

REF		SYSTEM
07125933 190	100	MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of Growth Differentiation Factor-15 (GDF-15) in human serum and plasma.

The Elecsys GDF-15 assay is intended as an aid in risk stratification of patients with Acute Coronary Syndrome (ACS) or Chronic Heart Failure (CHF).

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

GDF-15 is a member of the transforming growth factor β (TGF- β) cytokine superfamily.

GDF-15 levels increase sharply in response to pathological or physiological stress associated with inflammation, oxygen deficiency, tissue injury and remodelling as observed in cardiovascular diseases, as well as in some tumors and pregnancy.^{1,2} Induction of GDF-15 in response to cardiac injury has been shown to be cardioprotective to ischemia/reperfusion injury.² Levels of GDF-15 increase with the severity of cardiovascular diseases: elevated serum levels are found in stable coronary artery disease, ACS and heart failure (HF).²

Increasing evidence indicates that GDF-15 levels predict adverse outcomes of cardiovascular disease, independently from traditional risk factors such as previous myocardial infarction (MI), age, elevated levels of cardiac troponin T, N-terminal pro B-type natriuretic peptide, or high-sensitivity C-reactive protein. Increased GDF-15 levels are indicative of high mortality in patients with ST-segment elevation ACS (STE-ACS),³ non-ST-elevation ACS (NSTEMI-ACS)^{4,5,6} and HF.^{7,8} Higher levels of GDF-15 also identify NSTEMI-ACS patients at an elevated risk of recurrent MI^{4,6} or stroke.⁶

Studies have suggested a relationship between elevated GDF-15 levels and stroke mortality in atrial fibrillation.⁹ GDF-15 levels have been shown to correlate independently with a subsequent risk of serious bleeding complications in patients with atrial fibrillation treated with oral anticoagulants.⁹

GDF-15 provides prognostic information additional to that provided by established clinical risk scores. Adding GDF-15 levels to a troponin-modified Global Registry of Acute Coronary Events (GRACE) score further improves the prediction of 6-month all-cause mortality and non-fatal MI in patients with NSTEMI-ACS.¹⁰ GDF-15 levels may also assist in guiding therapeutic intervention: GDF-15 levels in patients with NSTEMI-ACS at admission predict those likely to benefit most from an intensive treatment regime.⁴ High levels of GDF-15 are also associated with increased risk of developing HF following an episode of ACS.² Therefore GDF-15 levels potentially allow to identify which ACS patients will benefit from more aggressive therapies aimed at reducing HF-related admissions.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Antigen in the sample (35 μ L), a biotinylated monoclonal GDF-15-specific antibody, and a monoclonal GDF-15-specific 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.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

The reagent rackpack is labeled as GDF-15.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-GDF-15-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-GDF-15 antibody (mouse) 1.5 μ g/mL; phosphate buffer 95 mmol/L, pH 6.0; preservative.
- R2 GDF-15-Ab~Ru(bpy)₃²⁺ (black cap), 1 bottle, 8 mL: Monoclonal anti-GDF-15 antibody (mouse) labeled with ruthenium complex 2.0 μ g/mL; phosphate buffer 95 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	8 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.

Li-heparin, K₂-EDTA and K₃-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within \pm 160 pg/mL + coefficient of correlation \geq 0.95.

Stable for 6 days at 15-25 °C, 8 days at 2-8 °C, 12 months at -20 °C (\pm 5 °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 samples and controls stabilized with azide.

Elecsys GDF-15

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] 07125941190, CalSet GDF-15, for 4 x 1.0 mL
- [REF] 04917049190, PreciControl Cardiac II, for 2 x 2.0 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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, 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.

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 by weighing recombinant GDF-15 into equine serum.

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 Cardiac II.

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 in pg/mL.

Interpretation of the results

With increasing age atherosclerosis and aging processes of the heart (e.g. fibrosis) result in cardiac dysfunction. Development of cardiac dysfunction is individually different and clinically asymptomatic in early stages.^{11,12,13} Elevated GDF-15 levels are more frequently found in apparently healthy individuals of higher ages and thus reflect the increasing frequency of cardiac stress and cardiovascular dysfunction.

GDF-15 values need to be interpreted in conjunction with the medical history, clinical findings and other information (e.g. imaging, laboratory findings, accompanying disorders, treatment effects).

Limitations - interference

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 65 g/L
IgM	≤ 10 g/L
IgA	≤ 16 g/L
Albumin	≤ 70 g/L

Criterion: Recovery within ± 80 pg/mL for GDF-15 concentrations ≤ 800 pg/mL or ± 10 % for concentrations > 800 pg/mL to 2000 pg/mL or ± 14 % for concentrations > 2000 pg/mL 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.

There is no high-dose hook effect at GDF-15 concentrations up to 150000 pg/mL.

Pharmaceutical substances

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

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

Special cardiac drugs

Drug	Concentration tested mg/L
Carvedilol	37.5
Clopidogrel	75.0
Digoxin	0.25

Drug	Concentration tested mg/L
Epinephrine (Adrenaline)	0.37
Insulin	0.84
Lidocaine	80.0
Lisinopril	10.0
Methylprednisolone	7.50
Metoprolol	15.0
Nifedipine	30.0
Phenprocoumon (Marcumar)	3.00
Propafenone	300
Retepase	1.12
Simvastatin	30.0
Spironolactone	75.0
Tolbutamide	1500
Torasemide	15.0
Verapamil	120

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

400-20000 pg/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 400 pg/mL. Values above the measuring range are reported as > 20000 pg/mL (or up to 100000 pg/mL for 5-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 350 pg/mL

Limit of Detection = 400 pg/mL

Limit of Quantitation = 400 pg/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ 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 the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with GDF-15 concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:5 (either automatically by the MODULAR ANALYTICS E170 or **cobas e** analyzers or manually). The concentration of the diluted sample must be ≥ 3500 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

Circulating levels of GDF-15 were determined in 739 samples from apparently healthy volunteers. The subjects were clinically well characterized and between 20 and 79 years old. All subjects were without

known cardiac risks, symptoms or medical history. Male and female gender was distributed equally and no difference was observed.

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

The descriptive statistics for GDF-15 concentrations in the reference group are summarized in the following table:

Age years	N	Mean pg/mL	SD pg/mL	Median pg/mL	95 th percentile pg/mL
20-< 30	127	514	273	429	831
30-< 40	120	564	223	500	852
40-< 50	125	660	266	614	1229
50-< 60	119	807	285	757	1466
60-< 70	122	937	306	866	1476
≥ 70	126	1187	547	1060	2199

GDF-15 values in cardiovascular diseases

GDF-15 derived risk stratification for CHF patients

A clinical performance study, based on samples from the HF ACTION sub study^{14,15} was designed to evaluate GDF-15 as a risk predictor and to compare its performance to established risk predictors. The primary endpoint was all-cause death. The secondary endpoints were cardiovascular (CV) death and heart failure (HF) hospitalization. Hierarchical Cox proportional hazards models were created where covariates were successively added for each of the endpoints. The following covariates were used: Demographics (Dem): age, sex, race, BMI (body mass index) and smoking status; Clinical parameters (Clin): etiology, NYHA class ≥ 3 , ejection fraction, aldosterone antagonist, ACEi/ARB (angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker), loop diuretic, beta blocker dose, hypertension, diabetes, heart rate, sodium, and glomerular filtration rate (GFR); Biomarkers: NT-proBNP, hsTnT. In total, 910 patients were included in the analysis and followed-up for 4 years.

Correlation of GDF-15 levels with NYHA classification in patients diagnosed with CHF

Increasing GDF-15 values (pg/mL) correlate with higher NYHA classification:

	NYHA functional class		
	NYHA II	NYHA III	NYHA IV
N* (%)	569 (62.5)	329 (36.2)	12 (1.3)
Mean (pg/mL)	1958	2712	5619
SD (pg/mL)	2000	2503	5559
Median (pg/mL)	1425	1985	3231
5 th percentile (pg/mL)	527	615	648
95 th percentile (pg/mL)	5151	6956	≥ 20000

*Total number (N) of the sub study population $n = 910$. No NYHA I patient present.

Relationship of log₂-transformed GDF-15 levels with all-cause death alone and with the addition of covariates

GDF-15 independently contributes to risk prediction of all-cause death in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI)* of log ₂ -transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	1.96 (1.70, 2.26)	< 0.0001	0.718 (0.672, 0.763)
GDF-15 + Dem	1.87 (1.58, 2.21)	< 0.0001	0.725 (0.679, 0.771)
GDF-15 + Dem + Clin	1.69 (1.39, 2.07)	< 0.0001	0.743 (0.700, 0.787)
GDF-15 + Dem + Clin + NT-proBNP	1.38 (1.11, 1.72)	0.0042	0.769 (0.728, 0.810)
GDF-15 + Dem + Clin + hsTnT	1.55 (1.25, 1.92)	< 0.0001	0.752 (0.709, 0.795)

Model	HR (95 % CI)* of log2-transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.34 (1.07, 1.69)	0.0120	0.771 (0.729, 0.812)

*CI = confidence interval

Relationship of log2-transformed GDF-15 levels with CV death alone and with the addition of covariates

GDF-15 independently contributes to risk prediction of CV death in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI) of log2-transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	2.10 (1.77, 2.48)	< 0.0001	0.731 (0.677, 0.785)
GDF-15 + Dem	2.01 (1.64, 2.46)	< 0.0001	0.747 (0.693, 0.801)
GDF-15 + Dem + Clin	1.84 (1.44, 2.34)	< 0.0001	0.787 (0.741, 0.834)
GDF-15 + Dem + Clin + NT-proBNP	1.48 (1.14, 1.94)	0.0038	0.810 (0.766, 0.854)
GDF-15 + Dem + Clin + hsTnT	1.71 (1.32, 2.21)	< 0.0001	0.793 (0.747, 0.838)
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.47 (1.12, 1.95)	0.0063	0.810 (0.766, 0.854)

Relationship of log2-transformed GDF-15 levels with HF hospitalization alone and with the addition of covariates

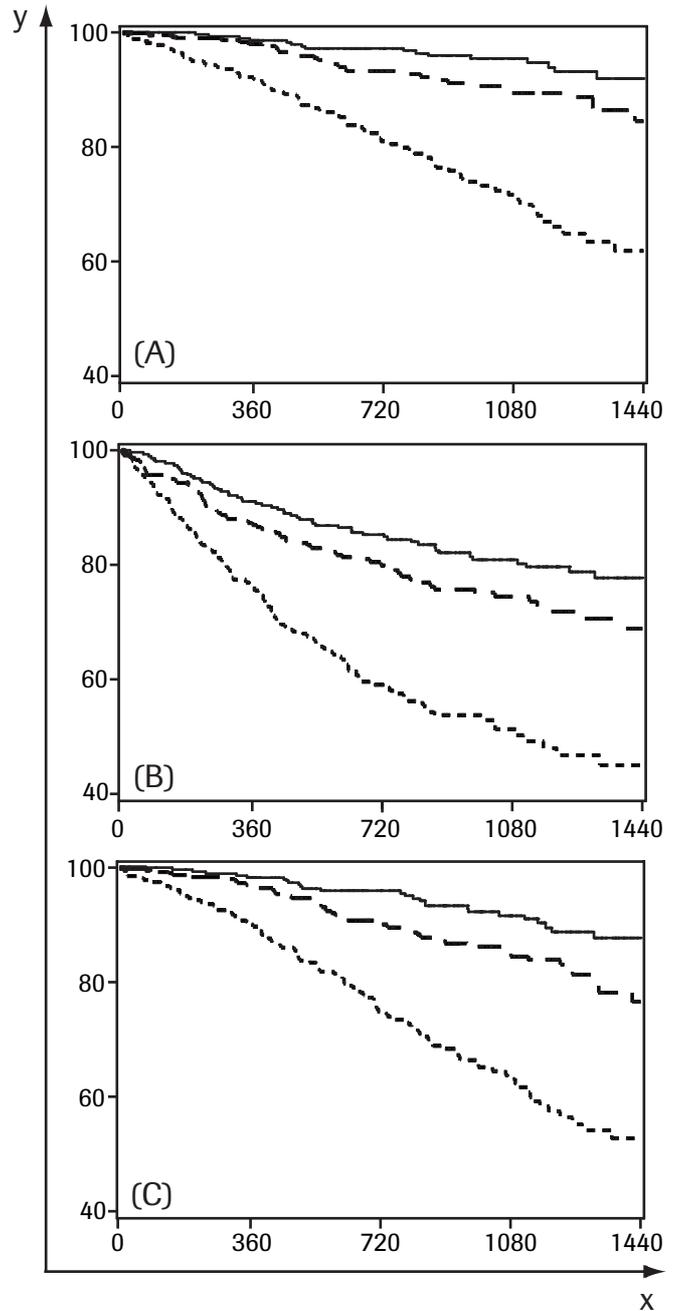
GDF-15 independently contributes to risk prediction of HF hospitalization in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI) of log2-transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	1.59 (1.41, 1.80)	< 0.0001	0.639 (0.599, 0.680)
GDF-15 + Dem	1.69 (1.47, 1.94)	< 0.0001	0.688 (0.653, 0.723)
GDF-15 + Dem + Clin	1.50 (1.26, 1.79)	< 0.0001	0.725 (0.690, 0.759)
GDF-15 + Dem + Clin + NT-proBNP	1.23 (1.02, 1.48)	0.0341	0.756 (0.725, 0.787)
GDF-15 + Dem + Clin + hsTnT	1.40 (1.17, 1.68)	0.0003	0.734 (0.700, 0.768)
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.20 (0.99, 1.46)	0.0591	0.758 (0.727, 0.789)

These results demonstrate that GDF-15 provides significant independent prognostic information in patients with HF for the period of 4 years.

Similar models were created with categorized GDF-15 levels to define cutoffs separating patient groups with high-, intermediate-, and low risk for all-cause and CV death as well as HF hospitalization. Creating two binary GDF-15 factors using the rounded tertiles 1200 pg/mL and 2300 pg/mL as cutoffs, i.e. comparing low versus intermediate/high GDF-15 levels and low/intermediate versus high GDF-15 levels, in two separate models, resulted in HRs of 3.10 (≥ 1200 pg/mL) and 3.40 (≥ 2300 pg/mL) for **all-cause death**, for **CV death** HRs were 3.55 (≥ 1200 pg/mL) and 3.81 (≥ 2300 pg/mL) and for **HF hospitalization** HRs were 2.06 (≥ 1200 pg/mL) and 2.50 (≥ 2300 pg/mL).

A similar model with a three category GDF-15 (low, intermediate and high) factor for **all-cause death** (see plot A below) resulted in HRs of 1.81 (intermediate vs. low) and 4.72 (high vs. low), for **CV death** (see plot B below) the HRs were 1.95 (intermediate vs. low) and 5.55 (high vs. low), and for **HF hospitalization** (see plot C below) the HRs were 1.38 (intermediate vs. low) and 2.94 (high vs. low).



x = days
 y = (A) % free from all-cause death
 (B) % free from CV death
 (C) % free from HF hospitalization

—: < 1200 pg/mL
 - - -: 1200-2299 pg/mL
 . . . : ≥ 2300 pg/mL

GDF-15 derived risk stratification for patients with Non-ST Elevation (NSTEMI) Acute Coronary Syndrome (ACS)

A clinical performance study using samples from a biomarker sub study of the MERLIN TIMI 36[®] trial in NSTEMI-ACS patients was designed to evaluate GDF-15 as a risk predictor and to compare its performance to established risk predictors. The primary endpoint was all-cause death and the secondary endpoints were cardiovascular (CV) death and the composite of CV death/myocardial infarction (MI). Hierarchical Cox proportional hazards models were created where covariates are added successively for each of the endpoints. The following covariates were used: Demographics (Dem): age, gender, BMI (body mass index), and smoking status; Clinical parameters (Clin): eGFR, history of HF, and TIMI risk score; Biomarkers: NT-proBNP, hsTnT. In total, 4330 patients were included in the analysis and followed-up for an average of 1 year.

Relationship of log₂-transformed GDF-15 levels with all-cause death alone and with the addition of covariates

GDF-15 independently contributes to risk prediction of all-cause death in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI) of log ₂ -transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	2.503 (2.209, 2.836)	< 0.0001	0.7397 (0.7061, 0.7732)
GDF-15 + Dem	2.109 (1.824, 2.439)	< 0.0001	0.7589 (0.7265, 0.7913)
GDF-15 + Dem + Clin	1.899 (1.614, 2.235)	< 0.0001	0.7823 (0.7513, 0.8132)
GDF-15 + Dem + Clin + NT-proBNP	1.462 (1.223, 1.749)	< 0.0001	0.8190 (0.7913, 0.8468)
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.475 (1.233, 1.764)	< 0.0001	0.8195 (0.7914, 0.8476)

Relationship of log₂-transformed GDF-15 levels with CV death alone and with the addition of covariates

GDF-15 independently contributes to risk prediction of CV death in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI) of log ₂ -transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	2.457 (2.151, 2.806)	< 0.0001	0.7333 (0.6974, 0.7693)
GDF-15 + Dem	2.071 (1.774, 2.418)	< 0.0001	0.7576 (0.7228, 0.7923)
GDF-15 + Dem + Clin	1.784 (1.498, 2.125)	< 0.0001	0.7898 (0.7574, 0.8223)
GDF-15 + Dem + Clin + NT-proBNP	1.373 (1.133, 1.665)	0.0012	0.8231 (0.7940, 0.8523)
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.386 (1.143, 1.681)	0.0009	0.8247 (0.7953, 0.8542)

Relationship of log₂-transformed GDF-15 levels with composite CV death/MI alone and with the addition of covariates

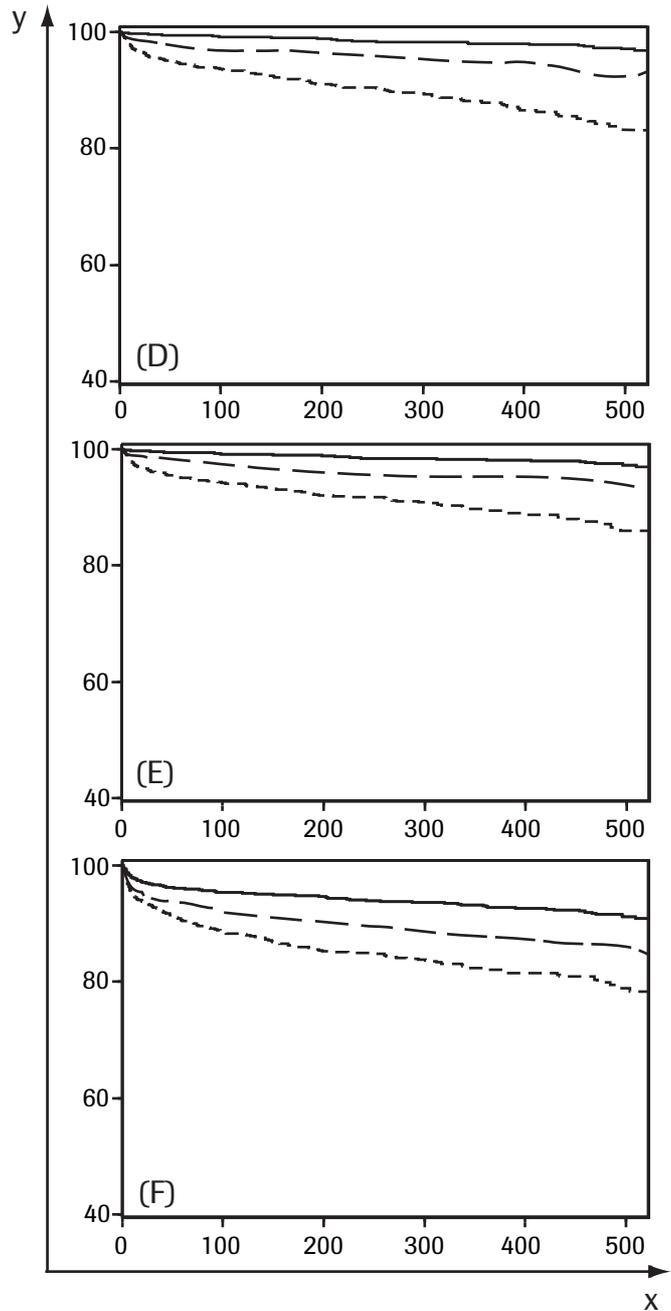
GDF-15 independently contributes to risk prediction of composite CV death/MI death in a Cox proportional hazard ratio (HR) adjusted for Dem, Clin and biomarkers:

Model	HR (95 % CI) of log ₂ -transformed GDF-15 levels	p-value	C-statistic 95 % CI
GDF-15 only	1.766 (1.600, 1.950)	< 0.0001	0.6328 (0.6062, 0.6595)
GDF-15 + Dem	1.584 (1.413, 1.777)	< 0.0001	0.6513 (0.6251, 0.6776)
GDF-15 + Dem + Clin	1.394 (1.232, 1.578)	< 0.0001	0.6831 (0.6569, 0.7093)
GDF-15 + Dem + Clin + NT-proBNP	1.183 (1.036, 1.351)	0.0132	0.7087 (0.6838, 0.7336)
GDF-15 + Dem + Clin + NT-proBNP + hsTnT	1.190 (1.042, 1.359)	0.0103	0.7110 (0.6864, 0.7356)

These results demonstrate that GDF-15 provides significant independent prognostic information in patients with NSTEMI-ACS for an average of 1 year.

Similar models were created with categorized GDF-15 levels to define cutoffs separating patient groups with high-, intermediate-, and low risk for all-cause and CV death as well as the composite of CV death/MI. Creating two binary GDF-15 factors using the rounded tertiles 1200 pg/mL and 1800 pg/mL as cutoffs, i.e. comparing low versus intermediate/high GDF-15 levels and low/intermediate versus high GDF-15 levels, in two separate models, resulted in HRs of 4.34 (≥ 1200 pg/mL) and 4.17 (≥ 1800 pg/mL) for **all-cause death**, for **CV death** HRs were 4.03 (≥ 1200 pg/mL) and 3.80 (≥ 1800 pg/mL) and for the composite of **CV death/MI** HRs were 2.16 (≥ 1200 pg/mL) and 2.13 (≥ 1800 pg/mL).

A similar model with a three category GDF-15 (low, intermediate and high) factor for **all-cause death** (see plot A below) resulted in HRs of 2.63 (intermediate vs. low) and 6.32 (high vs. low), for **CV death** (see plot B below) the HRs were 2.57 (intermediate vs. low) and 5.79 (high vs. low), and for the composite of **CV death/MI** (see plot C below) the HRs were 1.78 (intermediate vs. low) and 2.72 (high vs. low).



x = days
 y = (D) % free from all-cause death
 (E) % free from CV death
 (F) % free from CV death/MI

—: < 1200 pg/mL
 - - -: 1200-1799 pg/mL
 . . . : ≥ 1800 pg/mL

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-A3) 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 analyzer					
Sample	Mean pg/mL	Repeatability		Intermediate precision	
		SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	460	6.75	1.5	14.0	3.1
Human serum 2	1148	8.61	0.7	29.0	2.5
Human serum 3	1673	20.6	1.2	45.6	2.7
Human serum 4	4952	64.4	1.3	129	2.6
Human serum 5	9720	77.5	0.8	234	2.4
Human serum 6	18690	239	1.3	538	2.9
PC ^{b)} CARDII1	1329	13.9	1.0	31.7	2.4
PC CARDII2	7211	71.5	1.0	176	2.4

b) PC = PreciControl

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean pg/mL	Repeatability		Intermediate precision	
		SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	445	6.70	1.5	21.9	4.9
Human serum 2	1153	11.0	1.0	47.0	4.1
Human serum 3	1664	16.5	1.0	62.7	3.8
Human serum 4	4880	48.3	1.0	162	3.3
Human serum 5	9520	102	1.1	335	3.5
Human serum 6	18290	238	1.3	617	3.4
PC CARDII1	1280	20.4	1.6	58.5	4.6
PC CARDII2	7070	81.0	1.1	283	4.0

Analytical specificity

No significant cross-reactivity was found for Tumor necrosis factor- β (< 0.2 %; tested concentration 100 ng/mL) and C-reactive protein (< 0.001 %; tested concentration 200 mg/L).

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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:

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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