03045838500V23.0

Elecsys CA 15-3 II

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REF		Σ	SYSTEM
			cobas e 411
03045838122	03045838500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 332
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 052

Please note

The measured CA 15-3 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 15-3 assay method used. CA 15-3 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 15-3 assay procedure used while monitoring therapy, then the CA 15-3 values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunological in vitro assay for quantitative determination of CA 15-3 in human serum and plasma to aid in the management of breast cancer patients. In conjunction with other clinical and diagnostic procedures, serial testing with this assay is an aid

- in the early detection of recurrence in previously treated stage II and III breast cancer patients
- for monitoring response to therapy in metastatic breast cancer patients

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

Summary

The CA 15-3 (Cancer Antigen 15-3) is derived from glycoprotein Mucin-1 (MUC-1).[1] The CA 15-3 assay uses two monoclonal antibodies (MAb), 115D8 and DF3, in a sandwich assay to detect two antigenic sites associated with breast carcinoma cells. MAb 115D8 is directed against human milk fat globule membranes,[1,2,3] whereas MAb DF3 is directed against the membrane fraction from human breast cancer.[4]

The antigen is normally found in the luminal secretion of glandular cells and does not circulate in the blood. When cells become malignant and their basal membranes permeable, the antigen is detectable in serum. [5] Overexpression of MUC1 plays an important role in epithelial to mesenchymal transition; an important and complex phenomenon that determines cancer progression.[6] The guideline landscape for advanced disease monitoring was mapped in a review by Duffy et al.[7]
The low cost and minimally invasive CA 15-3 monitoring approach is mentioned in ASCO and the European Group on Tumor Markers (EGTM) guidelines, especially if there is non-measurable disease in conventional imaging.[8] The ESMO breast cancer guidelines suggest that tumour markers such as CA 15-3 may be useful to evaluate response to treatment, particularly in patients with non-measureable metastatic disease. A change in tumour markers alone should not be used to initiate a change in treatment.[7]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample are automatically prediluted 1:10 with Diluent Universal. The antigen (in 20 µL of prediluted sample), a biotinylated monoclonal CA 15-3-specific antibody, and a monoclonal CA 15-3-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.
- 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.

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

Reagents - working solutions

The reagent rackpack is labeled as CA 15-3 II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CA 15-3-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal antibody (115D8; mouse) 1.75 mg/L; phosphate buffer 20 mmol/L, pH 6.0; preservative.

R2 Anti-CA 15-3-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-CA 15-3 antibody (DF3; mouse) labeled with ruthenium complex 10 mg/L; phosphate buffer 100 mmol/L, pH 7.0; 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:	
P501	Dispose of contents/container to an approved waste disposal plant.

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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 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	5 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.

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 48 hours at 20-25 °C, 5 days at 2-8 °C, 90 days 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.

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 03045846122, CA 15-3 II CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials 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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials 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.

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 Elecsys CA 15-3 assay. This in turn has been standardized against the Enzymun-Test CA 15-3 method and CA 15-3 RIA by Fujirebio Diagnostics.

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).

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Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.9 mmol/L or < 3.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 409 nmol/L or < 100 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.

Typically, no high-dose hook effect[b)] can be observed at CA 15-3 concentrations up to 20000 U/mL. However, due to the heterogeneous nature of the CA 15-3 antigen a high-dose hook effect below this value cannot be completely excluded. In case of an unexpected low result, the sample should be diluted 1:10 (refer to chapter "Dilution") and tested again.

In vitro tests were performed on 28 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.

b) High-dose hook effect: A sample with a true concentration clearly above the measuring range, but found within the measuring range.

Limits and ranges

Measuring range

1.00-300 U/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 1.00 U/mL. Values above the measuring range are reported as > 300 U/mL (or up to 3000 U/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 1.00 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

Use Diluent Universal for automatic sample predilution. Samples with CA 15-3 concentrations above the measuring range despite predilution must be diluted 1:10 with Diluent Universal (either manually for all analyzers or automatically by the **cobas e** 601 and **cobas e** 602 analyzers). The concentration of the diluted sample must be > 30 U/mL.

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

After dilution by the analyzers, the **cobas e** 601 and **cobas e** 602 software automatically takes the dilution into account when calculating the sample concentration.

Expected values

• *Healthy subjects:*

Results of a reference range study using a panel of samples from 374 apparently healthy non-pregnant females (Roche study No. RD000788)

Percentile (%)	U/mL	Confidence interval (U/mL)
95	26.2	25.2-27.9
97.5	28.5	26.7-34.5
99	34.5	28.7-57.8

• *Patients with benign diseases and pregnant women:*

Relative distribution of CA 15-3 concentrations in patients with benign disease and pregnant women (Roche study No. B00P018)

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
Gastrointestinal	109	84	16	0	0
Breast	58	88	12	0	0
Gynecological diseases	42	83	12	5	0
Renal failure	37	81	19	0	0
Urological diseases	34	82	18	0	0
Bacterial infection	27	96	4	0	0
Pregnancy	34	97	0	3	0

• *Patients with malignant diseases (others than breast):*

Relative distribution of CA 15-3 concentrations in individuals with malignancy other than breast

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
Stomach-Ca[c)]	36	75	14	8	3
Hepatocellular-Ca	37	59	32	3	5
Lung-Ca	38	82	13	5	0
Ovarian-Ca	34	47	21	29	3
Gynecological-Ca	5	40	20	40	0
Prostate-Ca	48	79	17	4	0
Colorectal-Ca	40	93	8	0	0
Pancreatic-Ca	40	65	33	3	0

c) Ca = Carcinoma

• *Patients with breast cancer:*

Relative distribution of CA 15-3 concentrations in patients with breast malignancy. The staging of patients according to UICC criteria was performed at primary diagnosis before any treatment. The patients diagnosed with recurrent disease had developed metastases (M1).

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
UICC I	56	88	12	0	0
UICC II	126	85	13	2	0
UICC III	77	53	30	14	3
UICC IV	24	25	17	37	21
Recurrent Disease	75	15	25	36	24

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 accordance with 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:

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cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	38.0	0.81	2.1	1.38	3.6
Human serum 2	85.5	2.72	3.2	3.66	4.3
Human serum 3	179	4.56	2.6	6.60	3.7
PreciControl TM[d)]1	24.5	0.62	2.5	0.87	3.6
PreciControl TM2	67.6	2.48	3.7	2.83	4.2

d) TM = Tumor Marker

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	18.9	0.24	1.3	20.1	0.64	3.2
Human serum 2	76.4	1.07	1.4	79.0	3.08	3.9
Human serum 3	148	1.72	1.2	156	7.75	5.0
PreciControl TM1	20.3	0.24	1.2	21.3	0.89	4.2
PreciControl TM2	47.6	0.70	1.5	49.6	1.82	3.7

Method comparison

A comparison of the Elecsys CA 15-3 II assay (y) with the Elecsys CA 15-3 assay (x) using clinical samples gave the following correlations:

Number of samples measured: 52

Passing/Bablok regression[9]

Slope: 1.06 (95 % confidence range: 1.01-1.15)

Intercept: 2.66 (95 % confidence range: -0.99-5.97)

Coefficient of correlation: 0.965

The sample concentrations were between 6 and 280 U/mL.

Analytical specificity

The Elecsys CA 15-3 II tumor marker assay is based on the monoclonal 115D8 and DF3 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of test procedures using these antibodies cannot be assumed for test methods using other antibodies.

Clinical performance in follow-up

Patients diagnosed with breast cancer were examined in a retrospective study (at least 4 samples/patient during follow-up study) and classified as recurrence [yes/no] after no evidence of breast cancer or response to treatment [yes/no] after breast cancer metastasis based on the clinical information (medical imaging and other clinical investigations). The CA 15-3 concentrations were measured in parallel. The ROC (receiver-operating characteristics) analysis of relative CA15-3 change to determine breast cancer recurrence/ therapy response in metastasized breast cancer was done to show clinical accuracy at various cut-offs and to summarize the cutoff-independent clinical performance in a ROC plot and the related AUC (area under the curve).

Early detection of recurrence

Forty (40) patients treated for stage II or III breast cancer were followed for up to 1351 days (median 105 days). A total of 172 samples (median 4 samples per patient) were assessed for recurrence of disease over the follow-up period. Recurrence was defined as the presence of clinical symptoms in women with no evidence of disease at the beginning of the follow-up period. Eighteen (18) patients experienced recurrence of disease.

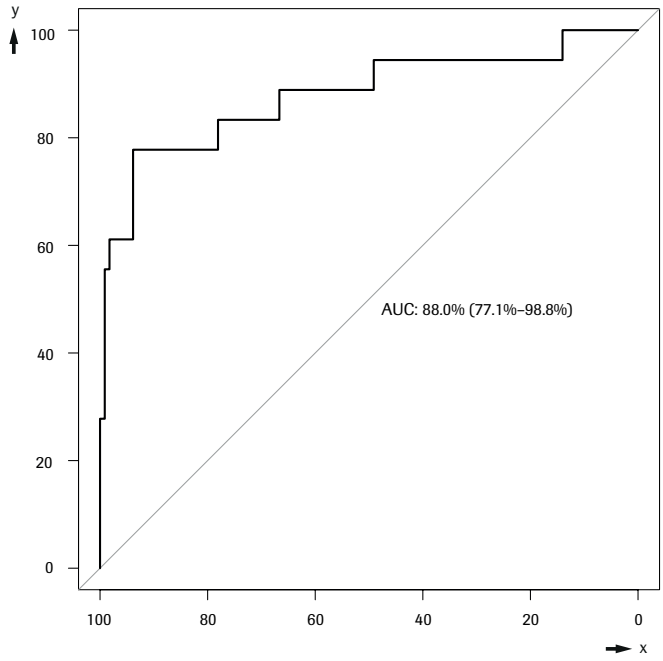
2 x 2 table for early detection of recurrence:

	recurrence	
CA15-3 increase > 25%	no	yes
no	93	4
yes	21	14

The corresponding results for positive predictive value (PPV) and negative predictive value (NPV) with the 95 % confidence interval for a cutoff of 25 % CA 15-3 increase as derived from the table are:

Positive predictive value: 40 % (24-58 %)

Negative predictive value: 90 % (90-99 %)



x = Specificity (%); y = Sensitivity (%)

Figure 1: ROC curve: breast cancer recurrence by relative change CA 15-3 to baseline

The area under the curve (AUC) was 0.8796 (95 % CI: 0.7709-0.9884)

Monitoring response to therapy

Fifteen (15) patients with metastatic breast cancer underwent treatment and response to therapy was assessed by clinical criteria. A total of 72 assessments (median 4 assessments per patient) were made. Fourteen (14) patients had a response to therapy.

2 x 2 table for response to therapy

	response	
CA15-3 decrease > 25 %	no	yes
no	25	19
yes	1	12

The corresponding results for positive predictive value (PPV) and negative predictive value (NPV) with the 95 % confidence interval as derived from the table are:

Positive predictive value: 92 % (64-100 %)

Negative predictive value: 57 % (41-72 %)

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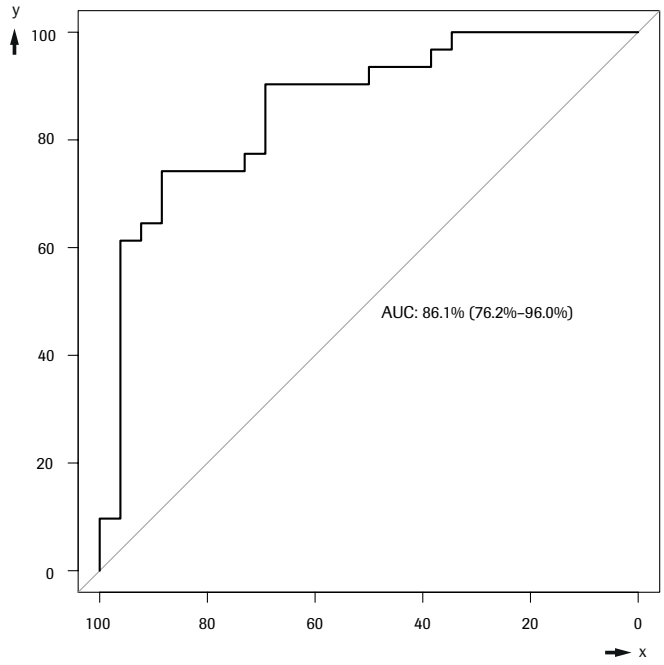

x = Specificity (%); y = Sensitivity (%)

Figure 2: ROC curve: breast cancer response to therapy by relative change CA 15-3 to baseline

The area under the curve (AUC) was 0.8610 (95% CI: 0.7623-0.9598).

References

1 Duffy MJ. CA 15-3 and related mucins as circulating markers in breast cancer. Ann Clin Biochem,1999;36:579-586.

2 Hilkens J, Buijs F, Hilgers J, et al. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland. Prot Biol Fluids 1982;29:813-816.

3 Hilkens J, Buijs F, Hilgers J, et al. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 1984;34:197-206.

4 Kufe D, Inghirami G, Abe M, et al. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumor. Hybridoma 1984;(3):223-232.

5 Sekine H, Ohno T, Kufe DW. Purification and characterization of a high molecular weight glycoprotein detectable in human milk and breast carcinomas. J Immunol 1985;135(5):3610-3615.

6 Ponnusamy MP, Seshacharyulu P, Lakshmanan I, et al. Emerging role of mucins in epithelial to mesenchymal transition. Curr cancer drug targets 2013;13(9):945-56.

7 Duffy MJ, Walsh S, McDermott EW, et al. Chapter One - Biomarkers in Breast Cancer: Where Are We and Where Are We Going?, In: Gregory S. Makowski, Editor(s), Advances in Clinical Chemistry, Elsevier, 2015;71: 1-23, ISSN 0065-2423, ISBN 9780128022566.

8 Cardoso F, et al. ESMO Guidelines for advanced breast cancer. Annals of Oncology,2018;29: 1634-1657.

9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

FUJIREBIO Diagnostics, Inc.

CA 15-3 is a registered trademark of Fujirebio Diagnostics, Inc.

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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