PAPP-A

Pregnancy-associated plasma protein A

cobas®

04854098 200 **100 tests**

• Indicates analyzers on which the kit can be used

Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•

English

Caution
The measured PAPP-A 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 PAPP-A assay method used. PAPP-A 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 PAPP-A assay procedure used while monitoring therapy, then the PAPP-A values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use
Immunoassay for the in vitro quantitative determination of pregnancy-associated plasma protein A in human serum.
The Elecsys PAPP-A assay is intended for the use as one component in combination with other parameters to evaluate the risk of trisomy 21 (Down syndrome) during the first trimester of pregnancy. Further testing is required for diagnosis of chromosomal aberrations.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary
Human pregnancy-associated plasma protein A (PAPP-A) is a large glycoprotein with a molecular weight of 200 kDa, which belongs to the metzincin superfamily of zinc peptidases.[1] PAPP-A was first isolated from the serum of pregnant women, where its concentration increases steadily until term. PAPP-A is produced by the trophoblast and secreted into the maternal serum, where it mainly circulates as a heterotetrameric 2:2 complex, together with two subunits of the proform of eosinophil major basic protein (proMBP).[2,3,4]
It is now well established that the PAPP-A concentration in the serum is a reliable marker for fetal aneuploidy. In a number of studies it could be confirmed that PAPP-A, in combination with free ßhCG and the sonografic determination of nuchal translucency (NT), is the serum marker of choice to identify women at increased risk of carrying a fetus affected with Down syndrome during the first trimester (week 11-14) of pregnancy.[5] Using this marker combination, detection rates of up to 70% (serum markers only) and 90% (combined with NT) have been described at a false positive rate of 5%.[6,7,8]
Median maternal serum PAPP-A levels in affected pregnancy are lower, compared to the median of non-affected pregnancies.[9]
Based on the maternal age, the risk for having a Down syndrome pregnancy can be calculated using a specific algorithm e.g. based on likelihood ratios.[10]

Test principle
Sandwich principle. Total duration of assay: 18 minutes.
- 1st incubation: 15 µL of sample, a biotinylated monoclonal PAPP-A-specific antibody and a monoclonal PAPP-A-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. 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
M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 Anti-PAPP-A-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti-PAPP-A antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L, pH 7.0; preservative.
R2 Anti-PAPP-A-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL: Monoclonal anti-PAPP-A antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 50 mmol/L, pH 7.4; 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 the formation of foam with 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 via the respective reagent barcodes.

Storage and stability
Store at 2-8°C.
Store the Elecsys PAPP-A 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	4 weeks
on the analyzers	3 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.
Do not use plasma.
Stable for 8 hours at 15-25°C, 3 days at 2-8°C, 3 months at -20°C.
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 patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
- Cat. No. 04854101, PAPP-A CalSet, for 4 x 1 mL
- Cat. No. 04899881, PreciControl Maternal Care, for 2 x 2 mL each of PreciControl Maternal Care 1, 2 and 3.
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

For risk calculation of trisomy 21:
- Cat No. 04854071, free βhCG, 100 tests
- Cat No. 04854080, free βhCG CalSet, for 4 x 1 mL
- A suitable software, e.g. Cat. No. 05126193, SsdwLab (V5.0 or later)

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Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, 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 manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before 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.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against a commercially available PAPP-A test, which in turn was standardized against the WHO standard preparation IRP 78/610.
Every Elecsys PAPP-A reagent set has a barcoded label containing the specific information required for calibration of the particular reagent lot. The pre-defined master curve is adapted to the analyzer by the use of Elecsys PAPP-A 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:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

For quality control, use Elecsys PreciControl Maternal Care 1, 2 and 3. Other suitable control material can be used in addition.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every 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 limits.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in mIU/L, IU/L or mIU/mL).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 205 µmol/L or < 12 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 123 nmol/L or < 30 ng/mL.
Criterion: Recovery within ± 10% of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.
There is no high-dose hook effect at PAPP-A concentrations up to 120000 mIU/mL.
In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.
In very rare cases, interference due to extremely high titers of antibodies against analyte-specific antibodies, streptavidin or ruthenium can occur. The test contains additives which minimize these effects.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

4-10000 mIU/L (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 4 mIU/L. Values above the measuring range are reported as > 10000 mIU/L (or up to 100000 mIU/L for 10-fold diluted samples).

Dilution

Samples with PAPP-A concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:10 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers or manually). The concentration of the diluted sample must be > 500 mIU/L. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values and clinical performance

The following results were obtained with the Elecsys PAPP-A assay:

1. Reference range study using a panel of samples from 500 healthy non-pregnant donors (Roche study No. R04P026)
< 7.15 mIU/L (95th percentile)

2. Perfomance evaluation study of the Elecsys PAPP-A assay and the Elecsys free βhCG assay in first trimester trisomy 21 risk assessment (Roche study No. B05P020, status July 2007)
Measurements with the Elecsys free βhCG assay and the Elecsys PAPP-A assay were conducted in 4 clinical centers in Belgium, Switzerland, and Germany. Median values (gestational weeks 11-14) were calculated from log-linear regression analysis of 3270 PAPP-A values for the middle of the respective week. Gestational age was calculated from ultrasound crown-to-rump length (CRL) according to Robinson.[11]

Gestational week	11	12	13	14
Number of samples	206	623	1384	1057
Median (mIU/L)	1337	1919	2926	4358

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. For prenatal testing it is recommended that the median values be re-evaluated periodically.

Clinical performance data

In total, 1079 samples from clinical routine with known outcome were examined. 32 out of the 1079 samples were from pregnancies with confirmed Down syndrome. All samples were measured in parallel with FMF (Fetal Medicine Foundation) certified PAPP-A and free βhCG tests. Risk calculation was performed using the software SsdwLab version 5.0. This software makes use of an algorithm described by Palomaki et al[12] by means of the mathematical calculations for Gaussian multivariate distribution as already published.[13] Risk analysis is based on maternal age, nuchal translucency as well as on the results of the biochemical parameters, corrected by different factors like e.g. maternal weight, smoking and ethnic background of the pregnant woman.

Individual risk calculation

The calculation of a woman's individual risk of carrying a single fetus affected by trisomy 21 was assessed without consideration of nuchal translucency (NT) data to demonstrate the performance of the biochemical methods. Maternal weight and smoking behavior were taken into account

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as correction factors. Concordance of risk analysis compared to a competitor method combination was examined using the cut-off value already established in the participating laboratory.[14,15]
It is the responsibility of the user to choose the cut-off which will apply for further procedures.

Concordance analysis data

A. Concordance analysis in unaffected pregnancies (n = 1047)

	Risk > cut-off (Roche*)	Risk < cut-off (Roche*)
Risk > cut-off (competitor**)	55 (5.2%)	26 (2.5%)
Risk < cut-off (competitor**)	6 (0.6%)	960 (91.7%)

In 1047 unaffected samples the Roche methods correctly classified 986 samples (specificity: 94.2%) in comparison to 966 (specificity: 92.3%) correctly classified by the competitor methods.

B. Detection rate in confirmed trisomy 21 pregnancies (n = 32)

	Risk > cut-off (Roche*)	Risk < cut-off (Roche*)
Risk > cut-off (competitor**)	21 (65.6%)	0
Risk < cut-off (competitor**)	3 (9.4%)	8 (25%)

In 32 affected samples the Roche methods showed a detection rate of 75% (24/32) in comparison to 65.6% (21/32) obtained with the competitor methods.

* Combination of results from the Elecsys PAPP-A assay and the Elecsys free βhCG assay
** Combination of results from the competitors PAPP-A and free βhCG methods

Specific performance data

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

Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS): 6 times daily for 10 days (n = 60). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers

		Within-run precision		Total precision	
Sample	Mean mIU/L	SD mIU/L	CV %	SD mIU/L	CV %
Human serum 1	283	6.7	2.4	6.6	2.3
Human serum 2	521	11.5	2.2	11.5	2.2
Human serum 3	4181	87.1	2.1	94.7	2.3
PC[b] Maternal Care 1	6630	111	1.7	133	2.0
PC Maternal Care 2	3361	55.0	1.6	58.7	1.8
PC Maternal Care 3	144	1.60	1.1	1.6	1.1

b) PC = PreciControl

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

		Within-run precision		Total precision	
Sample	Mean mIU/L	SD mIU/L	CV %	SD mIU/L	CV %
Human serum 1	280	5.55	2.0	7.67	2.8
Human serum 2	506	8.33	1.7	9.83	1.9
Human serum 3	4001	75.5	1.9	92.2	2.3
PC Maternal Care 1	6335	107	1.7	114	1.8
PC Maternal Care 2	3229	36.8	1.1	45.3	1.4
PC Maternal Care 3	141	1.94	1.4	2.72	1.9

Analytical sensitivity (lower detection limit)

< 4 mIU/L
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison

A comparison of the Elecsys PAPP-A assay (y) with a commercially available PAPP-A assay (x) using clinical samples gave the following correlations:
Number of samples measured: 627

Passing/Bablok[16]	Linear regression
y = 0.968x - 4.90	y = 0.988x - 64.9
τ = 0.918	r = 0.987

The sample concentrations were between approx. 150 and approx. 10000 mIU/L.

Analytical specificity

No cross reactivity against angiotensinogen and α2-macroglobulin detectable.

Functional sensitivity

< 20 mIU/L
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation 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 package inserts of all necessary components.

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Roche Diagnostics GmbH, D-68298 Mannheim