Elecsys CYFRA 21-1

cobas®

REF		Σ	SYSTEM
07299966190	07299966501	300	**cobas e** 402 **cobas e** 801

English

For use in the USA only

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.

System information

Short name	ACN (application code number)
CYFRA	10030

Intended use

Immunoassay for the in vitro quantitative determination of fragments of cytokeratin 19 in human serum and plasma (Li-heparin, K_2-EDTA and K_3-EDTA). 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 **cobas e** immunoassay analyzers.

Summary

Cytokeratins are structural proteins forming the subunits of epithelial intermediary filaments. Twenty different cytokeratin polypeptides have so far been identified. Due to their specific distribution patterns they are eminently suitable for use as differentiation markers in tumor pathology. Intact cytokeratin polypeptides are poorly soluble, but soluble fragments can be detected in serum.[1,2,3,4]

With the aid of two specific monoclonal antibodies (KS 19.1 and BM 19.21), CYFRA 21-1 measures a fragment of cytokeratin 19 having a molecular weight of approximately 30000 daltons.[5]

The main indication for CYFRA 21-1 is monitoring the course of non-small cell lung cancer (NSCLC).[2,5,6]

Good specificity is shown by CYFRA 21-1 relative to benign lung diseases (pneumonia, sarcoidosis, tuberculosis, chronic bronchitis, bronchial asthma, emphysema).[7,8]

Slightly elevated values (up to 10 ng/mL) are rarely found in marked benign liver diseases and renal failure. There is no correlation with sex, age or smoking.[9] The values are also unaffected by pregnancy.

The primary diagnosis of pulmonary carcinoma should be made on the basis of clinical symptomatology, imaging or endoscopic procedures and intraoperative findings.

An unclear circular focus in the lung together with CYFRA 21-1 values > 30 ng/mL indicate with high probability the existence of primary bronchial carcinoma.

High CYFRA 21-1 serum levels indicate an advanced tumor stage and a poor prognosis.[10] A normal or only slightly elevated value does not rule out the presence of a tumor.

Successful therapy is documented by a rapid fall in the CYFRA 21-1 serum level into the normal range. A constant CYFRA 21-1 value or a slight or only slow decrease in the CYFRA 21-1 value indicates incomplete removal of a tumor or the presence of multiple tumors with corresponding therapeutic and prognostic consequences. Progression of the disease is often shown earlier by increasing CYFRA 21-1 values than by clinical symptomatology and imaging procedures.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 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 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 CYFRA.

M Streptavidin-coated microparticles, 1 bottle, 13.2 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-cytokeratin 19-Ab~biotin, 1 bottle, 19.7 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~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 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.

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:

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.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

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, as well as Li-heparin and K_2-EDTA plasma tubes containing separating gel.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.3 ng/mL + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 12 weeks at -20 °C (± 5 °C). Freeze only once.

It is recommended that the samples be mixed by careful swirling or by placing on a roller mixer (maximum 5 minutes). 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.[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 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.

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.

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 11776452160, PreciControl Tumor Marker, for 4 x 3.0 mL or
- REF 07299001190, Diluent Universal, 36 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 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.

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 CYFRA 21-1 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 ng/mL or µg/L.

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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	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations of 0.1-1 ng/mL the deviation is ≤ ± 0.1 ng/mL. For concentrations > 1 ng/mL the deviation is ± 10 %.

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 CYFRA 21-1 concentrations up to 2000 ng/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	120
Cyclophosphamide	1000
Cisplatin	225
5-FU	500
Methotrexate	1000
Tamoxifen	50
Mitomycin	25
Carboplatin	1000
Etoposide	400
Paclitaxel	265
Clotrimazole	0.3
Dexamethasone	20
Leucovorin	750
Melphalan	15
Tarceva	150

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

0.5-100 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 0.5 ng/mL. Values above the measuring range are reported as > 100 ng/mL (or up to 500 ng/mL for 5-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.15 ng/mL

Limit of Detection = 0.3 ng/mL

Limit of Quantitation = 0.5 ng/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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a coefficient of variation of ≤ 20 %.

Dilution

Samples with CYFRA 21-1 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (either automatically by the analyzers or manually). The concentration of the diluted sample must be ≥ 20 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

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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**
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.

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 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 ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.872	0.026	3.0	0.030	3.4
Human serum 2	1.13	0.026	2.3	0.033	2.9
Human serum 3	3.56	0.043	1.2	0.052	1.4
Human serum 4	50.9	0.532	1.0	0.579	1.1
Human serum 5	94.8	0.974	1.0	1.18	1.2
PC[d)] Tumor Marker1	2.85	0.041	1.4	0.052	1.8
PC Tumor Marker2	26.8	0.244	0.9	0.351	1.3

d) PC = PreciControl

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 83 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	266
	Elevated	23	26	49
	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[e)] (%)**	**PPV[f)] (%)**
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

e) NPV = negative predictive value

f) PPV = positive predictive value

Method comparison

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

Number of samples measured: 121

Deming regression (unweighted)
Slope = 0.911 (95 % CI: 0.86, 0.96)
Intercept = -1.40 (95 % CI: -2.86, 0.06)
Pearson correlation coefficient
r = 0.955 (95 % CI: 0.94, 0.97)

The sample concentrations were between 0.56 and 96.39 ng/mL.

A comparison of the Elecsys CYFRA 21-1 assay on the **cobas e** 801 analyzer (y) with the Elecsys CYFRA 21-1 assay on the **cobas e** 601 analyzer (x) gave the following correlations (ng/mL):

Number of samples measured: 118

Passing/Bablok[12]	Linear regression
y = 1.00x - 0.120	y = 1.01x - 0.404
τ = 0.977	r = 0.999

The sample concentrations were between 0.519 and 96.9 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.[1]

References

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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 for reconstitution
GTIN	Global Trade Item Number

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