

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k100831

B. Purpose for Submission:

New device

C. Measurand:

CYFRA 21-1 (soluble cytokeratin 19 fragments)

D. Type of Test:

Quantitative, enzyme immunoassay

E. Applicant:

Fujirebio Diagnostics, Inc.

F. Proprietary and Established Names:

CYFRA 21-1 EIA Kit

G. Regulatory Information:

1. Regulation section:
21 CFR § 866.6010, Tumor – Associated antigen immunological test system
2. Classification:
Class II
3. Product code:
OVK, Cytokeratin fragments 21-1 EIA Kit
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The CYFRA 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum. 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.
2. Indication(s) for use:
Same as above
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
ELISA Plate Reader at 405 and 620 nm

I. Device Description:

Each CYFRA 21-1 EIA kit contains reagents for 96 tests.

Components in the CYFRA 21-1 EIA:

1. Streptavidin Microplate (12x8 breakable wells coated with streptavidin),
2. CFRA 21-1 Calibrator A (1x8 mL, phosphate buffered salt solution containing bovine serum albumin),
3. CYFRA 21-1 Calibrators B-F (5 vials, lyophilized calibrators contain CYFRA 21-1 antigen in a phosphate buffered salt solution containing bovine serum albumin),
4. CYFRA 21-1 Controls (2 vials, lyophilized controls contain CYFRA 21-1 antigen in a human serum matrix),

5. Biotin Anti-CYFRA 21-1 (1x15 mL, biotin anti-CYFRA 21-1 monoclonal antibody from mouse, approximately 1.25 µg/mL. Contains Tris-HCl buffered salt solution (pH 7.2), bovine serum albumin, blocking agents, detergent, an inert blue dye, and a non-azide antimicrobial preservative. To be mixed with Tracer before use.)
6. Tracer, HRP Anti-CYFRA 21-1 (1x0.75 mL , Stock Solution of HRP anti-CYFRA 21-1 monoclonal antibody from mouse, approximately 42µg/mL. Contains non-azide antimicrobial preservatives. To be mixed with biotin anti-CYFRA 21-1 prior to use.)
7. TMB HRP-Substrate (1x12 mL, contains buffered hydrogen peroxide and 3',3',5',5' tetra-methylbenzidine (TMB))
8. STOP Solution (1x15 mL, Contains 0.12 M hydrochloric acid)
9. Wash Concentrate (1x50 mL, Tris-HCl buffered salt solution with Tween 20)

J. Substantial Equivalence Information:

1. Predicate device name(s):
ARCHITECT CEA
2. Predicate 510(k) number(s):
k990774
3. Comparison with predicate:
Not applicable. Clearance is supported by serial monitoring clinical data.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Editions.

CLSI EP17-A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline - (2004)”

L. Test Principle:

The CYFRA 21-1 EIA is a solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of soluble fragments of cytokeratin 19. Calibrators, controls and patient samples are incubated together with biotinylated anti-CYFRA 21-1 MAb and horseradish peroxidase (HRP) labeled anti-CYFRA 21-1 mAb in streptavidin coated micro strips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color development is proportional to the amount of CYFRA 21-1 present in the samples. The color intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CYFRA 21-1 concentrations of unknown samples are then read from the calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. Precision/Reproducibility:
The precision evaluation was carried out in three laboratory sites. At sites 1

and 2, the study comprised of 2 kit lots, 20 days for each lot, 2 runs (separated by a minimum of 2 hours) per day and duplicate assays for a total of 160 assays. The third site differed in that 3 lots were used, 10 days for each lot (for a total of 30 days), 2 runs (separated by a minimum of 2 hours) per day, in duplicate, for a total of 120 assays.

For sites 1 and 2, sample panels consisting of pleural effusion from four (distinct) patients were spiked into normal human serum. For site 3 panels consisted of pleural effusion/serum mixtures, serum, or a calibrator/serum mixture were used. For this study a total of 8 panels were analyzed.

The precision analyses for Site 1 have determined that the total precision for both lots combined as well as individually is < 8.5% for the panels in this study. The total precision for the 4 panels in the study ranged from 2.8% to 5.4% and 3.5% to 6.4% for Lots 1 and 2, respectively, for their individual lot comparisons with 95% Upper Confidence Limits in the range of 3.3% to 6.4% for Lot 1 and 4.2% to 7.5% for Lot 2. The total precision for the 2 controls in the study ranged from 2.5% to 4.8% and 3.2% to 5.3% for Lots 1 and 2, respectively, for their individual lot comparisons with 95% Upper Confidence Limits in the range of 3.0% to 5.7% for Lot 1 and 3.7% to 6.3% for Lot 2. For the combined lot data, the total precision of the 4 panels ranged from 5.1% to 8.4% with 95% Upper Confidence Limits ranging from 5.8% to 9.4%. As a result, the CYFRA 21-1 EIA Kit meets the predetermined acceptance criteria of CV and supports the performance claims of the assay.

The precision analyses for Site 2 has determined that the total precision for both lots combined as well as individually is less than or equal to 10.6% for all controls and panels in this study. The total precision for the 4 panels in the study ranged from 4.6% to 7.9% and 5.1% to 10.6% for Lots 1 and 2, respectively, for their individual lot comparisons with 95% Upper Confidence Limits in the range of 5.4% to 9.3% for Lot 1 and 6.1% to 12.6% for Lot 2. The total precision for the 2 controls in the study ranged from 4.7% to 6.1% and 5.4% to 6.6% for Lots 1 and 2, respectively for their individual lot comparisons with 95% Upper Confidence Limits in the range of 5.6% to 7.2% for Lot 1 and 6.4% to 7.9% for Lot 2. For the combined lot data, the total precision of the 4 panels ranged from 6.2% to 10.5% with 95% Upper Confidence Limits ranging from 6.9% to 11.8%. As a result, the CYFRA 21-1 EIA Kit meets the predetermined acceptance criteria of CV and supports the performance claims of the assay.

For Site 3 the average total precision of the CYFRA 21-1 EIA Kit for the eight (8) panel members ≥ 0.5 ng/mL across 3 kit lots in this study was calculated to be in the range of 5.7% and 9.2% CV. As a result, the CYFRA 21-1 EIA Kit meets the predetermined acceptance criteria of $\geq 20\%$ total CV.

Additional imprecision at the lower range of the CYFRA 21-1 EIA Kit (0.31-

1.49ng/mL) was determined at the manufacturer’s site by testing each low panel in replicates of two (2), using two (2) lots of CYFRA 21-1 EIA Kits at two (2) separate times per day, for twenty (20) days. Three (3) technicians participated in the study. The order of the samples was changed for each run. (*n=80 per sample*). The total imprecision of the CYFRA 21-1 EIA Kit within this range was calculated to be in the range of 6.7% and 10.6% CV.

b. Linearity/assay reportable range:

A study was conducted to evaluate the dilution linearity within the CYFRA 21-1 EIA Kit assay range. CLSI guideline, EP6-A entitled “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline,” was used as a guide. Five (5) serum samples with elevated CYFRA 21-1 values were diluted with CYFRA 21-1 Calibrator A (Kit Sample Diluent) following the dilution scheme shown in the table below:

Dilution Number	Dilution Scheme	Dilution Factor
1	High Sample (H) - Elevated level of CYFRA 21-1	Neat
2	0.9 H + 0.1 CAL A	1/1.1
3	0.8 H + 0.2 CAL A	1/1.25
4	0.7 H + 0.3 CAL A	1/1.43
5	0.6 H + 0.4 CAL A	1/1.67
6	0.5 H + 0.5 CAL A	1/2
7	0.4 H + 0.6 CAL A	1/2.5
8	0.3 H + 0.7 CAL A	1/3.3
9	0.2 H + 0.8 CAL A	1/5
10	0.1 H + 0.9 CAL A	1/10
11	0.05 H + 0.95 CAL A	1/20
12	0.025 H + 0.975 CAL A	1/40
13	0.010 H + 0.990 CAL A	1/100

The diluted samples indicated in the table above were tested as unknowns with the CYFRA 21-1 EIA Kit, in replicates ranging from 4 to 8 per sample. Five (5) samples with elevated CYFRA 21-1 were tested. The results obtained from these samples are shown in the table below:

Sample	Mean Recovery (%)	%CV	Low	High	R²
1	102	4.2	92.8	108.1	0.9988
2	101.7	3.7	95.3	106.5	0.9985
3	97.7	4.1	92.7	102.3	0.9995
4	99.8	6.1	85.8	106.5	0.9986
5	98.9	6.7	86.7	111.1	0.9993

i. Reportable Assay range:

Based on the data in the table above, the nonlinearity calculated by weighted polynomial regression was $\leq 10\%$ across the measurement range of 0.5 to 50ng/mL. As a result the reportable range was set as 0.5 to 50 ng/mL.

ii. High Dose Hook Effect

A study was conducted to determine the interference that may be caused by extreme levels of analyte in the CYFRA 21-1 EIA Kit resulting in false low levels in the assay. Five (5) serum samples with elevated CYFRA 21-1 levels were used in this study. These samples were diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 with human serum. The dilution samples and the corresponding neat (undiluted) samples were tested as unknowns with the CYFRA 21-1 EIA Kit, in replicates of two (2) with two (2) CYFRA 21-1 EIA Kit lots. The CYFRA 21-1 EIA Kit Controls were tested and evaluated for each assay to determine assay validity.

The mean concentration was calculated from the sample values obtained for the two (2) replicates for each dilution and the corresponding neat (undiluted) sample. The concentrations of each of the five (5) elevated serum samples were calculated using the average of the values for the dilution series that fell within the range of the CYFRA 21-1 EIA Kit. The average concentration was used to evaluate the samples falling outside the range of the CYFRA 21-1 EIA Kit. NOTE: The theoretical Sample D concentration was calculated using the same dilutions as the other samples for consistency.

The hook concentration was determined as the highest concentration with an absorbance $\geq 10\%$ of the highest CYFRA 21-1 Calibrator. The absorbance of the highest CYFRA 21-1 Calibrator (CYFRA 21-1 Calibrator F), at both wavelengths, were used to calculate the limit of the hook concentration and are shown in the table below.

Table 24. Hook Effects

	CYFRA 21-1 KIT LOT Calibrator F = 50.4 ng/mL (O.D. of 2.931)		CYFRA 21-1 KIT LOT 2 Calibrator F = 47.6 ng/mL (O.D. of 2.656)	
Wavelength	620nm	405nm	620nm	405nm
Observed absorbance, CYFRA 21-1 Calibrator F	2.931	2.735	2.656	2.516
Limit of hook concentration 10% higher than observed absorbance	3.224	3.008	2.922	2.768

Results showed all elevated samples were above the limit of hook concentration. No high dose hook effect was observed in samples up to 1100 ng/mL CYFRA 21-1 antigen.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

i. Traceability:

There is no recognized reference standard for CYFRA 21-1. The concentration of the CYFRA 21-1 EIA primary calibrators has been assigned by using Roche CK19 antigen. The methods used for value assignment of the CYFRA 21 primary and secondary calibrators were provided.

ii. Calibrators:

Cytokeratin 19 (CK19) has a molecular weight of approximately 30,000 daltons. The CK19 (from a commercial source) used to prepare calibrators for the CYFRA 21-1 immunoassay, corresponds to the cytokeratin from the human cell line MCF-7.

The primary reference standards (calibrators) are held as a reference set from which the CYFRA 21-1 stock solution for the secondary reference sets of calibrators are determined using a calibrating factor. The secondary calibrators are used to manufacture and value assign all sets of calibrators to be included in CYFRA 21-1 EIA kits. (CYFRA 21-1 EIA Kit Calibrators).

The concentration of the CYFRA 21-1 EIA Primary Calibrators has been assigned based on the original Roche CK19 Antigen concentration. The CYFRA 21-1 EIA Primary Calibrators were held as a reference set from which the CK19 stock solution for the Secondary sets of calibrators was prepared using a calibration factor. Each CYFRA 21-1 EIA secondary calibrator level was manufactured gravimetrically using a CK19 antigen stock to the concentrations 0, 1.5, 5.0, 15, 25 and 50 ng/mL.

Calibrator set (1 Vial each) consists of CAL A to CAL F. CAL A (0 ng/mL) is ready-to-use and should also be used for dilution of samples. CAL B-F are lyophilized and require reconstitution before use. In addition, the CYFRA 21-1 concentration of CAL B-F is lot specific and is indicated on the label of each vial. Target concentrations for CAL B to F are 1.5, 5, 15, 25 and 50 ng/mL.

iii. *Kit Stability*

a) Freeze Thaw

1. For 1 cycle: 100.5% for Lot 1 and 100.8% for Lot 2. Individual percent recoveries ranged from 99.7-101.2% for Lot 1 and 99.3-102.3% for Lot 2.

2. For 3 cycles: 101.1% for Lot 1 and 99.8% for Lot 2. Individual percent recoveries ranged from 100.8-101.3% for Lot 1 and 98.1-101.6% for Lot 2.
3. For 5 cycles: 102.2% for Lot 1 and 100.2% for Lot 2. Individual percent recoveries ranged from 100-104.4% for Lot 1 and 99.6-100.7% for Lot 2.
4. For 10 cycles: 101.9% for Lot 1 and 99.8% for Lot 2. Individual percent recoveries ranged from 100.3-103.5% for Lot 1 and 99.2-100.4% for Lot 2.

The % CV within the replicates was $\leq 5.0\%$ for all conditions.

b) Stability at other temperatures

1. The average recovery of lyophilized CYFRA 21-1 EIA Kit Controls at 37°C for 4 weeks was 96.3% for Lot 1 and 97.0% for Lot 2. Individual percent recoveries ranged from 95.0-97.6% for Lot 1 and 96.7-97.2% for Lot 2.
2. The average recovery of reconstituted CYFRA 21-1 EIA Kit Controls at 2-8°C for 1 week was 97.3% for Lot 1 (after 4 weeks 92.6%) and 98.3% for Lot 2 (after 4 weeks 95.9%). Individual percent recoveries after 1 week ranged from 96.2-98.4% for Lot 1 and 97.8-98.8% for Lot 2.
3. The average recovery of reconstituted CYFRA 21-1 EIA Kit Controls at Room Temperature for 1 week was 96.6% for Lot 1 (after 4 weeks 90.5%) and 99.1% for Lot 2 (after 4 weeks 95.5%). Individual percent recoveries after 1 week ranged from 96.4-96.7% for Lot 1 and 99.0-99.2% for Lot 2.
4. The average recovery of reconstituted CYFRA 21-1 EIA Kit Controls at -20°C was 101.6% for Lot 1 and 98.6% for Lot 2 after 4 months. Individual percent recoveries ranged from 98.8-104.3% for Lot 1 and 97.3-99.9% for Lot 2.

The % CV within the replicates was $\leq 5.0\%$ for all conditions.

c) Real Time Stability

Two (2) lots of CYFRA 21-1 EIA Kits were used in the study. The study plan is described as follows: 2 Lots of CYFRA 21-1 EIA Kits are stored at 2-8°C, analyzed at Day 0, after 4, 7, 13, 19 and 25 months.

1. CYFRA 21-1 EIA Kit Calibrators, CYFRA 21-1 EIA Kit Controls and internal QC/QA controls are tested in replicates of four (4).
2. The CYFRA 21-1 EIA Kit is tested with the TMB included in the kit and with a new lot of TMB at each time point.

The acceptance criteria for the CYFRA 21-1 EIA Kit are predetermined to ensure that acceptable performance of each product is not compromised due to the product age and usage. The Real Time Stability studies met all acceptance criteria up to the 13 month time point.

d) Open Use Stability

One (1) lot of CYFRA 21-1 EIA Kits was set up for the study. Sufficient CYFRA 21-1 EIA Kits (including all components) were opened, closed, and then stored at the intended storage condition of 2-8°C. After reconstitution, CYFRA 21-1 EIA Kit Calibrators and CYFRA 21-1 EIA Kit Controls were stored at -70°C. After opening all components, the CYFRA 21-1 EIA Kits are analyzed at Day 0, after 4, 7, 13, and 19 months.

1. CYFRA 21-1 EIA Kit Calibrators, CYFRA 21-1 EIA Kit Controls and internal QC/QA controls are tested in replicates of four (4).
2. The CYFRA 21-1 EIA Kit is tested with the TMB included in the kit and with a new lot of TMB at each time point.
3. An unopened CYFRA 21-1 EIA Kit stored under normal conditions (2-8°C) is tested at Day 0 as a control.

The acceptance criteria for the CYFRA 21-1 EIA Kit are predetermined to ensure that acceptable performance of each product is not compromised due to routine product usage. The Open Use Stability studies met all acceptance criteria up to the 13 month time point.

e) Transport Simulation Stability

One (1) lot of CYFRA 21-1 EIA Kits was set up for the study. Sufficient CYFRA 21-1 EIA Kits were stressed at the conditions listed below. After each stress condition the CYFRA 21-1 EIA Kits were moved immediately to the next condition. After all cycles were completed the CYFRA 21-1 EIA Kits were stored at the intended storage condition of 2-8°C.

1. Store CYFRA 21-1 EIA Kits at 37°C for 24 ± 4 hours.
2. Transfer the CYFRA 21-1 EIA Kits to a -20 ± 10°C freezer for 24 ± 4 hours.
3. Transfer the CYFRA 21-1 EIA Kits to room temperature for 48 ± 4 hours.
4. Store CYFRA 21-1 EIA Kits at 2-8°C for the duration for the study.
5. After the stress cycle, the CYFRA 21-1 EIA Kits are analyzed at Day 0, after 4, 7, 13, 19 and 25 months.
6. CYFRA 21-1 EIA Kit Calibrators, CYFRA 21-1 EIA Kit Controls and internal QC/QA controls are tested in replicates of four (4).
7. An unstressed CYFRA 21-1 EIA Kit stored under normal conditions (2-8°C) is tested at Day 0 as a control.

The acceptance criteria for the CYFRA 21-1 EIA Kit are predetermined to ensure that acceptable performance of each product is not compromised due to the simulated shipping conditions. The Transport Stability studies met all acceptance criteria up to the 13 month time point.

d. *Detection limit:*

i. Limit of Blank (LoB)

The CYFRA 21-1 EIA Kit Calibrator A was run in replicates of twelve (12) over the course of four (4) days for a total of nine (9) assays using three (3) CYFRA 21-1 EIA kit combinations. Three (3) lots of CYFRA 21-1 EIA Kit Calibrator A were used in the study for a total of 108 replicates. The LoB was determined to be 0.06 ng/mL using the 95th percentile approach.

ii. Limit of Detection (LoD)

A five (5) member low level panel set (see panel table below) was run in replicates of twelve (12) over the course of four (4) days for a total of nine (9) assays using three (3) CYFRA 21-1 EIA kit combinations for a total of 108 replicates/panel member.

The above study was combined with the previous study where a three (3) member low level panel set (see panel table below) was run in replicates of two (2), using two (2) lots of CYFRA 21-1 EIA Kits at two (2) separate times per day, for twenty (20) days for a total 80 replicates/per panel member.

Panel Member	Mean (ng/mL)	Standard Deviation	Total n
LoD-1	0.16	0.050996	108
LoD-2	0.22	0.046342	108
LoD-3	0.28	0.040833	108
LoD-4	0.32	0.041549	108
LoD-5	0.40	0.028551	108
FDL4	0.31	0.03303	80
FDL5	0.16	0.034048	80
FDL6	0.07	0.024007	80
Total Standard Deviation (n=8)		0.037	

Using the formula $LoD = LoB + 1.645 * S.D. \text{ Panel set}$ [$LoD = 0.06 + (1.645 * 0.037) = 0.12 \text{ ng/mL}$], the Limit of Detection of the CYFRA 21-1 EIA was determined to be 0.12 ng/mL.

iii. Limit of Quantitation (LoQ)

The LoQ was determined by recalculating the data using the expanded set from the LoD study above and the definition of the analyte concentration at which the coefficient of variation is 17.78%. The expanded data set from which the LoD is defined shows that the assay standard deviation is 0.037 ng/mL over the range of values encompassing the LoQ. Thus, the LoQ is given by the equation $LoQ = 0.037 / 0.1778 = 0.21 \text{ ng/mL}$.

e. *Analytical specificity:*

The CYFRA 21-1 EIA assay mean assay specificity is $100 \pm 15\%$. Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera. The CLSI guideline EP7-A was used to design the interference experiments. The following substances and concentrations were tested and found not to interfere with the test.

Endogenous serum interferences test concentration

Triglycerides 30 mg/mL
 Billirubin 0.2 mg/mL
 Hemoglobin 5 mg/mL
 Total Protein 120 mg/mL

Chemotherapeutic drug interferences test concentration

Carboplatin 500 µg/mL
 Cisplatin 165 µg/mL
 Dexamethasone 10 µg/mL
 Doxorubicin 1.16 µg/mL
 Leucovorin 2.68 µg/mL
 Methotrexate 45 µg/mL
 Paclitaxel 3.5 ng/mL

Human anti-mouse antibody (HAMA) and Rheumatoid Factor (RF) interference

The CYFRA 21-1 EIA assay was evaluated using specimens with HAMA and Rheumatoid Factor (RF) to further assess the assay specificity. Six specimens positive for HAMA (80.7 to 291.3 ng/mL) and five specimens positive for RF (22.2 to 54.63 IU/mL) were evaluated for % recovery with CYFRA 21-1 antigen spiked into each specimen at approximately 5 and 25 ng/mL. Mean recovery results are summarized in the following table.*

Clinical condition	Highest Concentrations	Mean % recovery
HAMA	291.3 ng/mL	98
RF	54.63 IU/mL	101

- f. *Assay Cut-off:*
 See Clinical studies section below
- 2. Comparison studies:
 - a. *Method comparison with predicate device:*
 Not applicable. The predicate measures a different analyte. See clinical studies.
 - b. *Matrix comparison:*
 Not applicable. The assay is for serum samples only.
- 3. Clinical studies:

The effectiveness of the CYFRA 21-1 EIA as an aid in monitoring the course of disease in lung cancer patients was determined through a retrospective clinical study by correlating changes in CYFRA 21-1 levels in serial serum samples to changes in disease status. Remnant serial serum samples were obtained from a tertiary cancer center from lung cancer subjects who met the specified inclusion and exclusion criteria (see below).

Inclusion and exclusion criteria

The patient inclusion criteria are:

- Eighteen years of age or older
- Diagnosed with lung cancer
- Minimum of 3 serial draws available
- Appropriate clinical data
- Minimum 0.4 mL volume of serum available
- Normal appearance of sample

The exclusion criteria are:

- No diagnosis of lung cancer
- Less than 3 serial draws available
- Insufficient sample volume
- Multiple freeze-thaw cycles
- Icteric, lipemic, hemolytic, substantial particulates

Subject sample draws could be at various stages of the disease, including pre-treatment, during treatment, or standard of care follow-up. Subject samples could have been drawn either during therapy, or following the completion of therapy for the treatment of lung cancer. Subjects who had samples drawn at either time point with corresponding clinical data were included.

The clinical data collected for each subject included the following: patient ID, date of birth, gender, race/ethnicity, smoking status, including smoking history and date of smoking cessation, date of lung cancer diagnosis, histology, grade, and stage. For each subject sample draw, the following information was collected: chemotherapeutic treatment information (onset date, end date, regimen), imaging information (date, type, findings, disease status), physical exam date and findings, sample draw date, procedure information (type, date and findings), Clinical Disease Status, date of recurrence, and date of death (if subject expired).

Study Samples

The sample size was calculated to be approximately 100 subjects with a minimum of 3 draws per subject. A clinician assessment was made at each draw and each subject draw was categorized into 1 of the following subgroups:

1. Subjects with no evidence of disease (NED) for their lung cancer who are being monitored for recurrence in follow-up after therapy. This will include subjects who eventually recur while being followed and those that do not

recur during the follow-up period. NED is defined as a complete lack of clinical evidence of disease.

2. Subjects with evidence of residual but stable disease during or after therapy at visit. Stable Disease is defined as clinical evidence that the disease has not changed since last assessment.
3. Subjects with progressive disease while on therapy or subjects recurring while in follow-up after therapy. Progressive Disease is defined as clinical evidence of growth in the primary tumor or the appearance of new tumors since the last assessment.
4. Subjects with responsive disease while on therapy (responders). Responding Disease is defined as clinical evidence that there is a shrinking of the primary tumor and no evidence of new tumors.

A total of 437 samples for 104 subjects were obtained. All samples were tested for CYFRA 21-1 levels. A total of 414 samples from 100 subjects were used for the data analysis for disease progression. Twenty-three (23) samples from 4 subjects were excluded for the following reasons:

- 12 samples from 3 non-lung cancer patients
- 3 samples from 1 patient were excluded due to being drawn on the same date
- 7 samples had the same draw date as another sample
- 1 sample received in error

Sample Draw

There was a total of 414 draws for the 100 subjects resulting in a total of 314 observation pairs. The mean number of draws per subject was 4.1 ranging from 3 to 8 draws. The length of time over which the subjects were monitored ranged from 23 days to 2174 days (median of 198 days). The median interval between successive visits was 49 days ranging from 1 day to 1878 days. On average, each subject had blood drawn for CYFRA 21-1 testing at a baseline and 3 follow-up visits, spaced at an average of 101 days.

Number of Draws	Frequency	Percent (%)	Cumulative	
			Frequency	Percent (%)
3	46	46	46	46
4	23	23	69	69
5	15	15	84	84
6	7	7	91	91
7	5	5	96	96
8	4	4	100	100

Demographic Data

Of the 100 subjects, 57% were male. The mean age was 59 years old ranging from 34 to 82 years of age. The majority of subjects were Caucasian (88%).

Ethnicity	Frequency	Percent (%)
Asian	3	3
Black	6	6
Caucasian	87	87
Hispanic	4	4
Total	100	100

Disease Stage

For the 100 lung cancer cases, 95 were classified as non-small cell lung cancer (NSCLC) and 5 were classified as small cell lung cancer (SCLC). Ninety (90) of the NSCLC were further classified as adenocarcinoma (68), squamous cell carcinoma (19), and large cell carcinoma (3). Seventy-nine (79) of the 100 lung cancer cases had staging information as shown in the following table. In this study, only 5 patients had Stage I or II disease, and as a consequence the performance of CYFRA 21-1 has not been adequately assessed in these subpopulations.

Stage	Number of Patients
I	2
II	3
III	36
IV	38
Total (with Stage Information)	79
Unknown	17
Unstaged	4
Total Lung Cancer Cases	100

Summary statistics of analyte value and disease staging

The following table illustrates the summary statistics of the CYFRA 21-1 EIA results from all 414 draws compared to disease staging.

Stage	N	CYFRA 21-1 (ng/mL)				
		Minimum	1st Quartile	Median	3rd Quartile	Maximum
I	10	0.93	1.37	2.22	4.56	64.91
II	12	2.35	3.83	8.95	14.30	25.52
IIb	3	14.21	14.21	15.89	16.18	16.18
III	25	0.38	0.86	1.27	2.08	16.27
IIIa	54	0.77	1.73	3.20	10.43	74.59
IIIb	75	0.43	1.26	2.01	3.80	100.67
IV	150	0.41	1.41	2.63	12.41	88.38
UNK	66	0.60	1.52	2.10	4.43	48.90
Unstaged	19	0.47	0.71	0.89	1.40	3.67

The following table depicts the summary statistics of the ratio from the preceding

draw for all follow-up draws.

Stage	N	CYFRA 21-1 Ratio from Preceding Draw				
		Minimum	1st Quartile	Median	3rd Quartile	Maximum
I	8	0.37	0.67	1.05	1.85	12.72
II	10	0.64	0.68	0.97	2.11	4.64
IIb	2	0.89	N/A	0.94	N/A	0.98
III	19	0.59	0.77	0.98	1.30	5.89
IIIa	42	0.03	0.78	1.06	2.09	5.77
IIIb	56	0.10	0.70	1.05	1.45	38.85
IV	114	0.04	0.61	1.00	1.56	18.53
UNK	48	0.02	0.79	1.17	1.58	3.46
Unstaged	15	0.26	0.59	0.88	1.13	1.51

Clinical Performance Characteristics

a. *Clinical Sensitivity and Specificity:*

Analysis of Disease Progression

Progressive disease in this study was defined as clinical evidence of growth in the primary tumor or the appearance of new tumors since the last assessment. All imaging was reviewed and assessed by the ordering radiologist at the time of collection. The nurses reviewed the imaging reports and used the clinical information provided regarding tumor growth and overall evaluation.

The hypothesis was that progression leads to an increase in CYFRA 21-1, so the diagnostic rule investigated in the clinical study was to classify patients for disease progression if their CYFRA 21-1 level was statistically significantly higher at any visit than at the preceding visit and as not suspect if this was not the case. The success criterion was a total concordance probability of at least 65% with the 95% confidence interval (CI) being 67.5 to 78.2%. A positive change in CYFRA 21-1 was defined as a measurable increase in the value that was at least 50% greater than the previous value of the test. Observation pairs with both values below the normal reference range of 1.8 ng/mL were defined as no significant change. This level of change takes into account the variability of the assay and the biological variability and is based on the published report of Trapé et al. (Clinical Chemistry 51, pgs. 219-222, 2005).

Per-visit analysis

The cutoff value of 50% increase was used to categorize the successive CYFRA 21-1 readings into those that were and were not significantly elevated, and cross-tabulated against progression or no progression. The No Progression category included patients with clinical status of NED, stable and responding to treatment. The following table presents the data in a 2 x 2 format comparing the concordance between the CYFRA 21-1 value and the clinical assessment of disease progression. The Total values represent the total number of samples for that category. This table does not include the first

sample drawn for all 100 subjects as the calculations are based on the changes from visit to visit. (414 total draws minus the first draw (100 patients) = 314 total measurements). Results showed that 46% (39/85) of the patient samples with a positive change correlated with the disease progression while 87% (200/229) of the patient serial samples with no significant change in CYFRA 21-1 value correlated with no progression. The total concordance was 76% (239/314).

		Disease Progression		
		No Progression	Progression	Total
CYFRA 21-1 EIA Elevation	>50%	200	46	246
	≤50%	29	39	68
	Total	229	85	314

The table below summarizes statistics of the CYFRA 21-1 ratio for draws broken down by clinical disease status. These summary statistics conform to the expected pattern:

- The NED and Stable subjects have very similar summary statistics,
- Three quarters of the patient visits showing disease progression had an increase in CYFRA 21-1,
- Between a half and three quarters of visits in which the subject was responding to treatment, showed a decrease in CYFRA 21-1.

Category	Clinical Disease Status	n	Minimum	1 st Quartile	Median	3 rd Quartile	Maximum
No Progression	NED	41	0.151	0.695	0.894	1.472	7.378
	Stable	135	0.039	0.699	0.985	1.288	5.340
	Responding	53	0.031	0.500	0.891	1.199	2.812
Progression	Progression	85	0.020	0.998	1.366	2.454	38.85

ROC analysis showed the area under the curve was 0.72 with a standard error of 0.03. The cutoff of a 50% increase in CYFRA 21-1 gives a sensitivity of 45.9% with specificity 87.3%.

Using a 50% increase between serial samples the following table summarizes the performance measurements and 95% CIs:

Performance Measurement	Percent (%)	95% Confidence Interval
Sensitivity	45.9	34.8 - 58.3
Specificity	87.3	80.8 - 91.5
Total Concordance	75.8	70.2 -81.4
Positive Likelihood Ratio	3.62	2.21-5.94

Negative Likelihood Ratio	0.62	0.495-0.776
Positive Predictive Value	57.4	43.0-68.3
Negative Predictive Value	81.3	72.5- 85.5
Prevalence	27.1	27.1

With different cutoff values there are tradeoffs between sensitivity and specificity as illustrated in table below:

Percent (%) change in CYFRA21-1	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
30	52.9	84.3	55.6	82.8
40	48.2	85.6	55.4	81.7
50	45.9	87.3	57.4	81.3
60	44.7	88.2	58.5	81.1
70	43.5	89.5	60.7	81.0

When ratios of CYFRA 21-1 values over successive visits were analyzed by disease stage, results demonstrated that CYFRA 21-1 values were consistent with clinical finding of disease progression based on imaging, but not with disease status classified by staging (see below).

Stage	Patients	Pairs	Sens (%)	Spec (%)	Prev (%)	PPV (%)	NPV (%)
I	2	8	100	85.7	12.5	50	100
II	2	10	25	83.3	40.0	50	62.5
IIb	1	2	NA	100	NA	NA	100
III	7	19	50	100	21.1	100	88.2
IIIa	11	42	53.3	81.5	35.7	61.5	75.9
IIIb	18	56	23.1	83.7	23.2	30	78.3
IV	38	114	51.5	86.4	28.9	60.7	81.4
Unstaged	4	15	NA	100	13.3	NA	86.7
UNK	17	48	53.8	88.6	27.1	63.6	83.8
			(7/13)	(31/35)	(13/48)	(7/11)	(31/37)
Total	100	314	45.9	87.3	27.1	57.4	81.3

In summary, the clinical study results support the intended use of “ The CYFRA 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum. The assay is to be used as an aid in monitoring the 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.”

- b. Other clinical supportive data (when a. is not applicable):
Not applicable.
4. Clinical cut-off:
Not applicable for monitoring. CYFRA 21-1 value increases at least 50% higher than immediate previous sample.
5. Expected values/Reference range:
To determine the upper level of normal for the CYFRA 21-1 EIA assay 75 patients with benign lung disease and 120 patients with lung cancer were tested. The patient subgroups are listed in the table below:

Benign Lung Disease	Sample Size (n)	Lung Cancer	Sample Size (n)
Asthma	49	Bronchus or Lung (Unspecified)	105
COPD	21	Main Bronchus	5
Asthma and COPD	5	Lower Lobe	4
		Middle Lobe	1
		Upper Lobe, Bronchus or lung	4
		Overlapping Lesion of bronchus and Lung	1
Total	75	Total	120

The samples were tested in duplicate using the CYFRA21-1 EIA kit according to the proposed package insert. One (1) CYFRA 21-1 EIA Kit was used in the study. The individual sample replicates were evaluated using valid calibration curves and the mean of two (2) replicates was calculated. The samples used were obtained by commercial vendors that were collected under an IRB approval protocol. The primary approach to determine the upper limit of normal for the assay was a nonparametric one. In this, calculations of the 95th and 97.5th percentiles were performed for the overall set of the apparently healthy individuals.

Group	Sample Size	95th Percentile	97.5th Percentile
Apparently Healthy Individuals	240	1.813 ng/mL	2.237 ng/mL
Apparently Healthy Nonsmokers	120	2.168 ng/mL	2.265 ng/mL
Apparently Healthy Smokers	120	1.550 ng/mL	1.811 ng/mL
Apparently Healthy Females	125	1.798 ng/mL	2.691 ng/mL
Apparently Healthy Males	115	1.730 ng/mL	2.173 ng/mL

Based on this data it was determined that the CYFRA 21-1 EIA value that corresponded most closely to the 95th percentile for the overall group of apparently healthy individuals, that is 1.8 ng/mL, would be used for defining the upper limit of normal. It is recommended that each laboratory establish its own reference value for the population of interest.

875 serum patient specimens with various conditions were assessed using the CYFRA 21-1 EIA Kit to establish the reference ranges. The following patient cohorts were assembled to determine the distribution of the serum CYFRA 21-1 EIA Kit values in various benign and malignant conditions.

Distribution of CYFRA 21-1 Assay Values					
Subject Categories	Number of Subjects				
	Total number of subjects	CYFRA 21-1 Assay Values (ng/mL)			
		0-1.8	1.9-5.0	5.1 - 20	>20
Apparently Healthy Subjects					
All normal	240	228	12	0	0
Benign Disease or Conditions					
Lung	75	71	4	0	0
CHF	40	30	10	0	0
Liver	40	38	2	0	0
Kidney	40	4	32	4	0
Cancer Cases					
Lung	120	47	36	24	13
Bladder	40	17	3	12	8
Breast	40	32	6	1	1
Cervical	40	24	13	3	0
Esophageal SC	40	13	18	7	2
GI	40	25	10	4	1
Head and Neck	40	30	9	1	0
Prostate	40	37	1	2	0
Ovarian	40	23	10	5	2

Note: CHF Congestive heart failure; SC Squamous cell

In this study 95% of the healthy subjects had a CYFRA 21-1 assay value at or below 1.8 ng/mL.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.