antigen (tPSA) in human serum and plasma, is indicated for the measurement of total PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Prostate biopsy is required for diagnosis of prostate cancer. The test is further indicated for serial measurement of tPSA to aid in the management of cancer patients.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30000-34000 daltons) having a close structural relationship to the glandular kallikreins. It has the function of a serine proteinase.[1]

The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with protease inhibitors such as alpha-1-antichymotrypsin, alpha-2-macroglobulin, and other acute phase proteins.[2] Beside these complexes, about 30 % of the PSA present in blood occurs in the free form, but is proteolytically inactive.[3,4,5]

Elevated concentrations of PSA in serum are generally indicative of a pathologic condition of the prostate (prostatitis, benign hyperplasia or carcinoma).[6,7]

As PSA is also present in para-urethral and anal glands, as well as in breast tissue or with breast cancer, low levels of PSA can also be detected in sera from women. PSA may still be detectable even after radical prostatectomy.

The main areas in which PSA determinations are employed are the monitoring of progress and efficiency of therapy in patients with prostate carcinoma or receiving hormonal therapy.

The steepness of the rate of fall in PSA down to no-longer detectable levels following radiotherapy, hormonal therapy or radical surgical removal of the prostate provides information on the success of therapy.[8]

An inflammation or trauma of the prostate (e.g. in cases of urinary retention or following rectal examination, cystoscopy, coloscopy, transurethral biopsy, laser treatment or ergometry) can lead to PSA elevations of varying duration and magnitude.

The two monoclonal antibodies used in the Elecsys total PSA assay recognize PSA and PSA-ACT on an equimolar basis in the range of 10-50 % free PSA/total PSA which are the free PSA-ratios as seen in clinical practice.[9]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as TPSA.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin (gray cap), 1 bottle, 16 mL:
Biotinylated monoclonal anti-PSA antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-PSA-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 16 mL:
Monoclonal anti-PSA antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	4 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Lithium heparin, K_3-EDTA and sodium citrate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 days at 2-8 °C, 6 months at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all

available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04485220190, total PSA CalSet II, for 4 x 1 mL
- REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or

  REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170, **cobas e** 601 or **cobas e** 602 analyzer

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Stanford Reference Standard/WHO 96/670 (90 % PSA-ACT + 10 % free PSA).[10,11,12]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker or PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at tPSA concentrations up to 17000 ng/mL.

In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

It is known that in rare cases PSA isoforms do exist which may be measured differently by different PSA tests. Findings of this kind have occasionally been reported for PSA tests from various manufacturers.[13,14,15]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.003-100 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.003 ng/mL. Values above the measuring range are reported as > 100 ng/mL (or up to 5000 ng/mL for 50-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.003 ng/mL

The lower detection limit (LDL) is calculated as the concentration lying two signal standard deviations away from an analyte-free sample or from the lowest standard (within-run precision, n = 21).

Limit of Blank (LoB) and Limit of Detection (LoD)

LoB = 0.006 ng/mL

LoD = 0.014 ng/mL

Both Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank

total PSA

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Dilution

Samples with tPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 2 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The following data were established with the Elecsys total PSA assay on the Elecsys 2010 analyzer and can be transferred to MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers due to technical equivalence.

Expected values in normal healthy males

a) Studies in two clinical centers in the Netherlands and Germany with the Elecsys total PSA assay on sera from 244 healthy men of various age groups yielded the following results:

		tPSA (ng/mL)	
Age (years)	N	Median	95th percentile
< 40	45	0.57	1.4
40-49	42	0.59	2.0
50-59	107	0.75	3.1
60-69	41	1.65	4.1
≥ 70	9	1.73	4.4

b) The distribution of tPSA results was measured in a cohort of 395 normal healthy males aged 50-94 years (results of a study in the USA).

The subsequent table presents the tPSA values as measured on the Elecsys 2010 immunoassay analyzer.

		tPSA (ng/mL)	
Age (years)	N	Median	95th percentile
50-59	154	0.81	3.89
60-69	131	0.95	5.40
≥ 70	110	1.11	6.22

tPSA values in detection of prostate cancer

A multicenter cohort study was performed to demonstrate the effectiveness of the Elecsys total PSA assay when used in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men 50 years of age or older.

A total of 1121 serially accrued men 50 years of age or older participated in the study. The mean age of the cohort was 66.4 years (95 % confidence interval = 65.9 to 66.8 years).

Distribution of tPSA values by biopsy result and digital rectal examination result

Prostate biopsy result: benign

		tPSA (ng/mL)		
DRE result	N	Median	Minimum	Maximum
Normal	375	5.8	0.4	75.8
Pathological	355	4.9	0.3	29.6
Total	730	5.4	0.3	75.8

Prostate biopsy result: malignant

		tPSA (ng/mL)		
DRE result	N	Median	Minimum	Maximum
Normal	146	7.2	2.5	122.1
Pathological	245	7.8	0.5	778.5
Total	391	7.4	0.5	778.5

Utility of tPSA in detection of prostate cancer

As shown in the table below, within this cohort of 1121 males, 391 (34.9 %) prostate cancers were detected by biopsy. Abnormal digital rectal examination (DRE) results were reported for 245 (62.7 %) of the 391 prostate cancers while tPSA results above 4 ng/mL were reported for 336 (85.9 %) cancers for the Elecsys 2010 analyzer. Of the 391 men diagnosed with cancer, 379 (96.9 %) had either an abnormal DRE result or a tPSA value above 4.0 ng/mL.

The positive predictive value for the Elecsys total PSA assay on the Elecsys 2010 analyzer was 0.390 using 4.0 ng/mL as a cutoff (malign prostate biopsy + tPSA > 4.0 ng/mL: n = 336 / tPSA > 4.0 ng/mL: n = 862).

Results for digital rectal examination and tPSA as referred to prostate cancers detected by biopsy in a cohort of:

1121 males 50 years or older referred to an urologist for prostate evaluation.

	Total	DRE+[b]	PSA+[c]	PSA+ or DRE+	PSA+ and DRE+	PSA+ and DRE-[d]	PSA- and DRE+[e]
Total number	1121	600	862	1037	425	437	175
No. of malignant prostate biopsies	391	245	336	379	202	134	43
% positive biopsies	34.9	40.8	39.0	36.5	47.5	30.7	24.6

b) abnormal DRE

c) tPSA value > 4 ng/mL

d) normal DRE

e) tPSA value < 4 ng/mL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	1.12	0.02	1.4	1.12	0.04	3.2
Human serum 2	4.39	0.05	1.2	4.61	0.17	3.7
Human serum 3	27.8	0.46	1.7	27.5	0.75	2.7
PreciControl TM[f]1	3.27	0.04	1.3	3.25	0.05	1.4
PreciControl TM2	23.3	0.32	1.4	22.9	0.36	1.6

f) TM = Tumor Marker

Method comparison

A comparison of the Elecsys total PSA assay (y) with the Enzymun-Test PSA method (x) using clinical samples gave the following correlations:

Number of samples measured: 95

total PSA

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

Passing/Bablok[16]	Linear regression
y = 1.03x + 0.30	y = 1.02x + 0.60
τ = 0.950	r = 0.989

The sample concentrations were between approximately 0.1 and 50 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

PAP and ACT: none; PSA and PSA-ACT are recognized on an equimolar basis.

Functional sensitivity

0.03 ng/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

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For further information, please refer to the appropriate operator’s manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

CE 0123

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