Elecsys CA 19-9

cobas®

REF		Σ	SYSTEM
07027028190	07027028500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
CA 19-9	10019

Please note

The measured CA 19-9 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 CA 19-9 assay method used. CA 19-9 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 CA 19-9 assay procedure used while monitoring therapy, then the CA 19-9 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 CA 19-9 in human serum and plasma.

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

Summary

CA 19-9 (carbohydrate antigen 19-9 or sialylated Lewis (a) antigen) is a biomarker which is primarily used in the management of pancreatic cancer patients in addition to other diagnostic methods.[1] The CA 19-9 antibody binds to the Lewis (a) antigen on a mucin.[2,3] Elevated concentrations are frequently present in the blood of patients with various gastrointestinal conditions, such as pancreatic-, colorectal-, gastric-, hepatocellular- and cholangiocellular carcinomas.[4]

No data exist today which support the use of CA 19-9 in screening for malignancies[5] also concerning the fact that approximately 6 % of the population belong to the Lewis (a-/b-) blood group, lacking the antigenic determinant CA 19-9 and will therefore not release CA 19-9 even when a malignancy is present. This must be taken into account when interpreting the findings.[6]

Among non-malignant conditions, obstructive jaundice is frequently associated with increases in CA 19-9[7] and unspecific elevation of CA 19-9 in serum reflects both inflammatory hypersecretion and leakage of biliary mucins into serum.[8] CA 19-9 levels have also been reported in benign diseases like cystic fibrosis, hydronephrosis, and Hashimoto's thyroiditis.[9]

In addition, there is a strong correlation between the serum CA 19-9 concentration and the degree of cholestasis as well as the levels of alkaline phosphatase and bilirubin during acute liver failure, acute hepatitis or chronic liver diseases.[10,11] The common underlying mechanism for elevations in non-malignant conditions is probably inflammatory hypersecretion of CA 19-9 by epithelial cells.

In pancreatic cancer, levels > 100 U/mL are highly suggestive of unresectablity or metastatic disease and levels < 100 U/mL imply a likely resectable disease.[12]

The European Group of Tumor Markers (EGTM) advise that CA 19-9 may be used as a diagnostic aid and for monitoring therapy in patients with pancreatic adenocarcinoma.[13] CA 19-9 has been found to be prognostic for survival following resection of pancreatic ductal adenocarcinoma.[14]

In hepatobiliary carcinoma, CA 19-9 independently predicted a 2.6-fold increased mortality in a prospectively collected group of HCC patients in a multivariable analysis.[15] In colorectal cancer, CA 19-9 is described as an additional marker for disease monitoring in patients without an increase in CEA.[16]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample, a biotinylated monoclonal CA 19-9-specific antibody, and a monoclonal CA 19-9-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 II 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 **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as CA19-9.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CA 19-9-Ab~biotin, 1 bottle, 18.8 mL:
Biotinylated monoclonal anti-CA 19-9 antibody (mouse) 3 mg/L, phosphate buffer 100 mmol/L, pH 6.5; preservative.

R2 Anti-CA 19-9-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 21.0 mL:
Monoclonal anti-CA 19-9 antibody (mouse) labeled with ruthenium complex 4 mg/L; phosphate buffer 100 mmol/L, pH 6.5; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

Elecsys CA 19-9

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P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
on the analyzers	16 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, K_2-EDTA and K_3-EDTA plasma.

Do not use sodium citrate plasma.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 14 days at 2-8 °C, 5 days at 20-25 °C, 3 months at -20 °C (± 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 heat-inactivated samples.

Do not use samples and controls stabilized with azide.

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

Due to possible evaporation effects, samples and calibrators 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 11776215122, CA 19-9 CalSet, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Enzymun-Test CA 19-9 method.

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 **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker.

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 **cobas e** pack, 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.

Calculation

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

Limitations - interference

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

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 409 nmol/L or ≤ 100 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery ± 4.5 U/mL of initial value for samples ≤ 30 U/mL and within ± 15 % of initial value for samples > 30 U/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 CA 19-9 concentrations up to 500000 U/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 cancer drugs were tested. No interference with the assay was found.

Special cancer drugs

Drug	Concentration tested mg/L
Doxorubicin	75
Cyclophosphamide	1000
Cisplatin	225
5-Flourouracil	500
Methotrexate	1000
Tamoxifen	50
Mitomycin	25
Carboplatin	1000
Etoposide	400
Flutamide	1000
Taxol	5.5

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

2-1000 U/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 2 U/mL. Values above the measuring range are reported as > 1000 U/mL or up to 10000 U/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 1.5 U/mL

Limit of Detection = 2 U/mL

Limit of Quantitation = 9 U/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 ≥ 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 %.

An internal study was performed based on guidance from the CLSI, protocol EP17-A2. Limit of Blank and Limit of Detection were calculated to be the following:

Limit of Blank = 0.876 U/mL

Limit of Detection = 1.89 U/mL

For Limit of Quantitation ≥ 4 human serum samples were measured over 5 days with 5 replicates on one analyzer. With an intermediate precision of ≤ 20 % the Limit of Quantitation was 2.72 U/mL.

Dilution

Samples with CA 19-9 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 50 U/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.

Note: The CA 19-9 antigen tends to aggregate.[17] This may lead to non-linear dilution behavior in certain individual samples.

Expected values

In samples from 381 healthy test subjects (n = 187) and blood donors (n = 194), the following values were obtained:

27 U/mL (95th percentile)

34 U/mL (97.5th percentile)

39 U/mL (99th percentile)

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, samples and controls in a protocol (EP05-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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	4.00	0.106	2.7	0.119	3.0
Human serum 2	8.69	0.143	1.6	0.150	1.7
Human serum 3	21.0	0.235	1.1	0.287	1.4
Human serum 4	34.1	0.405	1.2	0.435	1.3
Human serum 5	498	5.90	1.2	7.15	1.4
Human serum 6	910	11.6	1.3	15.7	1.7
PreciControl TM[b)]1	22.2	0.278	1.3	0.361	1.6
PreciControl TM2	89.9	0.999	1.1	1.15	1.3

b) TM = Tumor Marker

Method comparison

a) A comparison of the Elecsys CA 19-9 assay, REF 07027028190 (**cobas e** 801 analyzer; y) with the Elecsys CA 19-9 assay, REF 11776193122 (**cobas e** 601 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 198

Passing/Bablok[18]	Linear regression
y = 0.968x - 0.359	y = 0.960x + 0.213
τ = 0.988	r = 0.999

The sample concentrations were between 4.00 and 981 U/mL.

b) A comparison of the Elecsys CA 19-9 assay, REF 07027028190 (**cobas e** 402 analyzer; y) with the Elecsys CA 19-9 assay, REF 07027028190 (**cobas e** 801 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 117

Elecsys CA 19-9

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Passing/Bablok[18]	Linear regression
y = 0.971x - 0.221	y = 0.975x - 0.760
τ = 0.988	r = 1.00

The sample concentrations were between 5.38 and 985 U/mL.

Analytical specificity

The Elecsys CA 19-9 tumor marker assay is based on the monoclonal 1116-NS-19-9 antibody which is only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of testing procedures using this antibody cannot be assumed for testing methods using other antibodies.

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.

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Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

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 0123

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