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Myoglobin STAT

cobas®

Myoglobin (STAT "Short Turn Around Time")

REF	Σ	SYSTEM
11820788 122	100	Elecsys 2010 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of myoglobin in human serum and plasma.

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

Summary

Myoglobin is a cytoplasmic protein in striated cardiac and skeletal musculature. It is involved in the transport of oxygen within the myocytes and also serves as an oxygen reservoir. Myoglobin has a molecular weight of 17.8 kD and is hence small enough to pass rapidly into the circulation following damage to myocytes.[1]

The determination of myoglobin in serum is an important factor in the diagnosis of acute myocardial infarction (AMI),[2,3] early reinfarction[1,4,5] and successful reperfusion following lysis therapy.[6,7,8] The myoglobin concentration rises already after approximately 2 hours following the occurrence of symptoms, and is therefore regarded as a very early marker of myocardial infarction. Depending on the therapeutic reperfusion measures taken, myoglobin reaches its maximum concentration in the circulation 4-12 hours after the commencement of infarction and falls back to normal levels after about 24 hours.[9]

Elevated myoglobin values can also occur after skeletal muscle damage and in cases of greatly restricted renal function.

The Elecsys Myoglobin STAT assay is based on the sandwich principle using two different monoclonal antibodies directed against human myoglobin.

Test principle

Sandwich principle. Total duration of assay: 9 minutes.

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

- 1st incubation: Antigen in the sample (15 µL), a biotinylated monoclonal myoglobin-specific antibody, and a monoclonal myoglobin-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.

cobas e 601 and **cobas e** 602 analyzers:

- During a 9 minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal myoglobin-specific antibody, a monoclonal myoglobin-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- 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 MYO-STAT.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-myoglobin-Ab~biotin (gray cap), 1 bottle, 10 mL:

Biotinylated monoclonal anti-myoglobin antibody (mouse) 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; preservative.

R2 Anti-myoglobin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:

Monoclonal anti-myoglobin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; 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-, Na-, NH_4^+-heparin, K_3-EDTA and sodium citrate plasma.

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 1 week at 2-8 °C, 3 months at -20 °C.[10]

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 > 0.09 %.

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.

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Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11820893122, Myoglobin STAT CalSet, 4 x 1 mL
- REF 04917049190, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 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
- Elecsys 2010 or **cobas e** analyzer

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

- 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 **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 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 prior to use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

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 an inhouse reference preparation.

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 8 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 ng/mL.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.869 mmol/L or < 1.4 g/dL), lipemia (Intralipid < 2200 mg/dL) and biotin (< 205 nmol/L or < 50 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 myoglobin concentrations up to 30000 ng/mL.

In vitro tests were performed on 50 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.

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

21-3000 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 21 ng/mL. Values above the measuring range are reported as > 3000 ng/mL (or up to 30000 ng/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: ≤ 21 ng/mL

The lower 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, repeatability study, n = 21).

Dilution

Samples with myoglobin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 50 ng/mL.

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

After dilution by the analyzers, the Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

In studies with the Elecsys Myoglobin STAT assay on 2162 healthy test subjects the following data were obtained:

	Number	2.5-97.5th Percentile
Men	1030	28-72 ng/mL
Women	1132	25-58 ng/mL

Data (status July 1999) combined from: Multicenter Evaluation of the Elecsys Myoglobin STAT assay, April 1999, and International Elecsys 1010 Study, Cardiac Markers, March 1999.

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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 a modified protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	33.9	0.57	1.7	0.72	2.1
Human serum 2	1016	17.7	1.8	22.3	2.2
Human serum 3	2468	54.6	2.2	63.6	2.6
PreciControl Card1	90.1	1.04	1.2	1.2	1.3
PreciControl Card2	1171	12.9	1.1	15.0	1.3

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	31.9	0.63	2.0	0.78	2.4
Human serum 2	60.5	0.92	1.5	1.17	1.9
Human serum 3	278	5.20	1.9	5.73	2.1
Human serum 4	603	7.28	1.2	10.3	1.7
Human serum 5	2245	41.0	1.8	53.2	2.4
PreciControl Card1	91.7	0.96	1.1	1.33	1.5
PreciControl Card2	1181	12.1	1.0	16.9	1.4

Method comparison

A comparison of the Elecsys Myoglobin STAT assay (y) with the Tina-quant Myoglobin test (x) using clinical samples gave the following correlations:

Number of paired values: 398

Passing/Bablok[11]	Linear regression
y = 1.01x - 0.13	y = 1.0x + 1.28
τ = 0.796	r = 0.996

The sample concentrations were between approximately 25 and 595 ng/mL.

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

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