Elecsys CA 19-9

English

For use in the USA only

System information
For cobas e 411 analyzer: test number 351
For MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: Application Code Number 054

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.
- Patients known to be genotypically negative for Lewis blood group antigens will be unable to produce the CA 19-9 antigen even in the presence of malignant tissue. Phenotyping for the presence of the Lewis blood group antigen may be insufficient to detect true Lewis antigen negative individuals. Even patients who are genotypically positive for the Lewis antigen may produce varying levels of CA 19-9 as the result of gene dosage effect.

Intended use
Immunocassay for the in vitro quantitative determination of CA 19-9 tumor associated antigen, in human serum and plasma. The assay is indicated for the serial measurement of CA 19-9 to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The test is useful as an aid in the monitoring of disease status in those patients having confirmed pancreatic cancer who have levels of CA 19-9 at some point in their disease process exceeding the median concentration determined for the apparently healthy cohort.

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%).5-7 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.5

The determination of CA 19-9 cannot be used for the early detection of pancreatic carcinoma.5,9,10

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.

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 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.

Reagents - working solutions
The reagent pack 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)3 (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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:
2-methyl-2H-isothiazol-3-one hydrochloride
EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.
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
Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes.

Li-heparin, K₂-EDTA and K₃-EDTA plasma. Do not use sodium citrate plasma.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95. Stable for 5 days at 20-25 °C, 14 days at 2-8 °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, calibrators and controls are at 20-25 °C prior to measurement.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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)

- 11776215122, CA 19-9 CalSet, for 4 x 1.0 mL
- 11776452160, PreciControl Tumor Marker, for 4 x 3.0 mL
- 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or cobas e analyzer

Additional materials for cobas e 411 analyzer:

- 11662988122, ProCell, 6 x 380 mL system buffer
- 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- 11930346122, Elecsys SysWash, 1 x 500 mL washer additive
- 11933159001, Adapter for SysClean
- 11706802001, AssayCup, 60 x 60 reaction cups
- 11707699001, AssayTip, 30 x 120 pipette tips
- 11800507001, Clean-Liner

Additional materials for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:

- 04868040190, ProCell M, 2 x 2 L system buffer
- 048680293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 003023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run initialization and rinsing during reagent change
- 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags

- RE 03023150001, WasteLiner, waste bags
- RE 03027851001, SysClean Adapter M
- Additional materials for all analyzers:
  - RE 11298500160, 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.

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). 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 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.

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 assay is unaffected by icterus (bilirubin < 1129 μmol/L or < 66 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 22 g/dL), lipemia (Intralipid < 1500 μg/dL) and biotin (< 100 ng/mL or < 409 nmol/L). Criterion: Recovery within ± 15 % 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 CA 19-9 concentrations up to 500000 IU/mL.

In vitro tests were performed on 27 commonly used pharmaceuticals. No interference with the assay was found.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of
Elecsys CA 19-9

concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titer 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 lower detection limit are reported as < 0.600 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

Lower detection limit of the test

Lower detection limit: < 0.600 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).

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 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 software automatically takes the dilution into account when calculating the sample concentration.

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

Expected values

Results of a clinical study conducted in the United States are as follows.

- Healthy Subjects:
  In serum samples from 403 apparently healthy test subjects, comprised of 200 females and 203 males, the Upper Limit of Normal (ULN) for this group, defined as the 97.5th percentile of the observed results, was 35 U/mL (95% confidence limits 30-42 U/mL). The median concentration of this cohort was 8.8 U/mL.

- Patients with benign and malignant diseases:

<table>
<thead>
<tr>
<th>Distribution of disease types (percentage of population)</th>
<th>Elecsys CA 19-9 assay values, U/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen classification</td>
<td>N</td>
</tr>
<tr>
<td>Apparently healthy</td>
<td>403</td>
</tr>
<tr>
<td>Benign disease</td>
<td>113</td>
</tr>
<tr>
<td>Uro-genital</td>
<td>145</td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>100</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>98</td>
</tr>
<tr>
<td>Cardiac</td>
<td></td>
</tr>
<tr>
<td>Malignant disease</td>
<td></td>
</tr>
<tr>
<td>Breast-Ovarian-Cervical</td>
<td>70</td>
</tr>
<tr>
<td>Colon/rectal</td>
<td>228</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>Elecsys CA 19-9 assay values, U/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen classification</td>
<td>N</td>
</tr>
<tr>
<td>Esophageal/ Gastric</td>
<td>29</td>
</tr>
<tr>
<td>Gall bladder/ Biliary</td>
<td>30</td>
</tr>
<tr>
<td>Liver</td>
<td>54</td>
</tr>
<tr>
<td>Lung</td>
<td>46</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>37</td>
</tr>
</tbody>
</table>

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); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

<table>
<thead>
<tr>
<th>Cobas e 411 analyzer</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean U/mL</td>
<td>SD U/mL</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>11.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>46.6</td>
<td>1.52</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>185</td>
<td>5.31</td>
</tr>
<tr>
<td>Precicontrol TM b)</td>
<td>19.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Precicontrol TM2</td>
<td>60.6</td>
<td>1.75</td>
</tr>
</tbody>
</table>

b) TM = Tumor Marker

MODULAR ANALYTICS E170, Cobas e 601 and Cobas e 602 analyzers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean U/mL</th>
<th>SD U/mL</th>
<th>CV %</th>
<th>Mean U/mL</th>
<th>SD U/mL</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum 1</td>
<td>5.20</td>
<td>0.10</td>
<td>1.9</td>
<td>5.57</td>
<td>0.45</td>
<td>8.0</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>30.2</td>
<td>0.47</td>
<td>1.6</td>
<td>30.6</td>
<td>0.72</td>
<td>2.3</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>379</td>
<td>9.27</td>
<td>2.5</td>
<td>371</td>
<td>10.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Precicontrol TM1</td>
<td>21.1</td>
<td>0.34</td>
<td>1.6</td>
<td>21.4</td>
<td>0.56</td>
<td>2.6</td>
</tr>
<tr>
<td>Precicontrol TM2</td>
<td>76.6</td>
<td>0.89</td>
<td>1.2</td>
<td>76.3</td>
<td>1.42</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Method comparison

A comparison of the Elecsys CA 19-9 assay (y) with an alternative RIA assay (x) using clinical samples gave the following correlations:

Number of samples measured: 1308

Passing/Bablok:

\[ y = 0.838x + 1.30 \]

\[ r = 0.858 \]

SD (md88) = 30.7

The sample concentrations were between 0.0 and 979 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.

Clinical performance data
The clinical utility of the Elecsys CA 19-9 Immunoassay test in monitoring the disease status in those patients having confirmed pancreatic cancer was evaluated using retrospective serum samples. CA 19-9 values were measured in 89 patients with histologically confirmed pancreatic cancer. These results were separated into groups that either demonstrated CA 19-9 values that did or did not correspond to the clinical course of disease. Serial measurements were analyzed on a per-patient basis as well as visit-to-visit basis. For each pair of serial measurements, an increase of > 15 % on the Elecsys CA 19-9 assay was considered to indicate progression, and an increase of ≤ 15 % was considered to indicate a lack of progression. The following tables show the overall correspondence of the serial CA 19-9 change with changes in clinical status.

Monitoring of pancreatic cancer patients for changes in disease status: Correspondence of serial CA 19-9 changes and clinical status (per-patient analysis)

<table>
<thead>
<tr>
<th>Change in CA 19-9</th>
<th>Progression</th>
<th>No progression</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 15 % increase</td>
<td>41</td>
<td>29</td>
<td>70</td>
</tr>
<tr>
<td>≤ 15 % increase</td>
<td>6</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>42</td>
<td>89</td>
</tr>
</tbody>
</table>

Concordance = (41+13)/89 = 60.7 % (95 % CI of 49.9 % - 70.9 %)

Negative Concordance = 13/42 = 30.9 % (95 % CI of 17.6 % - 47.1 %)

Positive Concordance = 41/47 = 87.2 % (95 % CI of 74.2 % - 95.2 %)

Monitoring of pancreatic cancer patients for changes in disease status: Correspondence of serial CA 19-9 changes and clinical status (per-visit analysis)

<table>
<thead>
<tr>
<th>Change in CA 19-9</th>
<th>Progression</th>
<th>No progression</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 15 % increase</td>
<td>54</td>
<td>65</td>
<td>119</td>
</tr>
<tr>
<td>≤ 15 % increase</td>
<td>34</td>
<td>120</td>
<td>154</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>185</td>
<td>273</td>
</tr>
</tbody>
</table>

Concordance = (54+122)/274 = 64.2 % (95 % CI of 58.4 % - 69.6 %)

Negative Concordance = 122/187 = 65.2 % (95 % CI of 58.8 % - 71.1 %)

Positive Concordance = 54/87 = 62.1 % (95 % CI of 49.3 % - 73.3 %)

References

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).

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
- SYSTEM
- REAGENT
- CALIBRATOR
- GTIN

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