# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAYONLY

## I Background Information:

### A 510(k) Number

K250925

### B Applicant

Fujirebio Diagnostics, Inc.

### C Proprietary and Established Names

ADVIA Centaur Cytokeratin Fragment 21-1

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| OVK | Class II | 21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System | IM - Immunology |

## II Submission/Device Overview:

### A Purpose for Submission:

New Device

### B Measurand:

CYFRA 21-1 (cytokeratin 19 fragments)

### C Type of Test:

Quantitative, electrochemiluminescence immunoassay

## III Intended Use/Indications for Use:

### A Intended Use(s):

See Indications for Use below.

## B Indication(s) for Use:

The ADVIA Centaur Cytokeratin Fragment 21-1 (CYFRA) assay is for in vitro diagnostic use in the quantitative measurement of cytokeratin 19 fragments in human serum and plasma (EDTA and lithium heparin) using the ADVIA Centaur XPT system.

The measurement of cytokeratin 19 is 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 information used for monitoring lung cancer.

## C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

The Instructions for Use of the device contains the following warning statement:

The concentration of CYFRA 21-1 in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CYFRA 21-1 assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining serial levels of CYFRA 21-1 is changed, the laboratory must perform additional testing to confirm baseline values.

## D Special Instrument Requirements:

Siemens ADVIA Centaur XPT system

# IV Device/System Characteristics:

## A Device Description:

The ADVIA Centaur Cytokeratin Fragment 21-1 (CYFRA) assay includes:

- ADVIA Centaur CYFRA ReadyPack primary reagent pack (100 tests/1 pack) consisting of:
  - Lite Reagent: Anti-CYFRA 21-1 monoclonal antibody (<0.001%) acridinium conjugate in buffer containing bovine serum albumin; surfactant; preservatives. 10.0 mL/reagent pack
  - Solid Phase: Anti-CYFRA 21-1 monoclonal antibody (<0.001%) coupled to magnetic microparticles in buffer containing bovine serum albumin; surfactant; preservatives. 17.5 mL/reagent pack
- ADVIA Centaur CYFRA master curve card

Materials Required but not provided:

- ADVIA Centaur Cytokeratin Fragment 21-1 Calibrator (CYFRA CAL)
- ADVIA Centaur Wash 1 (2 ×1500 mL); (2 ×2500 mL)

Optional Materials:

- ADVIA Centaur Tumor Marker Quality Control (TM QC)
- ADVIA Centaur Cytokeratin Fragment 21-1 Master Curve Material (CYFRA MCM)
- ADVIA Centaur Multi-Diluent 13

**B Principle of Operation:**

The ADVIA Centaur Cytokeratin Fragment 21-1 (CYFRA) assay is a fully automated sandwich immunoassay using acridinium ester chemiluminescent technology. The assay employs two anti-CYFRA 21-1 antibodies. The first antibody in the Lite Reagent, is a mouse monoclonal anti-CYFRA 21-1 (KS19.1) labeled with acridinium ester. The second antibody is a mouse monoclonal anti- CYFRA 21-1 (BM19.21) covalently coupled to paramagnetic microparticles in the Solid Phase. A direct relationship exists between the amount of CYFRA 21-1 present in the patient sample and the amount of relative light units (RLUs) detected by the system.

## V Substantial Equivalence Information:

**A Predicate Device Name(s):**

Elecsys CYFRA 21-1, Elecsys CYFRA 21-1 CalSet, PreciControl Tumor Marker

**B Predicate 510(k) Number(s):**

K160915

**C Comparison with Predicate(s):**

| Device & Predicate Device(s): | K250925 (Candidate Device) | K160915 (Predicate) |
|---|---|---|
| Device Trade Name | ADVIA Centaur CYFRA | Elecsys CYFRA 21-1 |
| **General Device Characteristic Similarities** | | |
| Intended Use/ Indications For Use | The ADVIA Centaur Cytokeratin Fragment 21-1 (CYFRA) assay is for in vitro diagnostic use in the quantitative measurement of cytokeratin 19 fragments in human serum and plasma (EDTA and lithium heparin) using the ADVIA Centaur XPT system.<br><br>The measurement of cytokeratin 19 | Immunoassay for the in vitro quantitative determination of fragments of cytokeratin 19 in human serum and plasma (Li-Heparin, K2-EDTA and K3-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 |

| Device & Predicate Device(s): | K250925 (Candidate Device) | K160915 (Predicate) |
|---|---|---|
| | is 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 information used for monitoring lung cancer. | patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.<br><br>The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers. |
| Analyte | CYFRA 21-1 | Same |
| Assay format | Quantitative | Same |
| Results interpretation | A positive change in CYFRA 21-1 is defined as an increase in the value that is at least 50% greater than the previous value of the test. | Same |
| Sample type | Human serum and plasma (Li-heparin, K2-EDTA, K3-EDTA) | Same |
| Assay operation | Automatic | Same |
| Detection Method | Chemiluminescence | Same |
| Calibrators | ADVIA Centaur CYFRA CAL: two levels (sold separately) | Elecsys CYFRA 21-1 CalSet: two levels (sold separately) |
| Controls | ADVIA Centaur TM QC: two levels (sold separately) | PreciControl Tumor Marker: two levels (sold separately) |
| **General Device Characteristic Differences** | | |
| Solid Phase | Anti-CYFRA 21-1 monoclonal antibody coupled to magnetic microparticles | Biotinylated monoclonal anti-cytokeratin 19 antibody and streptavidin-coated microparticles |
| Signal Conjugate | Anti-CYFRA 21-1 monoclonal antibody acridinium conjugate | Anti-cytokeratin 19 monoclonal antibody labeled with ruthenium complex |
| Instrument | ADVIA Centaur XPT system | Elecsys and cobas e analyzer |
| Sample volume | 50 µL | 20 µL |
| Measuring Interval | 0.49–100 ng/mL | 0.5–100 ng/mL |

## VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

- CLSI EP06, 2nd Edition, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07, 3rd Edition, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP25-ed2, Evaluation of Stability of In Vitro Medical Laboratory Test Reagents-Second Edition
- CLSI EP28-A3c, Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP34, 1st Edition, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking
- CLSI EP35, 1st Edition, Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures
- CLSI EP37, 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry
- CLSI EP39, 1st Edition, A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

1. Precision:

Precision studies including reproducibility study were performed in accordance with CLSI EP05-A3.

*a) Within-Laboratory Precision:*

Within-Laboratory precision was assessed using seven serum sample pools containing CYFRA 21-1 antigen concentrations spanning the analytical measuring interval at a single site using one ADVIA Centaur CYFRA reagent lot. The samples were tested in two replicates per run, two runs per day, for 20 days, resulting a total of 80 datapoints for each sample. The data were analyzed for repeatability (within-run), between-run, between-day, and within-laboratory precision. The mean (ng/mL), standard deviation (SD) (ng/mL) and percent coefficient of variation (%CV) are summarized in the table below.

| Sample | N | Mean (ng/mL) | Within-Run (Repeatability) | | Between-Run | | Between-Day | | Within-Laboratory | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 80 | 2.03 | 0.03 | 1.5 | 0.02 | 1.1 | 0.03 | 1.4 | 0.04 | 2.2 |
| 2 | 80 | 2.84 | 0.03 | 1.1 | 0.04 | 1.4 | 0.03 | 1.0 | 0.06 | 2.0 |
| 3 | 80 | 4.69 | 0.06 | 1.3 | 0.05 | 1.0 | 0.05 | 1.0 | 0.09 | 1.9 |
| 4 | 80 | 15.15 | 0.24 | 1.6 | 0.20 | 1.2 | 0.09 | 0.6 | 0.31 | 2.1 |
| 5 | 80 | 25.30 | 0.36 | 1.4 | 0.19 | 0.7 | 0.17 | 0.7 | 0.44 | 1.7 |
| 6 | 80 | 65.46 | 0.80 | 1.2 | 0.47 | 0.7 | 0.58 | 0.9 | 1.09 | 1.7 |
| 7 | 80 | 84.07 | 1.03 | 1.2 | 0.39 | 0.5 | 0.96 | 1.1 | 1.46 | 1.7 |

b) *Lot-to-Lot Precision:*

The lot-to-lot precision was evaluated using three lots of ADVIA Centaur CYFRA reagent. Seven serum sample pools, having CYFRA 21-1 concentrations across the analytical measuring interval. The samples were tested in three replicates per run, two runs per day, for five days, resulting in a total of 90 datapoints for each sample. The data were analyzed for repeatability (within-run), between-day, between-reagent lot, and total precision. The results are summarized in table below.

| Sample | N | Mean (ng/mL) | Within-Run (Repeatability) | | Between-Day | | Between-Lot | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %C | SD | %CV | SD | %CV |
| 1 | 90 | 1.96 | 0.03 | 1.5 | 0.01 | 0.1 | 0.04 | 0.3 | 0.05 | 0.4 |
| 2 | 90 | 2.77 | 0.03 | 1.2 | 0.01 | 0.0 | 0.03 | 1.2 | 0.05 | 1.9 |
| 3 | 90 | 4.62 | 0.06 | 1.4 | 0.04 | 0.8 | 0.04 | 0.9 | 0.09 | 1.9 |
| 4 | 90 | 14.97 | 0.19 | 1.3 | 0.08 | 0.5 | 0.24 | 1.6 | 0.32 | 2.1 |
| 5 | 90 | 35.06 | 0.47 | 1.3 | 0.0 | 0.0 | 0.29 | 0.8 | 0.71 | 2.0 |
| 6 | 90 | 66.96 | 0.91 | 1.4 | 0.0 | 0.0 | 1.33 | 2.0 | 1.67 | 2.5 |
| 7 | 90 | 86.80 | 1.09 | 1.3 | 0.0 | 0.0 | 2.11 | 2.4 | 2.50 | 2.9 |

c) *Site-to-Site Reproducibility:*

The site-to-site reproducibility was evaluated by testing seven serum sample pools with CYFRA 21-1 concentrations across the analytical measuring interval at three sites using one lot of reagents. Each sample was tested in three replicates per run, two runs per day for five days, resulting a total of 90 datapoints for each sample. Data were analyzed for within-run, between-run, between-site and total reproducibility. The results are summarized in the table below.

| Sample | N | Mean (ng/mL) | Within-Run | | Between-Run | | Between-Day | | Between-Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 90 | 1.95 | 0.04 | 2.0 | 0.01 | 0.1 | 0.03 | 1.7 | 0.07 | 3.4 | 0.09 | 4.4 |
| 2 | 90 | 2.76 | 0.05 | 1.8 | 0.05 | 1.8 | 0.00 | 0.0 | 0.04 | 1.4 | 0.08 | 2.9 |
| 3 | 90 | 4.65 | 0.08 | 1.6 | 0.07 | 1.6 | 0.06 | 1.3 | 0.00 | 0.0 | 0.12 | 2.6 |
| 4 | 90 | 15.23 | 0.28 | 1.8 | 0.11 | 0.7 | 0.11 | 0.7 | 0.39 | 2.6 | 0.50 | 3.3 |

| Sample | N | Mean (ng/mL) | Within-Run | | Between-Run | | Between-Day | | Between-Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 5 | 90 | 25.48 | 0.50 | 2.0 | 0.19 | 0.7 | 0.23 | 0.9 | 0.44 | 1.7 | 0.73 | 2.9 |
| 6 | 90 | 65.84 | 1.09 | 1.7 | 0.33 | 0.5 | 0.85 | 1.3 | 0.44 | 0.7 | 1.48 | 2.3 |
| 7 | 90 | 84.18 | 1.38 | 1.6 | 0.00 | 0.0 | 1.13 | 1.3 | 1.14 | 1.4 | 2.11 | 2.5 |

2. Linearity:

*a) Linearity*:

The linearity of the ADVIA Centaur CYFRA was determined in accordance with CLSI EP06-ED2. Three high serum samples pools (HSSP) with targeted concentrations of 103 – 110 ng/mL CYFRA 21-1 were used to prepare three series (of 15 concentrations each) by diluting the HSSP with a low serum sample pool (LSSP) from apparently healthy donors to concentrations below the LoQ. Each dilution was tested in 12 replicates with the ADVIA Centaur CYFRA. The 'measured value' (mean CYFRA 21-1 ng/mL) ranged from 0.27 – 109 ng/mL and was compared to its predicted value using a weighted linear regression analysis for each series. The %deviation from linearity was calculated. The results are summarized in the table below.

| Sample Set | Range (ng/mL) | Slope (95% CI) | Intercept (95% CI) | $R^2$ | % Deviation from Linearity* |
|---|---|---|---|---|---|
| 1 | 0.25–108.6 | 1.00 (1.00 to 1.01) | 0.01 (-0.00 to 0.02) | 1.00 | -3.3% to 4.4% |
| 2 | 0.30–111.3 | 1.02 (1.005 to 1.038) | 0.023 (0.002 to 0.044) | 1.00 | -9.1% to 5.7% |
| 3 | 0.26–107.6 | 0.91 (0.861 to 0.956) | 0.014 (-0.031 to 0.059) | 0.99 | -8.4% to 11.0%^ |

*Minimum and maximum deviation from linearity

^%Deviation from linearity of 11.0% for sample Set at a concentration of 77.58 ng/mL.

The study results support the linearity of the claimed analytical measuring interval (AMI): 0.49 – 100.00 ng/mL.

*b) Dilution:*

A dilution study for the ADVIA Centaur CYFRA was performed on the ADVIA Centaur XPT analyzer using five human samples spiked to analyte concentrations above the analytical measuring interval (433 ng/mL) and diluted automatically on the instrument with the Siemens Multi-Diluent 13 at a 1:5 dilution. The same set of samples prepared manually was used as reference. For automated dilution, between-lot, within-lot, between-instrument, between-day, within-run, between-run, within-run, within-lab, and total imprecision %CV were ≤ 10% for all panels. The mean % recovery based on value assigned concentration for each tested sample concentration was within 100 ±10%.

*c) High Dose Hook Effect (Prozone):*

The high dose hook effect of the ADVIA Centaur CYFRA was assessed on the ADVIA Centaur XPT analyzer in accordance with CLSI EP34, 1st Edition. Three serum samples

were spiked to achieve CYFRA 21-1 concentration of 5000 ng/mL. Each sample was diluted by serial two-fold dilutions and analyzed in triplicate using two ADVIA Centaur CYFRA reagent lots on two ADVIA Centaur XPT analyzers. The results showed that no hook effect was observed with the ADVIA Centaur CYFRA assay in serum samples with CYFRA 21-1 concentration up to 2,500 ng/mL.

3. Analytical Specificity/Interference:

Interference study was performed according to CLSI EP07 (3rd Edition) and EP37 (1st Edition) to determine the effect of various endogenous and exogenous substances on the ADVIA Centaur CYFRA assay. Three-levels serum samples with CYFRA 21-1 target concentrations at 4 ng/mL, 24 ng/mL and 30 ng/mL, were spiked with (or without) varying levels of interference substance and were tested in three replicates on ADVIA Centaur XPT analyzer. The percent interference (% Interference) for CYFRA 21-1 in the serum sample was calculated as the observed interference divided by the mean measurand value of the control sample and multiplied by 100. Non-significant interference was defined as % interference within ±10% of CYFRA 21-1 for the following endogenous, exogenous substance and cancer drugs tested:

*a) Endogenous Interference:*

No significant interference was noted for samples containing the following potential endogenous interferents, up to the concentration listed in the table below.

| Substance | Concentration |
|---|---|
| Bilirubin, conjugated | 66 mg/dL |
| Bilirubin, unconjugated | 66 mg/dL |
| Hemoglobin | 1,500 mg/dL |
| Total Protein | 15 g/dL |
| Chyle | 3,000 mg/dL |
| Rheumatoid Factor | 1200 IU/mL |
| HAMA | 805 µg/L |

*b) Exogenous Substance Interference:*

No significant interference was noted for samples containing the following potential exogenous interferents, up to the concentration listed in the table below.

***Commonly used pharmaceuticals***:

| Exogenous Substance | Concentration | Exogenous Substance | Concentration |
|---|---|---|---|
| Acetaminophen | 200 mg/L | Ibuprofen | 500 mg/L |
| Acetylcysteine | 553 mg/L | Intralipid | 1500 mg/dL |
| Acetylsalicylic Acid | 1000 mg/L | Levodopa | 20 mg/L |
| Ampicillin-Na | 1000 mg/L | Methyldopa | 20 mg/L |
| Ascorbic acid | 300 mg/L | Metronidazole | 200 mg/L |
| Biotin | 3.5 mg/L | Phenylbutazone | 400 mg/L |
| Cyclosporine | 5 mg/L | Rifampicin | 60 mg/L |
| Doxycycline | 50 mg/L | Theophylline | 100 mg/L |
| Heparin | 5000 U/L | | |

***Cancer Drugs**:*

| Exogenous Substance | Concentration | Exogenous Substance | Concentration |
|---|---|---|---|
| 5-Fluorouracil | 500 mg/L | Leucovorin | 750 mg/L |
| Bevacizumab | 750 mg/L | Mitomycin | 25 mg/L |
| Carboplatin | 1000 mg/L | Melphalan | 15 mg/L |
| Cefoxitin | 2500 mg/L | Methotrexate | 1000 mg/L |
| Cisplatin | 45 mg/L | Nivolumab | 225 mg/L |
| Clotrimazole | 0.3 mg/L | Paclitaxel | 265 mg/L |
| Cyclophosphamide | 1000 mg/L | Pembrolizumab | 150 mg/L |
| Dexamethasone | 20 mg/L | Rituximab | 750 mg/L |
| Doxorubicin | 120 mg/L | Tarceva | 30 mg/L |
| Etoposide | 400 mg/L | Tamoxifen | 50 mg/L |

c) *Cross Reactivity*:

Cross reactivity of the ADVIA Centaur CYFRA assay was studied. No cross reactivity of the assay for the sample containing the following materials at the concentration listed in the table below:

| Substance | Test Concentration |
|---|---|
| α-fetoprotein (AFP) | 810 ng/mL |
| Human Chorionic Gonadotropin (β-hCG) | 517 mlU/mL |
| CA 125 | 4000 U/mL |
| CA 15-3 | 550 U/mL |
| CA 19-9 | 4167 U/mL |
| Carcinoembryonic Antigen (CEA) | 500 ng/mL |

4. Assay Reportable Range:

The ADVIA Centaur CYFRA 21-1 assay reportable range without dilution is the claimed analytical measuring interval (AMI): 0.49–100 ng/mL. For sample with CYFRA 21-1 level > 100.00 ng/mL, automated dilutions of 1:5 was validated up to 433 ng/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) *Traceability:*

There is no recognized reference standard for CYFRA 21-1. This device is traceable to internal reference standard manufactured using highly purified material.

b) *Sample stability:*

Sample stability was evaluated using the claimed serum and plasma sample types which were prepared from the pooled native whole blood with native samples or with the purified CYFRA 21-1 antigen to cover the various concentration levels. All samples were tested at various timepoint under the storage conditions: 23–27°C, 2–8°C, −20°C. At

each testing point, the recovery was calculated. The resulting data support the claim that serum, Li-heparin, K2-EDTA and K3-EDTA plasma specimens are stable for seven (7) days at 23–27°C, 30 days at 2–8°C, 12 months at -20°C, and up to three freeze thaw cycles.

*c) Reagent Stability:*

The stability of the ADVIA Centaur CYFRA reagent kit was determined according to CLSI EP25-Ed2.

i) Shelf-life:
The real-time stability was performed for the three reagent lots stored at 2–8°C. Four serum samples (with CYFRA 21-1 at 1.95, 2.94, 13.7 and 58.36 ng/mL) and two ADVIA Centaur Tumor Marker Quality Controls (3.3 and 33 ng/mL) were tested in three replicates for each lot at baseline and two replicates at each subsequent monthly testing timepoint. The test results for each sample at each timepoint were compared to the baseline value. The real-time stability study data support that the ADVIA Centaur CYFRA reagent kits have a shelf-life up to 18 months when stored at 2–8 °C.

ii) On-board stability:
On-board reagent (in use) stability was evaluated for the reagent stored open on the instrument at 2–8°C and reference reagents were stored off the instrument at 2–8°C by testing five serum sample panels (with CYFRA 21-1 at 1.64, 2.87, 14.20, 60.09 and 334.08 ng/mL) and two controls (3.35 and 33.32 ng/mL) on one ADVIA Centaur XPT instrument. The results showed that the ADVIA Centaur CYFRA Reagent ReadyPacks remained on-board the instrument for 34 days (on-board reagent evaluation) with no more than 10% drift from baseline for the Test Pack and with no need for recalibration. The study supports the 31 days on-board reagent stability without recalibration.

iii) Transport Stability:
Transport stability of the ADVIA Centaur CYFRA was determined by testing four serum pooled samples (with CYFRA 21-1 concentrations of 1.5, 3, 15, and 70 ng/ml) using two lots of reagents stored under the following simulated shipping conditions (ambient temperature). At each testing point, sample panels were tested in six replicates along with calibrators and controls, on each lot. The study showed that the ADVIA Centaur CYFRA kit can be shipped at ambient temperature (15°C to 30°C) and is stable up to two days.

6. Detection Capability:

The limit of blank (LoB), limit of detection (LoD) and limit of Quantitation (LoQ) were determined in accordance with CLSI EP17-A2.

LoB: Four analyte-free serum sample pools prepared by stripping serum samples of CYFRA 21-1 were tested in 10 replicates per run, two runs per day for three days using three reagent lots on one ADVIA Centaur XPT instrument. For each lot, the LoB was determined as the 95th percentile of the measurements of blank samples based on a total of 60 measurements and found to be 0.48, 0.36 and 0.36 ng/mL for three lots. The claimed LoB is 0.48 ng/mL.

LoD: Six low serum sample pools were run in two replicates per run, two runs per day for three days using three reagent lots on one ADVIA Centaur XPT instrument. For each lot, the LoD value was calculated based on the LoB + 1.645 x SD of the replicates for the samples and found to be 0.49, 0.37 and 0.38 ng/mL for three lots. The claimed LoD is 0.49 ng/mL.

LoQ: The within laboratory precision data from the LoD were also used to determine the LoQ based on precision profile for each lot. The LoQ is defined as the highest value of the sample having <20% CV within-laboratory precision across all lots. The LoQ is determined to be 0.49 ng/mL.

7. Assay Cut-Off:

See clinical cut-off

## B Comparison Studies:

1. Method Comparison with Predicate Device:

The method comparison study was performed to compare the ADVIA Centaur CYFRA assay on ADVIA Centaur XPT analyzer to the predicate device, the Elecsys CYFRA 21-1 on the Roche cobas e 411 analyzer. A total of 120 samples with analyte concentration ranging from 1.18 – 97.19 ng/mL were tested using three lots (Lot A, B, C) of the candidate reagent and one lot of the predicate reagent on one respective instrument. Samples within the analytical measuring interval (AMI) for both the predicate and candidate devices were used for the Deming regression analysis. The results are summarized as follows:

| Lot | N | Range (ng/mL) | Slope (95% CI) | Intercept (95% CI) | $R^2$ |
|---|---|---|---|---|---|
| A | 115 | 1.00 – 91.61 | 0.989 (0.962 to 1.017) | -0.306 (-0.457 to -0.155) | 0.975 |
| B | 115 | 0.96 – 91.49 | 0.997 (0.968 to 1.026) | -0.474 (-0.667 to -0.281) | 0.9736 |
| C | 115 | 0.98 – 90.05 | 0.979 (0.951 to 1.005) | -0.378 (-0.553 to -0.204) | 0.975 |

2. Matrix Comparison:

The matrix equivalency study was performed according to CLSI EP35, 1st Edition to demonstrate that serum and plasma (Li heparin, K2-EDTA, K3-EDTA) matrices yield comparable values as serum with the ADVIA Centaur CYFRA assay. Thirty-nine blood donor matched sample sets were collected into six vacutainer tube types, including Red Top (RT), Serum Separator Tube (SST), Plasma Li-Heparin Separator Tube (LiHepST), Plasma Lithium Heparin (Li-Hep), Plasma K2EDTA (K2EDTA), and Plasma K3EDTA (K3EDTA). In addition, 22 blood donor matched sample sets were prepared by spiking with CYFRA 21-1 antigen to create samples with CYFRA 21-1 concentrations at 10 (n=5), 25 (n=7), 50 (n=4) and 75 (n=6) ng/mL. Samples were tested and a Weighted Deming regression analysis was performed. The results are summarized in the following table:

| Tube Type | N | Range (ng/mL) | Slope (95% CI) | Intercept (95% CI) | r |
|---|---|---|---|---|---|
| SST vs. Serum | 61 | 0.80–79.10 | 1.01 (0.99 to 1.02) | -0.01 (-0.03 to 0.01) | 1.00 |
| LiHepST vs. Serum | 61 | 0.82–83.53 | 1.00 (0.98 to 1.01) | 0.02 (0.00 to 0.04) | 1.00 |
| Li-Hep vs. Serum | 61 | 0.80–80.83 | 1.00 (0.98 to 1.01) | 0.03 (0.01 to 0.05) | 1.00 |
| K2-EDTA vs. Serum | 61 | 0.81–82.81 | 0.99 (0.98 to 1.01) | 0.01 (-0.01 to 0.03) | 1.00 |
| K3-EDTA vs. Serum | 61 | 0.84–77.98 | 0.96 (0.95 to 0.98) | 0.03 (0.00 to 0.05) | 1.00 |

## C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A prospective clinical study was conducted to evaluate CYFRA 21-1 values in 93 lung cancer patients during the course of disease and therapy using the ADVIA Centaur CYFRA assay on ADVIA Centaur XPT analyzer.

The subject inclusion and exclusion criteria are as follows:

Inclusion criteria:

- Female or Male
- 22 years of age or older
- Histologic/pathologic confirmation of lung cancer
- Any stage of disease: newly diagnosed, no evidence of disease (NED), stable, disease progression/recurrence and responding disease
- Any treatment time point: treatment naïve, currently receiving or completed therapy for squamous cell lung cancer including active monitoring
- Individuals with a history of malignant disease other than lung cancer that was resected greater than 5 years ago and are currently in remission are eligible
- Appropriate informed consent or remnant sample documentation

Exclusion criteria:

- Less than 22 years of age
- No histologic/pathologic confirmation of lung cancer
- Any concurrent malignancy other than basal or squamous cell skin cancers or in-situ cervical cancer

The clinical data collected for each subject included the following, when available: subject ID, date of birth, gender, race/ethnicity, date of cancer diagnosis, histology, grade, and stage. For each subject sample draw, the following information was collected, if available: 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). The clinical disease status was determined by reviewing a compilation of procedures and clinical notes which may have included but were not limited to: imaging of various modalities, physical exam, physician transcription notes, and/or other laboratory data.

The clinical disease status at the time of each sample collection was defined as the following:

- No evidence of disease (NED): NED is defined as a complete lack of clinical evidence of disease as determined by the treating physician.
- Stable disease: Stable Disease is defined as clinical evidence that the disease has not changed since the last assessment as determined by the treating physician.
- Progressive Disease: Progressive Disease is defined as clinical evidence of growth in the primary tumor or the appearance of new tumors since the last assessment as determined by the treating physician.
- Responsive disease (RD): RD is defined as clinical evidence that there is a shrinking of the primary tumor and no evidence of new tumors as determined by the treating physician.

A total of 462 serial samples from 93 subjects were obtained and tested for CYFRA 21-1 levels. Of the 93 subjects, 39 (41.8%) were male and 54 (58.1%) were female. The mean age was 66 years ranging from 38 to 89 years. The majority of subjects were Caucasian (n=79, 84.9%), the remaining subjects included nine (9.7%) African American, one (1.1%) Asian, and two (2.2%) Hispanic. The length of time (in days) over which the subjects were monitored ranged from 122 to 967 (median of 550). The median interval (in days) between successive visits was 109, ranging from 8 to 501.

For the 93 lung cancer cases, 75 were classified as non-small cell lung cancer (NSCLC) and 16 were classified as small cell lung cancer (SCLC). The histopathology distribution of the subjects is summarized below:

| Histologic Subtype | n (%) |
|---|---|
| **Non-Small Cell** | **77 (82.8%)** |
| Adenocarcinoma | 48 (51.6%) |
| Large Cell Carcinoma | 2 (2.2%) |
| Not Otherwise Specified | 3 (3.2%) |
| Squamous Cell Carcinoma | 24 (25.8%) |
| **Small Cell** | **16 (17.2%)** |
| **Total** | **93 (100%)** |

Of 93 subjects, one subject did not have stage information (Unknown), 15 were stage I, 16 were stage II, while 61 (66%) were stage III or IV.

For the data analysis, all 462 serial samples from 93 subjects were tested for CYFRA 21-1 levels. At each follow-up visit, the percentage change of the CYFRA 21-1 was calculated for the percent change by comparing the test result to the result obtained from the previous visit. An increase in CYFRA 21-1 of at least 50% over the previous visit, along with a requirement that the current CYFRA 21-1 value was above the upper limit of the Reference Interval (for

non-smokers, determined to be 2.77 ng/mL in the Reference Interval/Expected Values Study), as an indicator of disease progression for the results from the ADVIA Centaur CYFRA. The clinical performance of the test was assessed by comparing to the test results to the clinical disease status categorized as progression (Progressive Disease) or non-progression (NED, Stable Disease or RD).

The ratio of CYFRA 21-1 values obtained using the ADVIA Centaur CYFRA assay at each visit to that at the preceding visit was calculated and referred to as the visit-to-visit CYFRA 21-1 ratio. Summary statistics of the distribution of the ratio by clinical disease status are shown in the following table for ADVIA Centaur CYFRA.

| Clinical Disease Status | N | Visit-to-Visit Ratio of ADVIA Centaur CYFRA | | | | |
|---|---|---|---|---|---|---|
| | | Minimum | 1st Quartile | Median | 3rd Quartile | Maximum |
| NED | 84 | 0.44 | 0.76 | 0.95 | 1.20 | 2.87 |
| Stable | 190 | 0.11 | 0.82 | 1.01 | 1.31 | 5.19 |
| Responding | 43 | 0.14 | 0.68 | 1.00 | 1.27 | 2.76 |
| Progression | 52 | 0.46 | 0.87 | 1.16 | 1.69 | 4.10 |

- Most of the visits showing progression had an ADVIA Centaur CYFRA 21-1 ratio above one, but the remaining three groups were centered close to 1. Summary statistics showed the NED, stable, and responding subjects have similar summary statistics and half of the subject visits showing disease progression had an increase in the ADVIA Centaur CYFRA values.

The following tables represent the number of all clinical follow-up visits (n=369) for 93 subjects at which a clinical evaluation and the percentage change of the subjects at these clinical evaluations using a threshold of a ≥ 50.0% increase in CYFRA 21-1 from previous visit and a current CYFRA 21-1 value of > 2.77 ng/mL.:

| ADVIA Centaur CYFRA | Progression | Responding | Stable | NED | Total |
|---|---|---|---|---|---|
| > 2.77 ng/mL (≥ 50% change) | 16 | 2 | 23 | 9 | 50 |
| > 2.77 ng/mL (< 50% change) | 14 | 10 | 45 | 15 | 84 |
| ≤ 2.77 ng/mL | 22 | 31 | 122 | 60 | 235 |
| Total | 52 | 43 | 190 | 84 | 369 |

| ADVIA Centaur CYFRA | Progression | Non-Progression | Total |
|---|---|---|---|
| > 2.77 ng/mL (≥ 50% change) | 16 | 34 | 50 |
| > 2.77 ng/mL (< 50% change) | 14 | 70 | 84 |
| ≤ 2.77 ng/mL | 22 | 213 | 235 |
| Total | 52 | 317 | 369 |

For evaluation of clinical sensitivity and specificity of the ADVIA Centaur CYFRA, the clinical disease status was condensed into two categories: progression and no-progression. Subjects with progression contained those monitoring events defined as progressive disease. Subjects with no-progression contained those monitoring events defined as NED, stable disease and responding disease. A positive change in CYFRA 21-1 was defined as

measurable increase in the value that was at least 50% greater than the previous value of the test when the CYFRA 21-1 result was outside the reference range of 2.77 ng/mL.

| | | Clinical Disease Status | | |
|---|---|---|---|---|
| | | **Progression** | **Non-Progression** | **Total** |
| **ADVIA Centaur CYFRA** | **≥ 50.0%; > 2.77 ng/mL** | 16 | 34 | 50 |
| | **< 50.0%; ≤ 2.77 ng/mL** | 36 | 283 | 319 |
| | **Total** | 52 | 317 | 369 |
| | | **Estimate** | **95% CI** | |
| Sensitivity | | 30.8% | 19.6 – 42.0% | |
| Specificity | | 89.3% | 85.9 – 92.4% | |
| Positive Predictive Value (PPV) | | 32.0% | 21.7 – 42.8% | |
| Negative Predictive Value (NPV) | | 88.7% | 87.1 – 90.4% | |
| Positive Likelihood Ratio (PLR) | | 2.87 | 1.69 – 4.56 | |
| Negative Likelihood Ratio (NLR) | | 0.78 | 0.65 – 0.91 | |
| Prevalence | | 14.1% | | |

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

| Percent (%) Change in ADVIA Centaur CYFRA | Sensitivity (%) | Specificity (%) | NPV (%) | PPV (%) |
|---|---|---|---|---|
| ≥ 30.0% | 34.6 | 84.5 | 88.7 | 26.9 |
| ≥ 40.0% | 34.6 | 86.4 | 89.0 | 29.5 |
| ≥ 50.0% | 30.8 | 89.3 | 88.7 | 32.0 |
| ≥ 60.0% | 28.8 | 90.9 | 88.6 | 34.1 |
| ≥ 70.0% | 25.0 | 92.7 | 88.3 | 36.1 |

The summary statistics of the ratio of the ADVIA Centaur CYFRA results from the preceding draw for all follow-up draws by disease stages is shown in the following table:

| Stage | Subjects | Pairs | Visit-to-Visit Ratio of ADVIA Centaur CYFRA | | | | |
|---|---|---|---|---|---|---|---|
| | | | Min | 1st Quartile | Median | 3rd Quartile | Max |
| IA | 10 | 38 | 0.4 | 0.7 | 1.0 | 1.2 | 2.6 |
| IB | 5 | 20 | 0.5 | 0.8 | 0.9 | 1.1 | 2.5 |
| IIA | 4 | 13 | 0.5 | 0.7 | 0.9 | 1.1 | 2.3 |
| IIB | 12 | 49 | 0.3 | 0.9 | 1.1 | 1.4 | 3.4 |
| IIIA | 16 | 59 | 0.1 | 0.8 | 1.0 | 1.4 | 5.2 |
| IIIB | 12 | 49 | 0.4 | 0.8 | 1.0 | 1.2 | 2.9 |
| IIIC | 1 | 3 | 0.9 | 1.1 | 1.2 | 1.3 | 1.4 |
| IV | 32 | 132 | 0.1 | 0.8 | 1.1 | 1.3 | 3.5 |
| Unknown | 1 | 6 | 0.5 | 0.8 | 0.9 | 1.3 | 1.7 |
| All | 93 | 369 | 0.1 | 0.8 | 1.0 | 1.3 | 5.2 |

The following table summarized the diagnostic performance of the ADVIA Centaur CYFRA 21-1 values over successive visits by disease stage:

| Stage | Subjects | Pairs | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| I | 15 | 58 | 0.0% | 88.7% | 0.0% | 90.4% |
| II | 16 | 62 | 16.7% | 83.9% | 10.0% | 90.4% |
| III | 29 | 111 | 38.5% | 89.8% | 33.3% | 91.7% |
| IV | 32 | 132 | 35.7% | 92.3% | 55.6% | 84.2% |
| Unknown | 1 | 6 | * | 83.3% | 0.0% | 100.0% |

* For this one subject, there were no instances of PD, only six NPD occurrences.

**D Clinical Cut-Off:**

An absolute cutoff is not applicable for monitoring. CYFRA 21-1 value increases at least 50% higher than immediate previous sample are considered a significant change when the ADVIA Centaur CYFRA result was above 2.77 ng/mL (the upper limit of the Reference Interval for non-smokers, determined in the Reference Interval/Expected Values Study), as an indicator of disease progression.

**E Expected Values/Reference Range:**

A reference interval for healthy adults (smokers and nonsmokers) was established in accordance with CLSI Document EP28-A3c on the ADVIA Centaur XPT System along with subjects with conditions and diseases that may result in elevated/depressed CYFRA 21-1 antigen levels. Samples were collected prospectively from apparently healthy subjects (N= 239) and included males and females ≥ 22 years old without any chronic medical conditions or on chronic medications (women using hormones for menopause or on birth control were eligible). The tested set included 104 females and 135 males, ages 22 to 87 years, with an average age of 43 years and median age of 42 years including 119 smokers and 120 nonsmokers. The sample set included 176 Caucasian, 18 African American, 62 Hispanic, 19 Asian, 10 American Indian or Alaskan Native, and 16 other/unknown ethnicities.

The lower (2.5$^{th}$ percentile) and upper (97.5$^{th}$ percentile) reference interval limits were determined from the distribution of the ordered measurments of the apparently healthy subjects. The reference interval (2.5$^{th}$ percentile – 97.5$^{th}$ percentile) is summarized in the table below:

| Group | N | Median (ng/mL) | Reference Interval (ng/mL) |
|---|---|---|---|
| **All (22 – 87 years)** | 239 | 1.36 ng/mL | 0.75 – 7.64 |
| Smoker (22 – 71 years) | 119 | 1.56 ng/mL | 0.78 – 5.69 |
| Non-Smoker (22 – 87 years) | 120 | 1.22 ng/mL | 0.71 – 2.77 |

For CYFRA 21-1 values in different disease groups (benign and malignant), the distribution in percentage (%) of CYFRA 21-1 assay values determined in nine (9) clinical centers in the U.S. along with commercially available samples with the ADVIA Centaur CYFRA 21-1 assay in eight hundred sixty-eight (868) serum specimens is summarized in the following table:

| | N | n% > 2.77 ng/mL | Mean (ng/mL) | 25th Percentile (ng/mL) | Median | 75th Percentile (ng/mL) |
|---|---|---|---|---|---|---|
| ***Benign conditions*** | | | | | | |
| Breast diseases | 40 | 2.5 | 1.26 | 0.92 | 1.11 | 1.38 |
| Liver diseases | 40 | 30.0 | 2.70 | 1.36 | 1.88 | 3.02 |
| Lung diseases | 75 | 17.3 | 2.05 | 1.32 | 1.71 | 2.16 |
| Congestive heart failure | 38 | 21.1 | 2.43 | 1.50 | 1.78 | 2.57 |
| Renal diseases | 40 | 45.0 | 4.41 | 1.90 | 2.54 | 3.59 |
| Bladder | 40 | 20.0 | 1.89 | 1.00 | 1.64 | 2.47 |
| Breast | 45 | 20.0 | 2.88 | 1.31 | 1.76 | 2.29 |
| ***Cancers*** | | | | | | |
| Cervical | 39 | 20.5 | 2.67 | 1.04 | 1.38 | 2.19 |
| Colorectal | 40 | 42.5 | 6.72 | 1.89 | 2.51 | 3.65 |
| Esophageal Squamous Cell | 40 | 22.5 | 2.27 | 1.38 | 1.72 | 2.48 |
| Head & Neck | 39 | 43.6 | 5.68 | 1.74 | 2.45 | 4.99 |
| Treatment-naïve NSCLC | 119 | 47.9 | 7.48 | 1.62 | 2.59 | 5.89 |
| Treatment-naïve SCLC | 113 | 41.6 | 6.31 | 1.53 | 2.19 | 4.66 |
| Ovarian | 40 | 52.5 | 13.62 | 1.40 | 2.91 | 9.06 |
| Prostate | 40 | 30.0 | 4.32 | 1.50 | 2.29 | 3.01 |
| Testicular | 40 | 12.5 | 2.98 | 0.95 | 1.32 | 1.58 |
| Stomach | 40 | 25.0 | 6.87 | 1.44 | 1.68 | 2.79 |
| NSCLC Females | 41 | 41.5 | 8.06 | 1.39 | 2.26 | 3.47 |
| NSCLC Males | 78 | 51.3 | 7.17 | 1.70 | 2.85 | 6.46 |
| SCLC Females | 34 | 44.1 | 5.00 | 1.48 | 2.19 | 4.29 |
| SCLC Males | 78 | 39.7 | 6.89 | 1.60 | 2.19 | 4.83 |

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

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