Elecsys CYFRA 21-1

cobas®

REF		Σ	SYSTEM
11820966119	11820966500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 370
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 063

Please note

The measured CYFRA 21-1 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 CYFRA 21-1 assay method used. CYFRA 21-1 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 CYFRA 21-1 assay procedure used while monitoring therapy, then the CYFRA 21-1 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 fragments of cytokeratin 19 in human serum and plasma.

The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.

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

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 11820966122 for the Elecsys CYFRA 21-1 assay. The last 3 digits -122 have been replaced by -119 for logistic purposes

Summary

Cytokeratins are structural proteins forming the subunits of epithelial intermediary filaments. Twenty different cytokeratins have so far been identified, CYFRA 21-1 which is a fragment of cytokeratin 19, being the most prominent one. Intact cytokeratin polypeptides are poorly soluble, but soluble fragments like CYFRA 21-1, are frequently released into the blood of cancer patients and can be detected in serum.[1] Although being expressed in different organs, its major occurrence is in the lung. CYFRA 21-1 can be considered as the biomarker of choice for non-small cell lung cancer (primarily for squamous cell and large cell carcinoma subtypes).[2,3,4,5,6] In adenocarcinoma of the lung, a combination of CYFRA 21-1 and Carcinoembryonic antigen (CEA) has been found to be the most useful.[7,8]

The main indication for CYFRA 21-1 is monitoring the course of NSCLC.[9] Successful therapy is accompanied by a rapid decrease of serum levels to the normal range.[9,10]

Increased CYFRA 21-1 levels have also been described in non-malignant diseases (i.e. pneumonia, sepsis)[11,12,13] and renal dysfunction.[14] Therefore evaluation of renal function (i.e. by measuring serum creatinine levels) should be considered in cases of high CYFRA 21-1 levels that are not consistent with the diagnostic and clinical characteristics of the patient.

With the use of two specific monoclonal antibodies (KS 19.1 and BM 19.21), the Elecsys CYFRA 21-1 assay measures a fragment of cytokeratin 19 having a molecular weight of approximately 30000 Da.[15]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal cytokeratin 19-specific antibody, and a monoclonal cytokeratin 19-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 or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as CYFRA.

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

R1 Anti-cytokeratin 19-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-cytokeratin 19 antibody (KS 19.1; mouse) 1.5 mg/L, phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-cytokeratin 19-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-cytokeratin 19 antibody (BM 19.21; mouse) labeled with ruthenium complex 2 mg/L; phosphate buffer 100 mmol/L, pH 7.2; 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.

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	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-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

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 7 days at 20-25 °C, 30 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.[16]

It is recommended that the samples be mixed by careful swirling or by placing on a roller mixer (max. 5 min). Homogenization of samples using electric vibration mixers must be limited to a maximum of 5 seconds. Longer mixing times lead to lower values being found.

Contamination of the sample with saliva leads to falsely elevated results.[16]

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 11820974322, CYFRA 21-1 CalSet, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 07360070190, PreciControl Lung Cancer, 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 Enzymun-Test CYFRA 21-1 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 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 Lung Cancer or 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 ng/mL or µg/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.93 mmol/L or < 1.5 g/dL), lipemia (Intralipid < 1500 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 CYFRA 21-1 concentrations up to 2000 ng/mL.

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.

Limits and ranges

Measuring range

0.100-500 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.100 ng/mL. Values above the measuring range are reported as > 500 ng/mL (or up to 1000 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: ≤ 0.10 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 CYFRA 21-1 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 250 ng/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.

Expected values

Normal CYFRA 21-1 values are expected to be ≤ 2.37 ng/mL.

The following table shows the results of three separate cohorts. The first study represents the distribution of expected results from 240 apparently healthy men and women equally divided into smokers and nonsmokers. The second study represents the distribution of expected results from 195 benign disease conditions other than cancers, and the third study represents the distribution of expected results from different cancers, including lung cancer.

Elecsys CYFRA 21-1 distribution of values by cohort

	No. of subjects	0.3-2.37 ng/mL	2.38-5.0 ng/mL	5.01-20.0 ng/mL	20.01-100 ng/mL	> 100 ng/mL
Apparently healthy	**240**					
All normals	240	228	12	0	0	0
Nonsmokers	120	111	9	0	0	0
Smokers	120	117	3	0	0	0
Normal females	125	119	6	0	0	0
Nonsmokers	63	59	4	0	0	0
Smokers	62	60	2	0	0	0
Normal males	115	109	6	0	0	0
Nonsmokers	57	52	5	0	0	0
Smokers	58	57	1	0	0	0
Benign conditions	**195**					
Benign lung disease	75	70	5	0	0	0
CHF[b)]	40	29	11	0	0	0
Benign kidney disease	40	8	24	8	0	0
Benign liver disease	40	35	4	1	0	0
Cancer	**440**					
Lung cancer	120	53	33	27	5	2
Bladder cancer	40	13	9	12	5	1
Breast cancer	40	32	5	3	0	0
Cervical cancer	40	28	11	1	0	0
ESCC[c)]	40	21	12	6	1	0
GI tract cancer	40	23	10	6	1	0
Head and neck cancer	40	29	11	0	0	0
Prostate cancer	40	37	1	2	0	0
Ovarian cancer	40	25	8	5	2	0

b) CHF = Congestive heart failure

c) ESCC = Esophageal squamous cell carcinoma

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Clinical performance data

The effectiveness of the Elecsys CYFRA 21-1 assay as an aid in monitoring of disease status in lung cancer patients was determined by assessing changes in CYFRA 21-1 levels in serial serum samples from 83 patients compared to changes in disease status. A total of 398 samples were measured, including 86 baseline values and 315 monitoring values.

Subjects had ≥ 3 blood draws over time with a minimum follow-up time of 30 days and no less than 7 days between consecutive blood draws. A positive change in CYFRA 21-1 was defined as an increase in the value that was at least 50 % greater than the previous value of the test. This level of change takes into account the analytical variability of the assay. 44.1 % (26/59) of the patient samples with a positive change correlated with the disease progression while 91.0 % (233/256) of the patient serial samples with no significant change in CYFRA 21-1 value correlated with no progression. The following table presents the data:

Elecsys CYFRA 21-1 elevation vs. disease progression				
		Disease progression		
		No progression	Progression	Total
Elecsys CYFRA 21-1 elevation	Not elevated	233	33	267
	Elevated	23	26	48
	Total	256	59	315

No set cutoff exists for CYFRA 21-1. The clinical performance of other percent changes in serial samples are presented below. Clinicians may choose to use these other values to enhance the sensitivity or specificity of the assay, depending on their needs.

Cutoff values and corresponding performance measurements				
Percent (%) change in Elecsys CYFRA 21-1	Sensitivity (%)	Specificity (%)	NPV[d] (%)	PPV[e] (%)
30	49.2	87.1	88.1	46.8
40	44.1	89.8	87.5	50.0
50	44.1	91.0	87.6	53.1
60	39.0	91.4	86.7	51.1
70	35.6	93.4	86.3	55.3

d) NPV = negative predictive value
e) PPV = positive predictive value

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:

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	2.68	0.06	2.1	0.13	4.7
Human serum 2	6.86	0.14	2.0	0.23	3.3
Human serum 3	21.5	0.36	1.7	0.66	3.1
PreciControl TM[d]1	5.04	0.10	2.0	0.12	2.4
PreciControl TM2	29.9	0.49	1.6	0.63	2.1
PreciControl LC[e]1	2.90	0.046	1.6	0.129	4.5
PreciControl LC2	27.2	0.281	1.0	0.569	2.1

d) TM = Tumor Marker
e) LC = Lung Cancer

cobas e 601 and **cobas e** 602 analyzers						
	Repeatability			Intermediate precision		
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	2.60	0.04	1.5	2.65	0.07	2.7
Human serum 2	5.51	0.06	1.2	5.51	0.13	2.3
Human serum 3	57.0	0.62	1.1	56.0	1.10	2.0
PreciControl TM1	5.06	0.11	2.1	5.12	0.13	2.6
PreciControl TM2	33.4	0.53	1.6	33.9	0.65	1.9

cobas e 601 and **cobas e** 602 analyzers					
	Repeatability			Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PreciControl LC1	2.97	0.068	2.3	0.146	4.9
PreciControl LC2	27.5	0.336	1.2	0.497	1.8

Method comparison

A comparison of the Elecsys CYFRA 21-1 assay (y) with the Enzymun-Test CYFRA 21-1 method (x) using clinical samples gave the following correlations:

Number of samples measured: 76

Passing/Bablok[17]	Linear regression
y = 0.98x - 0.30	y = 0.95x - 0.10
τ = 0.941	r = 0.993

The sample concentrations were between 1.0 and 44 ng/mL.

Analytical specificity

The monoclonal anti-cytokeratin 19 antibodies recognize a fragment of the cytokeratin 19 peptide. There is no cross-reactivity with cytokeratins 8 and 18.[18]

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 (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

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