

CA 19-9

Carbohydrate Antigen 19-9



REF		SYSTEM
11776193 122	100	Elecsys 2010 MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English

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

Summary

The CA 19-9 values measured are defined by the use of the monoclonal antibody 1116-NS-19-9. The 1116-NS-19-9-reactive determinants on a glycolipid having a molecular weight of approximately 10000 daltons are measured. This mucin corresponds to a hapten of Lewis-a blood group determinants and is a component of a number of mucous membrane cells.^{1, 2}

3-7 % of the population have the Lewis a-negative/b-negative blood group configuration and are unable to express the mucin with the reactive determinant CA 19-9. This must be taken into account when interpreting the findings.³

Mucin occurs in fetal gastric, intestinal and pancreatic epithelia. Low concentrations can also be found in adult tissue in the liver, lungs, and pancreas.^{3,4}

CA 19-9 assay values can assist in the differential diagnosis and monitoring of patients with pancreatic carcinoma (sensitivity 70-87 %).^{5,6,7} There is no correlation between tumor mass and the CA 19-9 assay values. However, patients with CA 19-9 serum levels above 10000 U/mL almost always have distal metastasis.³

The determination of CA 19-9 cannot be used for the early detection of pancreatic carcinoma.^{8,9,10}

In hepatobiliary carcinoma the CA 19-9 values provide a sensitivity of 50-75 %. The concomitant determination of CA 72-4 and CEA is recommended in cases of gastric carcinoma. In colorectal carcinoma, determination of CEA alone is adequate; only in rare CEA-negative cases the determination of CA 19-9 can be useful.³

As the mucin is excreted exclusively via the liver, even slight cholestasis can lead to clearly elevated CA 19-9 serum levels in some cases. Elevated CA 19-9 values are also found with a number of benign and inflammatory diseases of the gastrointestinal tract and the liver, as well as in cystic fibrosis.^{3,6,7}

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 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/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 CA19-9.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-CA 19-9-Ab~biotin (gray cap), 1 bottle, 10 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)₃²⁺ (black cap), 1 bottle, 10 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.

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	8 weeks
on MODULAR ANALYTICS E170, cobas e 601 and cobas e 602	6 weeks
on Elecsys 2010 and cobas e 411	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-, NH₄⁺-heparin and K₃-EDTA plasma. Do not use sodium citrate plasma.

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Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within $\pm 2x$ analytical sensitivity (LDL) + coefficient of correlation > 0.95 .

Stable for 30 days at 2-8 °C, 3 months at -20 °C.¹¹

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] 11776215122, CA 19-9 CalSet, for 4 x 1 mL
- [REF] 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 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, MODULAR ANALYTICS E170 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 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] 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 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.

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 Enzymun-Test CA 19-9 method.

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 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 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 (either in U/mL or kU/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin $< 1129 \mu\text{mol/L}$ or $< 66 \text{ mg/dL}$), hemolysis (Hb $< 1.4 \text{ mmol/L}$ or $< 2.2 \text{ g/dL}$), lipemia (Intralipid $< 1500 \text{ mg/dL}$) and biotin ($< 100 \text{ ng/mL}$ or $< 409 \text{ nmol/L}$).

Criterion: Recovery within $\pm 15 \%$ of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. $> 5 \text{ mg/day}$) 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 CA 19-9 concentrations up to 500000 U/mL.

In vitro tests were performed on 27 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

0.600-1000 U/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as $< 0.600 \text{ U/mL}$. Values above the measuring range are reported as $> 1000 \text{ U/mL}$ (or up to 10000 U/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: $< 0.60 \text{ U/mL}$

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, $n = 21$).

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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 MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers 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 MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Note: The CA 19-9 antigen tends to aggregate.¹² This may lead to non-linear dilution behaviour 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, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	11.1	0.40	3.6	0.45	4.1
Human serum 2	46.6	1.52	3.3	1.75	3.8
Human serum 3	185	5.31	2.9	5.42	2.9
PreciControl TM ^b 1	19.2	0.85	4.4	0.93	4.8
PreciControl TM2	60.6	1.75	2.9	2.28	3.8

b) TM = Tumor Marker

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers							
		Repeatability			Intermediate precision		
Sample	Mean U/mL	SD U/mL	CV %	Mean U/mL	SD U/mL	CV %	
Human serum 1	5.20	0.10	1.9	5.57	0.45	8.0	
Human serum 2	30.2	0.47	1.6	30.6	0.72	2.3	
Human serum 3	379	9.27	2.5	371	10.0	2.7	
PreciControl TM1	21.1	0.34	1.6	21.4	0.56	2.6	
PreciControl TM2	76.6	0.89	1.2	76.3	1.42	1.9	

Method comparison

A comparison of the Elecsys CA 19-9 assay (y) with the Enzymun-Test CA 19-9 method (x) using clinical samples gave the following correlations:

Number of samples measured: 78

Passing/Bablok¹³ Linear regression
 $y = 0.99x + 0.87$ $y = 0.99x + 2.68$
 $\tau = 0.766$ $r = 0.944$

The sample concentrations were between approximately 4.5 and 216 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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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

ms_11776193122V22.0

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cobas®



Volume after reconstitution or mixing

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