ProGRP

cobas®

REF	Σ	SYSTEM
06505961 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1120
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 557

Please note

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

Intended use

Immunoassay for the quantitative determination of ProGRP in human plasma and serum. The assay is used to aid in the differential diagnosis in lung cancer[1] and in the management of patients with small cell lung cancer in conjunction with other clinical methods. The results must be interpreted in conjunction with other methods in accordance with standard clinical management guidelines.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Gastrin-releasing peptide (GRP) is an important regulatory molecule that is implicated in a number of physiological and pathophysiological processes in humans. It is a gut hormone and the mammalian counterpart of amphibian bombesin, originally isolated from porcine stomach[2] and widely distributed throughout the mammalian nervous system as well as the gastrointestinal and pulmonary tract.[3] Its 148 amino acid preproprotein, following cleavage of a signal peptide, is further processed to produce the 27 amino acid GRP and the 68 amino acid ProGRP. Due to its short half-life of 2 minutes it is not possible to measure GRP in blood.[4] Therefore, an assay for the measurement of ProGRP (31-98), a carboxy-terminal region common to three types of human ProGRP splice variants, was developed and it was proven that serum ProGRP (31-98) levels serve as a reliable biomarker in small cell lung cancer (SCLC) patients.[2,5,6,7] The Elecsys ProGRP assay measures ProGRP (31-98) in plasma and serum.

ProGRP and neuron-specific enolase (NSE) are two molecules which are associated with neuroendocrine derived tissues and tumors. Increased levels of ProGRP have been reported in several neuroendocrine-derived tumor types, including small cell lung cancer, carcinoids, undifferentiated large cell carcinomas of the lung with neuroendocrine features, medullary thyroid carcinoma,[8] other neuroendocrine malignancies,[8] and in a subset of androgen-independent prostate cancer with neuroendocrine features.[9]

ProGRP in benign diseases:
ProGRP serum concentrations between 2 and 50 pg/mL are considered normal in the literature.[10] However, in a study on patients with benign diseases (excluding those with renal failure) including liver diseases, abnormal ProGRP serum levels > 50 pg/mL were found in 2.5 % of the patients. All levels were < 80 pg/mL. Renal failure was the only source of important increases in levels of this biomarker.[11] Elevated levels of ProGRP are highly specific for SCLC, and ProGRP has been reported to be the most sensitive biomarker for SCLC compared to benign diseases of the lung.[12] A clinical study with the Elecsys ProGRP assay (described in more detail in the section "Expected values") confirmed the high specificity for SCLC in differential diagnosis.

ProGRP in lung cancer:
ProGRP has been reported as a specific biomarker for SCLC, but abnormal levels may be found in a small subset of non-small cell lung cancer (NSCLC) patients. These concentrations are significantly lower than the ProGRP serum levels found in SCLC patients.[7] ProGRP serum levels correlate with tumor stage.[10]

ProGRP in differential diagnosis of lung cancer:
High levels of ProGRP (> 120 pg/mL) found in lung cancer patients (without renal failure) indicate a high probability of SCLC.[13]

ProGRP in malignancies other than lung cancer:
Elevated ProGRP serum levels are mainly found in patients with SCLC or neuroendocrine tumors.[14] An elevated ProGRP level in patients with well-differentiated neuroendocrine tumours indicates a primary tumour in the lung and is a factor for poor prognosis.[15] Slightly elevated serum ProGRP levels were found in patients with other malignancies without renal failure, but 99.7 % of them had levels < 100 pg/mL. Using a cutoff of 150 pg/mL as one of the criteria, ProGRP predicted a diagnosis of SCLC with a sensitivity of 72.5 %.[11]

ProGRP in monitoring SCLC patients:
Several investigators have reported that ProGRP is helpful in therapy monitoring of SCLC patients and for the detection of recurrent disease.[16,17] A clinical study with the Elecsys ProGRP assay (described in more detail in the section "Expected values") confirmed the utility of this biomarker in therapy- and follow-up monitoring with different therapy regimens.

ProGRP is suggested as the biomarker of choice in SCLC. This is supported by

- the sensitivity of ProGRP in SCLC, as well as its specificity in other malignancies,
- the normal values seen in most diseases, excluding renal failure and
- the absence of false positive results resulting from hemolysis, in addition to the increased discrimination between the normal range and the levels found in SCLC patients.

NSE can be a complementary biomarker in SCLC and combining NSE and ProGRP results in enhanced precision in the histological diagnosis, prognosis, and follow-up.[18]

ProGRP is elevated in early stage SCLC. However as the incidence of SCLC in the general population is low, Elecsys ProGRP assay testing is not recommended as a screening procedure in the general population.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal ProGRP-specific antibody, and a monoclonal ProGRP-specifc antibody labeled with a ruthenium complex[a)] 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 or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as ProGRP.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-ProGRP-Ab~biotin (gray cap), 1 bottle, 9 mL:

Biotinylated monoclonal anti-ProGRP antibody (mouse) 3.5 mg/L; phosphate buffer 40 mmol/L, pH 7.0; preservative.

R2 Anti-ProGRP-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 9 mL:

Monoclonal anti-ProGRP antibody (mouse) labeled with ruthenium complex 2.0 mg/L; phosphate buffer 40 mmol/L, pH 7.0; 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	42 days

Specimen collection and preparation

ProGRP can be degraded in serum by endogenous proteases which are generated during the clotting process. Therefore ProGRP is considered to be more stable in plasma than in serum. Plasma is the preferred sample material according to literature.[19] **However, it is possible to use serum for performing the Elecsys ProGRP assay as the antibodies in the assay bind in a region which is less susceptible to cleavage by proteases.**[1]

Only the specimens listed below were tested and found acceptable.

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

Li-heparin, K_2-EDTA and K_3-EDTA plasma as well as Li-heparin plasma tubes containing separating gel.

Criterion: Slope 0.9-1.1 + intercept ≤ ± 6 pg/mL, coefficient of correlation ≥ 0.95.

Stable for 72 hours at 2-8 °C, 9 hours at 20 °C, 12 weeks at -20 °C. The samples may be frozen twice.

(Acceptance criteria: For serum and plasma: < 60 pg/mL ± 6 pg/mL > 60 pg/mL ± 10 %. Additionally, for serum for up to 10 % of samples: < 60 pg/mL ± 18 pg/mL > 60 pg/mL ± 30 %.)

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 06505970190, ProGRP CalSet, for 4 x 1 mL
- REF 06505988190, PreciControl ProGRP, for 4 x 1 mL
- REF 07360070190, PreciControl Lung Cancer, for 4 x 3 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/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 (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: 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 ARCHITECT ProGRP assay from Abbott Diagnostics.

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 1 month (28 days) 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 Lung Cancer or PreciControl ProGRP.

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.

If necessary, repeat the measurement of the samples concerned.

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

Note: The controls are not barcode-labeled and therefore have to be run like external controls. All values and ranges have to be entered manually. Please refer to the section "QC" in the operator's manual or to the online help of the instrument software.

Non-barcode labeled controls: Only one target value and range for each control level can be entered in the analyzer. The reagent lot-specific target values have to be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run.

The exact lot-specific target values and ranges are printed on the enclosed (or electronically available) value sheet in the reagent kit or PreciControl kit.

Please make sure that the correct values are used.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in pg/mL.

Limitations - interference

The effect of the following substances and pharmaceuticals on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Criterion: Recovery ± 6 pg/mL of intial value ≤ 60 pg/mL and within ± 10 % of initial value > 60 pg/mL.

Substances

Compound	Concentration tested
Albumin	≤ 200 g/L
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Biotin	≤ 35 ng/mL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1.0 g/dL
IgA	≤ 10 g/L
IgG	≤ 17 g/L
IgM	≤ 2.9 g/L
Intralipid	≤ 2000 mg/dL
Rheumatoid factors	up to 540 IU/mL

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.

There is no high-dose hook effect at ProGRP concentrations up to 100000 pg/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

Commonly used pharmaceuticals

Pharmaceutical	Concentration tested
Acetaminophen	200 µg/mL
Acetylcystein	150 µg/mL
Acetylsalicylic acid	1000 µg/mL
Ampicillin-Na	1000 µg/mL
Ascorbic acid	300 µg/mL
Cefoxitin	2500 µg/mL
Cyclosporine	5 µg/mL
Doxycyclin	50 µg/mL
Heparin	5000 U/L
Ibuprofen	500 µg/mL
Levodopa	20 µg/mL
Methyldopa	20 µg/mL
Metronidazole	200 µg/mL
Phenylbutazone	400 µg/mL
Rifampicin	60 µg/mL
Theophylline	100 µg/mL

In addition, the following special drugs were tested. No interference with the assay was found.

Special drugs used in cancer treatment

Drug	Concentration tested
Avastin	750 µg/mL
Carboplatin	600 µg/mL
Cisplatin	180 µg/mL
Cyclophosphamide	500 µg/mL
Dexamethasone	20 µg/mL
Docetaxel	112.5 µg/mL
Doxorubicin	72 µg/mL
Epoetin	25 mU/L
Erlotinib	150 µg/mL
Etoposide	300 µg/mL
Gefitinib	250 µg/mL
Gemcitabine hydrochloride	1500 µg/mL
Ifosfamide	2400 µg/mL
Lomustine	172.5 µg/mL
Methotrexate	150 µg/mL
Metoclopramide	7.5 µg/mL
Neupogen	0.9 µg/mL
Paclitaxel	330 µg/mL
Topotecan hydrochloride	2.25 µg/mL
Vincristine sulfate	3.0 µg/mL
Vinorelbine tartrate	53.1 µg/mL

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.

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

3.00-5000 pg/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.00 pg/mL. Values above the measuring range are reported as > 5000 pg/mL (or up to 50000 pg/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

The Elecsys ProGRP assay is designed to have the following lower limits of measurement:

Limit of Blank = 2.00 pg/mL

Limit of Detection = 3.00 pg/mL

Limit of Quantitation = 7.00 pg/mL with a total allowable error of ≤ 30 %

The Limit of Blank, Limit of Detection and Limit of Quantitation 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 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 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 30 %.

A study was performed based on guidance from the CLSI, Protocol EP17-A2, using 5 equine serum samples and diluted human plasma samples each for Limit of Blank and Limit of Detection respectively. The samples were tested in 6 runs over 3 days on 2 analyzers resulting in n = 60 values. Limit of Blank and Limit of Detection were calculated to be 1.60 pg/mL and 2.23 pg/mL respectively. For Limit of Quantitation 3 human plasma samples were diluted and measured in 6 runs over 3 days on 2 analyzers. At a total allowable error of ≤ 30 % the Limit of Quantitation was 3.99 pg/mL.

Linearity

The Elecsys ProGRP assay is linear across the measuring range from 3.00-5000 pg/mL. Samples were prepared according to CLSI EP6-A by diluting 3 serum and 3 plasma samples each with Diluent MultiAssay in multiple steps ranging from > 5000 pg/mL downwards to Limit of Blank.

Dilution

Samples with ProGRP concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 500 pg/mL.

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

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

A study in Europe and China with the Elecsys ProGRP assay on 1085 samples from apparently healthy adults (607 females, 478 males) aged between 20 and 79 years yielded the following results (Roche study No. RD001525 and RD000788).

As renal failure is known to elevate ProGRP levels,[20] only patients with an eGFR (estimated Glomerular Filtration Rate) ≥ 30 calculated according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula[21] were taken into account in this study.

ProGRP values in serum and plasma (Europe and China combined):

	ProGRP (pg/mL)			
	5th percentile	Median	95th percentile (95 % CI[b)])	97.5th percentile (95 % CI)
Serum n = 1010	22.1	40.1	68.3 (64.2-74.4)	77.7 (74.4-86.5)
Li-heparin plasma n = 698	25.7	41.4	68.0 (63.7-74.5)	77.0 (73.0-101.1)
EDTA plasma n = 844	22.8	36.4	59.5 (55.8-63.4)	67.5 (63.4-76.9)

b) CI = confidence interval

ProGRP values (serum and plasma combined) according to study region:

	ProGRP (pg/mL)			
	5th percentile	Median	95th percentile (95 % CI)	97.5th percentile (95 % CI)
China n = 146	28.3	42.7	65.7 (59.9-86.5)	74.4 (65.2-107.1)
Europe n = 939	21.5	39.5	66.3 (62.8-72.6)	77.7 (74.5-92.1)

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

ProGRP values in apparently healthy individuals and different types of benign and malignant disorders

The distribution in percentage (%) of ProGRP assay values determined in 4 clinical centers in Europe and 2 clinical centers in China with the Elecsys ProGRP assay in 2767 serum samples is summarized in the table below:

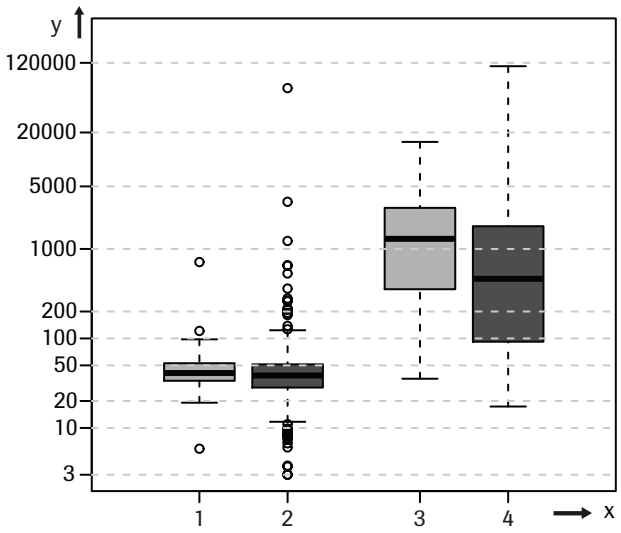
		Elecsys ProGRP values (pg/mL)				
	Total	< 3.0	3.0-50	50.1-100	100.1-200	> 200
		N (%)	N (%)	N (%)	N (%)	N (%)
Apparently healthy						
Smokers	192	0 (0)	153 (79.7)	39 (20.3)	0 (0)	0 (0)
Past smokers	74	0 (0)	57 (77.0)	16 (21.6)	1 (1.4)	0 (0)
Never smokers	618	0 (0)	474 (76.7)	136 (22.0)	8 (1.3)	0 (0)
Benign conditions						
Benign lung diseases	100	0 (0)	76 (76.0)	23 (23.0)	1 (1.0)	0 (0)
Renal diseases	9	0 (0)	5 (55.6)	3 (33.3)	1 (11.1)	0 (0)
Other benign diseases[c)]	143	0 (0)	113 (79.0)	28 (19.6)	2 (1.4)	0 (0)
Cancer						
SCLC	206	0 (0)	31 (15.0)	20 (9.7)	19 (9.2)	136 (66.0)
NSCLC	853	0 (0)	619 (72.6)	209 (24.5)	12 (1.4)	13 (1.5)
NSCLC/SCLC mix	8	0 (0)	2 (25.0)	2 (25.0)	0 (0)	4 (50.0)
Mesothelioma	28	0 (0)	25 (89.3)	3 (10.7)	0 (0)	0 (0)
Thyroid medullary	15	0 (0)	2 (13.3)	1 (6.7)	0 (0)	12 (80.0)
Neuroendocrine carcinoma	22	0 (0)	9 (40.9)	5 (22.7)	3 (13.6)	5 (22.7)
Breast	52	0 (0)	40 (76.9)	12 (23.1)	0 (0)	0 (0)
Ovarian	36	0 (0)	25 (69.4)	9 (25.0)	1 (2.8)	1 (2.8)
Prostate	31	0 (0)	18 (58.1)	9 (29.0)	4 (12.9)	0 (0)

		Elecsys ProGRP values (pg/mL)				
	Total	< 3.0	3.0-50	50.1-100	100.1-200	> 200
		N (%)	N (%)	N (%)	N (%)	N (%)
Colorectal	64	0 (0)	46 (71.9)	15 (23.4)	3 (4.7)	0 (0)
Other malignancies[d)]	115	0 (0)	86 (74.8)	25 (21.7)	4 (3.5)	0 (0)

c) Other benign diseases contain liver-, metabolic-, autoimmune- and inflammatory diseases. Benign lung diseases contain pneumonia, asthma, COPD and tuberculosis.

d) Other malignancies contain renal-, liver-, pancreatic-, gastrointestinal-, stomach- and cervical tumors and lymphoma.

Use of ProGRP for the primary differential diagnosis in lung cancer
The ability of ProGRP to distinguish SCLC from NSCLC was investigated in a study on 1059 patients in 5 centers in Europe and China (206 SCLCs and 853 NSCLCs), and ProGRP levels were correlated with biopsy proven histology. The distribution of values is shown in the box-plot and the 2 x 2 table below:



x = 1: NSCLC, China (n = 105),
2: NSCLC, Europe (n = 748),
3: SCLC, China (n = 37),
4: SCLC, Europe (n = 169)
y = ProGRP serum (pg/mL)

	NSCLC	SCLC	N
ProGRP ≤ 80.1 pg/mL	811	45	856 (80.8 %)
ProGRP > 80.1 pg/mL	42	161	203 (19.2 %)
N	853 (80.5 %)	206 (19.5 %)	1059 (100 %)

The cutoff value for a specificity of 95 % (based on NSCLC cohort) was 80.1 pg/mL. The correlation of Elecsys ProGRP values and stage for 189, 853, 100 patients with SCLC, NSCLC, and benign lung diesease, respectively is shown in the following table:

Clinical condition	N	5th percentile pg/mL	Median ProGRP pg/mL	95th percentile pg/mL
Stage I-II SCLC	11	17.9	75.9	1215
Stage III SCLC	66	32.9	545	3277
Stage IV SCLC	112	35.3	748	21410
Stage I-IV NSCLC	853	16.7	38.7	80.1
Benign lung disease	100	14.8	36.0	80.8

The sensitivity of ProGRP in patients with stage I-IV SCLC vs. NSCLC and vs. benign lung diseases at the prespecified specificity of 95 % as well as the area under the curve (AUC) values are shown in the table below.

	Cut-off pg/mL	Sensitivity %	AUC (95 % CI)
SCLC vs. NSCLC	80.1	78.2	0.898 (0.868-0.928)
SCLC vs. benign lung diseases	80.8	78.2	0.913 (0.882-0.943)
SCLC vs. other malignancies	191	66.0	0.864 (0.830-0.898)

Monitoring of therapy response in patients diagnosed with SCLC
The utility of the Elecsys ProGRP assay as an aid in monitoring of therapy response in SCLC patients was determined in a clinical study in 6 centers in Europe and China involving a total of 1209 blood collections from 314 patients in first- and second-line therapy (mainly platinum-doublet regimens and topotecan respectively). Imaging (CT-scan) was performed according to local standards. ProGRP levels were drawn around each imaging timepoint. Pretreatment ProGRP levels were correlated to ProGRP levels obtained at the time of maximum tumor response seen on imaging ("best response"; n = 215 patients).

The following table gives an overview of demographic variables in this analysis:

Variable		Europe	China	All sites
	N	145	70	215
Age	Mean	62.7	61.0	62.1
	Min/Max	36/83	27/83	27/38
Gender	Female	67	18	85
	Male	78	52	130
Ethnicity	Caucasian	128	0	128
	Chinese	0	70	70
Smoking habits	Current smokers	63	40	103
	Past smokers	39	13	52
	Never smokers	3	17	20
	No specifications	40	0	40
Treatment line at best response	1st-line therapy	88	57	145
	2nd-line therapy	36	7	43
	3rd-line and other therapy	21	6	27

A negative change in ProGRP was defined as percent decrease at time of best response compared to pretreatment levels. The following table displays respective sensitivity and specificity using various cutoff levels for

percent ProGRP decrease. Non-responders are defined as patients with tumor progression seen on imaging, as opposed to patients with tumor control ("responders").

Cutoff[e] % ProGRP	Sensitivity[f] %	Specificity[g] %	PPV[h] %	NPV[i] %
-50	82.8	65.6	27.3	96.1
-60	89.7	61.8	26.8	97.5
-70	89.7	55.4	23.9	97.2
-80	93.1	48.9	22.1	97.8
-90	96.6	39.8	20.0	98.7

e) Change from baseline to best response

f) Correctly detected non-responders

g) Potentially spared CT-scans in responders

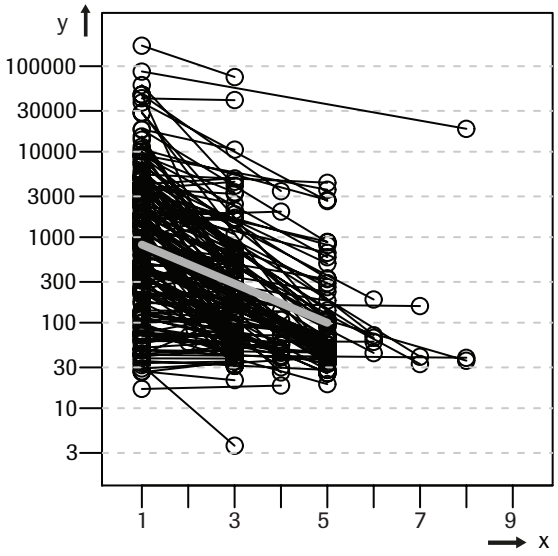
h) PPV = positive predictive value

i) NPV = negative predictive value

NPV and PPV are based on the prevalences of responders and non-responders observed in this clinical study.

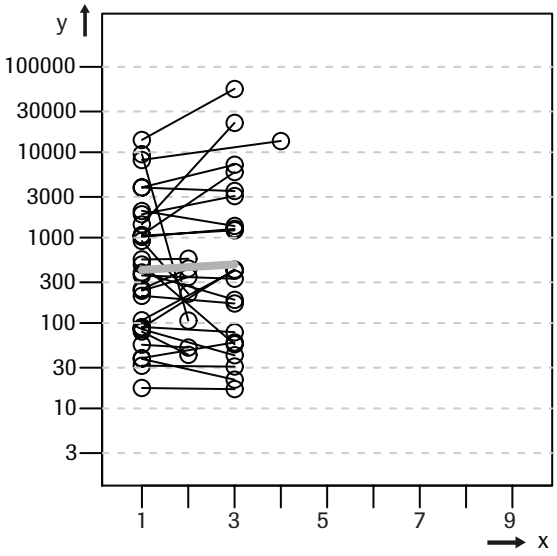
The following graph shows the changes in ProGRP levels from pretreatment to time of maximum response. The gray line represents the mean change for all patients included.

Responders, n = 186



x = visits, y = ProGRP (pg/mL)

Non-responders, n = 29

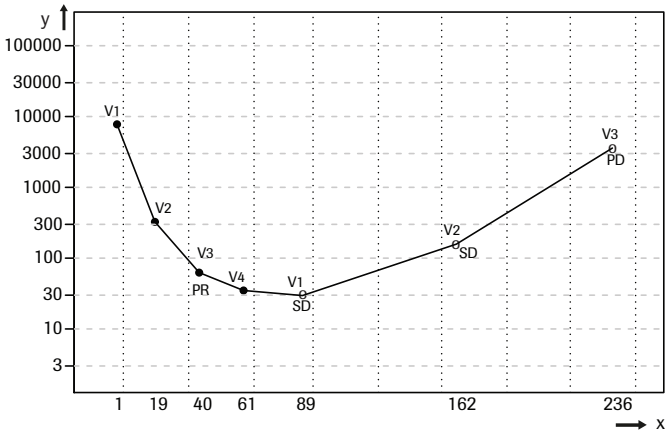


x = visits, y = ProGRP (pg/mL)

Follow-up monitoring of primarily treated SCLC patients

The utility of the Elecsys ProGRP assay as an aid in follow-up monitoring after chemotherapy is shown by an example below. The graph depicts the ProGRP values in a patient over time during first line treatment (carboplatin/etoposide regimen) and subsequent follow-up.

Female patient, 72 years
UICC (Union Internationale Contre le Cancer) stage: IV, TNM (Classification of Malignant Tumours) stage: T4_N3_M1b



x = days, y = ProGRP (pg/mL)

V = visit, PR = partial remission, SD = stable disease, PD = progressive disease

• = first-line therapy, o = follow-up

The following has to be taken into consideration

- If the ProGRP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Report the results in conjunction with the sample type.
- Increased concentrations of ProGRP have been observed in patients with renal dysfunction. There is a significant correlation between serum ProGRP levels and serum creatinine concentrations in patients with renal dysfunction.[20] The evaluation of serum creatinine levels should be considered in cases of high ProGRP levels that are not consistent with diagnostic and clinical characteristics of the patient.

- ProGRP levels, regardless of value, should not be interpreted as absolute evidence for the presence or absence of malignant disease. In patients with suspected or known cancer, other appropriate tests and procedures must also be considered for diagnosis and subsequent management.
- The concentration of ProGRP in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.

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, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human plasma 1	6.65	0.171	2.6	0.281	4.2
Human plasma 2	57.8	0.574	1.0	1.41	2.4
Human plasma 3	468	3.45	0.7	10.5	2.2
Human plasma 4	2420	18.8	0.8	52.0	2.1
Human plasma 5	4520	41.4	0.9	109	2.4
PC[j] ProGRP1	35.7	0.250	0.7	1.14	3.2
PC ProGRP2	609	4.59	0.8	20.3	3.3
PC LC[k]1	61.1	0.901	1.5	2.10	3.4
PC LC2	822	7.83	1.0	13.8	1.7

j) PC = PreciControl

k) LC = Lung Cancer

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human plasma 1	7.20	0.270	3.7	0.491	6.8
Human plasma 2	61.0	0.577	0.9	1.83	3.0
Human plasma 3	478	5.03	1.1	17.7	3.7
Human plasma 4	2510	23.8	0.9	83.8	3.3
Human plasma 5	4180	73.0	1.7	157	3.8
PC ProGRP1	39.4	0.457	1.2	1.62	4.1
PC ProGRP2	678	5.19	0.8	24.1	3.6
PC LC1	63.9	0.828	1.3	2.16	3.4
PC LC2	872	6.63	0.8	19.1	2.2

Method comparison

A comparison of the Elecsys ProGRP assay on the **cobas e** 601 analyzer (y) with a manual ProGRP ELISA (x) gave the following correlations:

Number of serum samples measured: 153

Passing/Bablok[22]	Linear regression
y = 1.49x + 4.38	y = 1.40x + 13.2
τ = 0.800	r = 0.988

The sample concentrations were between approximately 3 and 4384 pg/mL.

A comparison of the Elecsys ProGRP assay on the **cobas e** 601 analyzer (y) with an automated ProGRP method (x) gave the following correlations:

Number of K_2-EDTA plasma samples measured: 166

Passing/Bablok[22]	Linear regression
y = 0.997x - 2.68	y = 1.00x - 4.83
τ = 0.852	r = 0.996

The sample concentrations were between approximately 3 and 4068 pg/mL.

Analytical specificity

The specificity of the Elecsys ProGRP assay is designed to have ≤ 1 % cross-reactivity when tested with gastrin-releasing peptide (GRP) at a concentration of 100 ng/mL. A study was performed with the Elecsys ProGRP assay based on guidance from the CLSI, Protocol EP7-A2. Aliquots of human plasma, containing ProGRP across the range from 34 to 107 pg/mL, were supplemented with GRP at a concentration of 400 ng/mL and tested for ProGRP. Cross-reactivity of GRP was calculated to be less than 0.01 %.[l]

l) Representative data; results in individual laboratories may vary from these data.

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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.

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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
GTIN	Global Trade Item Number

CE

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