

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

**A. 510(k) Number:**

k080469

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Cancer Antigen 15-3 (CA 15-3)

**D. Type of Test:**

Quantitative, Enzyme Linked Fluorescent assay (ELFA)

**E. Applicant:**

bioMérieux, Inc.

**F. Proprietary and Established Names:**

VIDAS® CA

**G. Regulatory Information:**

1. Regulation section:  
21 CFR 866.6010 Tumor-associated antigen immunological test system
2. Classification:  
Class II
3. Product code:  
MOI, System, Test, Immunological, Antigen, Tumor
4. Panel:  
Immunology (82)

**H. Intended Use:**

1. Intended use(s):  
VIDAS® CA 15-3 is an automated quantitative test for use on the VIDAS® instruments for the quantitative measurement of CA 15-3 reactive antigenic determinants in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay). The VIDAS® CA 15-3 is indicated for the serial measurement of CA 15-3 reactive antigenic determinants as an aid in the monitoring of patients previously diagnosed with breast cancer for disease progression or response to therapy in conjunction with other clinical methods. The VIDAS CA® 15-3 assay can also be used as an aid in the detection of recurrence in previously treated Stage II and III breast cancer patients.
2. Indication(s) for use:  
Same as Intended Use.
3. Special conditions for use statement(s):  
Prescription use only.
4. Special instrument requirements:  
VIDAS® or mini VIDAS® analyzer

**I. Device Description:**

Each VIDAS CA 15-3 kit contains reagents sufficient for 30 tests. The kit is comprised of 30 CA 15-3 reagent strips (10 wells per strip), 30 CA 15-3 Solid Phase Receptacle (SPR) (coated with anti-CA 15-3 mouse monoclonal 115D8 antibodies and also serves as the pipetting device), CA 15-3 control (C1)(1 ml, ready-to-use), CA 15-3 calibrator (S1)(1.5 mL, ready-to-use with bovine albumin, DF3 antigenic determinants (human origin), and sodium

azide), CA 15-3 diluent (5 mL, ready-to-use vial with calf serum and sodium azide), one MLE (Master Lot Entry) card (contains C1 value range and SI dose value and relative fluorescence value range), clip seal, and package insert.

#### Description of the CA 15-3 Reagent Strip

| Wells     | Reagents   |
|-----------|--|
| 1         | Sample well.   |
| 2 - 3 - 4 | Empty wells.   |
| 5         | Conjugate: Alkaline phosphatase labeled monoclonal DF3 antibody + 0.9 g/L sodium azide (400 µL).   |
| 6 - 7     | Wash buffer: Tris (0.1 mol/L, pH 7.4) + NaCl (0.1 mol/L) + Tween (0.05%) + 0.9 g/L sodium azide (600 µL).  |
| 8         | Diluent: Tris (0.1 mol/L) + NaCl (0.1 mol/L) + calf serum (5 %) + 0.9 g/L sodium azide (400 µL).   |
| 9         | Wash buffer: Tris (0.1 mol/L, pH 7.4) + NaCl (0.1 mol/L) + Tween (0.05 %) + 0.9 g/L sodium azide (600 µL).   |
| 10        | Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine (DEA*) (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL). |

#### J. Substantial Equivalence Information:

1. Predicate device name(s):  
Tosoh Medical, Inc. ST AIA Pack BRCA
2. Predicate K number(s):  
k010796
3. Comparison with predicate:

| Similarities    |   |                   |
|-----------------|---|-------------------|
| Item            | Device  | Predicate         |
| Specimen        | Serum   | Serum             |
| Analyte         | CA 15-3   | CA 27.29 (CA15-3) |
| Assay Principle | Two antibody “sandwich” assay One antibody is bound to a solid phase and the second antibody is in liquid form and is labeled with fluorescent compound | Same              |
| Assay Technique | Enzyme-linked fluorescent assay (ELFA)  | Same              |
| Enzyme label    | Alkaline phosphatase  | Same              |
| Substrate       | 4-methylumbelliferyl phosphate (4MUP)   | Same              |

| Differences  |   |   |
|--------------|---|---|
| Item         | Device  | Predicate   |
| Intended Use | For the serial measurement of CA 15-3 reactive antigenic determinants as an aid in the monitoring of patients | For the quantitative measurement of CA27.29 in human serum used as an aid in monitoring response to therapy |

| Differences                                       |   |  |
|---|---|--|
| Item  | Device  | Predicate  |
|   | previously diagnosed with breast cancer for disease progression or response to therapy in conjunction with other clinical methods. The VIDAS CA 15-3 assay can also be used as an aid in the detection of recurrence in previously treated Stage II and III breast cancer patients. | for patients with Stage IV (metastatic) breast cancer as well as determining early recurrence in Stage II and Stage III breast cancer patients who were previously treated and free of disease. Serial testing for patient CA27.29 assay values should be used in conjunction with other clinical methods used for monitoring response to therapy in patients with Stage IV metastatic breast cancer and for detecting early recurrence in Stage II and Stage III disease. |
| Antibody  | Mouse monoclonal 115D8 and DF3 antibodies   | Mouse monoclonal antibodies to CA 15-3   |
| Measurement range                                 | 2.00 – 365.00 U/mL  | 2.0 – 400 U/mL   |
| Sample Volume                                     | 100 µL  | 20 µL  |
| Positive change from previous value (significant) | 12%   | 10%  |
| Traceability/Standardization                      | Master curve for each kit lot and each calibrator lot are traceable to working standards established by bioMérieux, Inc. and value assigned by the Fujirebio Diagnostics, Inc. radioimmunoassay method  | Each calibrator lot are traceable to internal reference standards  |
| Instrument platforms                              | Vidas and mini Vidas instruments  | TOSOH AIA Nex·IA and AIA-600 II Immunoassay analyzers  |

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

CLSI EP5-A2; Evaluation of Precision Performance of quantitative measurement methods; Approved Guideline – Second Edition.

CLSI EP6-A; Evaluation of linearity of quantitative measurement procedures: A statistical approach; Approved Guideline

EP09-A2 Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline

CLSI EP17-A; Protocols for the determination of limits of detection and limits of quantitation; Approved Guideline.

**L. Test Principle:**

The assay principle combines a 2-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is cycled in and out of the SPR several times. This operation enables the monoclonal 115D8 antibody fixed onto the interior wall of the SPR to capture the reactive antigenic determinants present in the sample. Unbound components are eliminated during the washing steps. Alkaline phosphatase labeled monoclonal DF3 antibody is then incubated in the SPR where it binds with the DF3 reactive antigenic determinants. Unbound conjugate is then eliminated during the washing steps. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of CA 15-3 reactive antigenic determinants present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

**M. Performance Characteristics (if/when applicable):**1. Analytical performance:a. *Precision/Reproducibility:*

Precision and Reproducibility were carried out at three sites. Three pooled samples covering the assay range were tested in duplicate, 2 runs per day for a period of 20 days, with 2 reagent lots. Two separate calibrations were performed on each lot followed by 10 testing days per calibration. A total of 40 values were generated per sample, site, and lot, and the precision and reproducibility is summarized in the table below. The mean value (U/mL) for each pool appears in parentheses.

|              |             | % CV                   |       |                       |       |                       |       |
|--------------|-------------|------------------------|-------|-----------------------|-------|-----------------------|-------|
|              |             | Pool A<br>(270.0 U/mL) |       | Pool B<br>(67.7 U/mL) |       | Pool C<br>(21.4 U/mL) |       |
|              |             | Lot 1                  | Lot 2 | Lot 1                 | Lot 2 | Lot 1                 | Lot 2 |
| Site 1       | Day-to-Day  | 2.06                   | 2.07  | 1.23                  | 1.92  | 0.74                  | 2.97  |
|              | Inter-assay | 0.00                   | 0.25  | 1.4                   | 0.00  | 0.60                  | 1.19  |
|              | Intra-assay | 3.35                   | 3.53  | 3.2                   | 3.77  | 3.59                  | 3.80  |
|              | Total       | 3.93                   | 4.10  | 3.7                   | 4.23  | 3.71                  | 4.97  |
| Site 2       | Day-to-Day  | 2.26                   | 3.38  | 1.79                  | 2.64  | 1.12                  | 3.93  |
|              | Inter-assay | 0.00                   | 0.64  | 0.19                  | 0.99  | 2.07                  | 2.24  |
|              | Intra-assay | 3.11                   | 4.00  | 3.42                  | 4.55  | 3.59                  | 3.76  |
|              | Total       | 3.84                   | 5.28  | 3.87                  | 5.36  | 4.29                  | 5.89  |
| Site 3       | Day-to-Day  | 2.45                   | 2.36  | 1.20                  | 2.23  | 2.60                  | 2.36  |
|              | Inter-assay | 1.22                   | 0.60  | 0.52                  | 0.00  | 1.05                  | 1.28  |
|              | Intra-assay | 2.10                   | 3.65  | 3.44                  | 3.44  | 2.29                  | 4.06  |
|              | Total       | 3.45                   | 4.39  | 3.68                  | 4.10  | 3.62                  | 4.87  |
| Across sites |             | 4.16                   |       | 4.16                  |       | 4.56                  |       |

**Lot-to-Lot**

Two reagent lots were compared as described in the Precision/Reproducibility section, above, and the results described in the table below.

| Source                | Pool A<br>(270 U/mL) | Pool B<br>(67.7 U/mL) | Pool C<br>(21.4 U/mL) |
|-----------------------|----------------------|-----------------------|-----------------------|
|                       | CV (%)               | CV (%)                | CV (%)                |
| Between-site          | 0.00                 | 0.00                  | 1.92                  |
| Between-lot           | 3.17                 | 2.10                  | 2.01                  |
| Between-recalibration | 2.81                 | 2.03                  | 2.36                  |
| Between-day           | 0.76                 | 0.00                  | 1.08                  |
| Between-run           | 1.71                 | 1.93                  | 2.23                  |
| Within-run            | 3.09                 | 3.36                  | 3.32                  |
| Total                 | 5.57                 | 4.85                  | 5.52                  |

An additional determination for precision between lots was provided to show the total precision calculated from the QC release data of sixty-eight manufactured lots. Lot-to-lot, instrument-to-instrument, and run-to-run variability were included in the estimation. The total CV was calculated to be 4.03% – 6.06% with a median CV of 4.90%. The data from these lots are as follows:

| Sample<br>(N=12) | Mean<br>(U/mL) | SD<br>(U/mL) | CV<br>(%) | # lots sample<br>tested in |
|------------------|----------------|--------------|-----------|----------------------------|
| X13              | 7.24           | 0.44         | 6.06%     | 68                         |
| X14              | 11.48          | 0.63         | 5.46%     | 68                         |
| X15              | 17.96          | 1.02         | 5.68%     | 68                         |
| X16              | 18.75          | 0.81         | 4.30%     | 68                         |
| X12              | 21.94          | 1.3          | 5.93%     | 68                         |
| X23              | 22.05          | 0.92         | 4.17%     | 68                         |
| X22              | 37.72          | 1.84         | 4.86%     | 68                         |
| X21              | 46.1           | 2.44         | 5.29%     | 68                         |
| X17              | 64.85          | 3.15         | 4.86%     | 68                         |
| X18              | 81.78          | 3.98         | 4.87%     | 68                         |
| X19              | 91.59          | 3.69         | 4.03%     | 68                         |
| X20              | 102.54         | 5.05         | 4.93%     | 68                         |

*b. Linearity/assay reportable range:*

Three samples with naturally high CA 15-3 values (> 400 U/mL) and one naturally low sample (~ 55 U/mL) were serially diluted to 1/20 with the CA 15-3 diluent. Linearity and recovery after dilution were studied according to a protocol based on the recommendations of the document CLSI® EP06-A and is shown to be linear over the entire measuring range.

| Sample<br>Actual Conc.<br>(U/ml) | Dilution | Calculated Conc.<br>(U/mL) | Measured Conc.<br>(U/mL) | Recovery<br>% |
|----------------------------------|----------|----------------------------|--------------------------|---------------|
| Sample 1<br><b>54.78</b>         | 1        | 54.78                      | 54.78                    | 100           |
|                                  | 9/10     | 49.3                       | 48.28                    | 97.9          |
|                                  | 8/10     | 43.82                      | 44.24                    | 100.9         |
|                                  | 7/10     | 38.35                      | 37.38                    | 97.5          |
|                                  | 6/10     | 32.87                      | 33.12                    | 100.8         |
|                                  | 5/10     | 27.39                      | 27.01                    | 98.6          |
|                                  | 4/10     | 21.91                      | 21.55                    | 98.3          |
|                                  | 3/10     | 16.43                      | 17.46                    | 106.2         |
|                                  | 1/10     | 5.48                       | 5.71                     | 104.2         |
|                                  | 1/20     | 2.74                       | 2.76                     | 100.6         |
| Sample 2<br><b>452.06</b>        | 8/10     | 361.7                      | 361.7                    | 100           |
|                                  | 7/10     | 316.44                     | 310.21                   | 98            |
|                                  | 6/10     | 271.24                     | 276.96                   | 102           |
|                                  | 5/10     | 226.03                     | 212.48                   | 94            |
|                                  | 4/10     | 180.82                     | 168.00                   | 93            |
|                                  | 3/10     | 135.62                     | 125.17                   | 92            |
|                                  | 1/10     | 45.21                      | 44.09                    | 98            |
|                                  | 1/20     | 22.60                      | 21.61                    | 96            |
| Sample 3<br><b>606.97</b>        | 5/10     | 303.49                     | 285.86                   | 94            |
|                                  | 4/10     | 242.79                     | 230.66                   | 95            |
|                                  | 3/10     | 182.09                     | 193.03                   | 106           |
|                                  | 1/10     | 60.70                      | 61.22                    | 101           |
|                                  | 1/20     | 30.35                      | 31.95                    | 105           |
| Sample 4<br><b>498.06</b>        | 6/10     | 298.84                     | 325.37                   | 109           |
|                                  | 5/10     | 249.03                     | 263.03                   | 106           |
|                                  | 4/10     | 199.22                     | 207.70                   | 104           |
|                                  | 3/10     | 149.42                     | 147.97                   | 99            |
|                                  | 1/10     | 49.81                      | 56.23                    | 113           |
|                                  | 1/20     | 24.90                      | 27.17                    | 109           |

#### High Dose Hook Effect:

A serum sample with high CA 15-3 (approximately 13,000 U/mL) was tested with three separate manufactured lots. The undiluted sample gave a Relative Fluorescence Value (RFV) signal near or above the approximate VIDAS fluorescent signal saturation point. The sample was diluted to 1/28 with sample diluent and the data plotted to demonstrate the RFV signal relative to the calculated concentration. No high dose hook effect was observed for CA 15-3 concentrations up to 13,000 U/mL.

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

Traceability: Assay calibrators are traceable to working standards established by bioMérieux, Inc. with values assigned by Fujirebio Diagnostic Inc. radioimmunoassay method.

Stability of calibration curve: The calibration frequency was assessed using three VIDAS CA 15-3 kit lots, six instruments (2 per kit lot), 8 working standards, the lot-

specific assay calibrator (S1) and control (C1), and 5 internal control sera and performed over an 8 week period. The first week seven (7) runs were performed on the 3 VIDAS #1 instruments, one per kit lot, using the working standards to establish the Master Curve for the kit lots. An additional 15 runs were performed on the remaining 3 instruments (#2) using the working standards and lot-specific S1 in duplicate, and 5 internal control sera and lot-specific C1 in singlet. The runs were repeated on weeks 2-8, one run per week on the two VIDAS #2 instruments. The assay was calibrated every 14 and 28 days. The concentrations obtained by total calibration were not significantly different from those obtained by systematic recalibration or 14 day/28 day recalibration indicating the assay can be recalibrated with the kit lot-specific calibrator, tested in duplicate, at a frequency of 14 days.

Calibrator and control stability is 12 months when stored at 2-8°C. Reconstituted calibrator and control stability is 2 weeks when stored at 2-8°C or 7 months when stored at  $-25 \pm 6^{\circ}\text{C}$ .

Freeze/thaw calibrator stability: One lot of control and calibrator was tested. The acceptance criterion was target concentration plus 3 standard deviations. Frozen calibrator was demonstrated to be able to undergo up to five freeze/thaw cycles..

*d. Detection limit:*

Limits of blank, detection, and quantitation were determined using 2 kit lots on 2 instruments (one per lot) using CLSI protocol EP17-A. Five low CA 15-3 samples were tested using two lots on two VIDAS instruments. Tested CA 15-3 values ranged from approximately 0.79 to 1.38 U/mL. Acceptance criteria for the limit of blank, detection, and quantitation were described as follows:

- Limit of blank (LOB) – highest measurement result which has a 95% probability to be observed for a blank sample. It is the 95<sup>th</sup> percentile of a blank distribution
- Limit of detection (LOD) – lowest amount of analyte that can be detected with 95% probability, though not quantified at an exact value.
- Limit of quantitation (LOQ) – lowest actual amount of analyte that can be reliably detected and at which total error meets lab requirements for accuracy.

The results support a claim for LOB, LOD and LOQ of less than 2 U/mL. Therefore, the lowest value for the range of the assay is 2 U/mL.

*e. Analytical specificity:*

Hemoglobin, triglyceride, bilirubin, human albumin, rheumatoid factor (RF), HAMA, and 27 anti-cancer and over-the-counter (OTC)-drug interferents were evaluated for interference when added to three human serum samples containing very low, low, and moderate concentrations of CA 15-3 (approximately 7, 32, and 217 U/mL, respectively). The stock solutions of HAMA, RF, and albumin interferents were prepared by initially dissolving the HAMA and RF in human plasma and the albumin in water, prior to spiking into the CA 15-3 containing serum samples. Each sample was divided into two aliquots and one spiked with the potential interferents and the other spiked with buffer only. Three intermediate levels were prepared by variable

mixing of the two aliquots. Aliquots of each concentration were tested in single replicates in three runs. None of the potential interferents (see table below) were found to significantly influence this assay where acceptance was defined as the mean  $\pm$  2SD relative to the blank.

| Interferent       | Concentrations tested |
|-------------------|-----------------------|
| Hemoglobin        | 0 – 312 $\mu$ mol/L   |
| Triglyceride      | 0 – 30 g/L            |
| Bilirubin         | 0 – 513 $\mu$ mol/L   |
| Human albumin     | 0 – 150 mg/mL         |
| Rheumatoid factor | 0 – 100.5 IU/mL       |
| HAMA              | 0 – 912.5 mg/mL       |

A CA 15-3 positive serum pool with a CA 15-3 concentration of  $67.7 \pm 10.38$  U/mL was spiked with each drug listed in the table below, at a final concentration of at least 3X its therapeutic dose. No significant interference was noted for tested drugs and all % recoveries were within the acceptance range of 90-110%.

| Tested interfering drugs |                      |
|--------------------------|----------------------|
| 5-fluorouracil           | Acetaminophen        |
| N-acetyl-L-cysteine      | Acetylsalicylic acid |
| Ampicillin               | Ascorbic acid        |
| Bleomycin                | Carboplatin          |
| Cefoxitin                | Cisplatin            |
| Cyclophosphamide         | Cyclosporine         |
| Dactinomycin             | Doxocycline          |
| Doxorubicin              | Etoposide            |
| Ibuprofen                | Levodopa             |
| Methotrexate             | Metronidazole        |
| Mitomycin C              | Naprosyn             |
| Paclitaxel               | Phenylbutazone       |
| Rifampicin               | Vinblastine          |
| Vincristine              |                      |

#### Cross-reactivity:

Cross-reactivity with  $\beta$ -hCG, AFP, CEA, CA 19-9, CA 125, Prostate-specific antigen (PSA), and Prostatic Acid Phosphatase (PAP) was assessed using calibrators from the respective TOSOH assays. A human serum-based diluent served as a blank. All samples gave no CA 15-3 value, which was the acceptance criterion for this analysis. The samples and concentrations tested are:

- $\beta$ -hCG - 206 mIU/mL
- AFP - 201 ng/mL
- CEA - 49.6 ng/mL
- CA 19-9 - 423 U/mL
- PSA - 52 ng/mL
- PAP – 19.3 ng/mL
- CA 125 - 1100 U/mL



*f. Assay cut-off:*

The sponsor defines a 12% percentage change in CA 15-3 values as significant for patients being serially monitored. The significant change is 2.5 times the total imprecision of the CA 15-3 assay across sites, lots, and concentration (expressed as %CV = 4.30%). The sponsor chose this value to ensure that the change in CA 15-3 value is not attributed to assay variation.

2. Comparison studies:

*a. Method comparison with predicate device:*

A study consisting of 1035 paired samples representing normal, benign, and malignant conditions were included. The benign conditions included urogenital, GI tract/lung, breast, diabetes, heart disease/hypertension, and benign liver disease. Malignant conditions included treated cancers of the lung, liver, colorectal, breast, ovarian, uterine and cervix, and “other” cancers (gall bladder, gastric, pancreatic, etc.). CA 15-3 results were assayed using the VIDAS<sup>®</sup> CA 15-3 (Y) assay and the predicate assay (X). The results were analyzed by Deming regression analysis and are presented below.

$$Y = 0.96X - 1.94$$

95% Confidence interval for the intercept: - 4.90 to 1.01 U/mL

95% Confidence interval for the slope: 0.83 to 1.09

*b. Matrix comparison:*

Not applicable since only serum specimens are utilized.

3. Clinical studies:

*a. Clinical Sensitivity and Specificity:*

Not applicable.

*b. Other clinical supportive data (when a. is not applicable):*

The samples utilized in this study were from subjects with breast cancer obtained from retrospective sample banks at M.D. Anderson Cancer Center. Serial sets must include a minimum of 3 draws (4 draws or more desired) per subject. Samples were blood draws performed at or after diagnosis throughout as much of the clinical course as possible. Clinical information which detailed the disease status for each sample and types of any therapy received with the dates of administration was collected. Initially 80 evaluable serial sets were collected from subjects with confirmed breast cancer (Stage I – IV), yielding 353 individual samples. CA 15-3 values were obtained from a total of 273 evaluable observation pairs and the change between visits for serial samples was compared to the change in disease state. The average age of the 80 subjects was 49 years (median 48.0 yrs, SD = 10.9 yrs). Caucasian subjects comprised 78% of the sample set, Hispanic 12.5%, and Asian 1.8%. The average number of observation pairs per subject was 3.41, the median number of observation pairs per subject was 3, and the average number of draws per subject was 4.41.

The outcome of interest was defined as progression of disease from time point  $i$  (clinical visit  $i$ ,  $i=1$  to  $n-1$ ) to a succeeding time point  $j$  (clinical visit  $j$ ,  $j=i+1$  to  $n$ ). The number of clinical visits for which samples and data are available is defined as  $n$ .

The visit number made by a study subject is at the time of diagnosis or after diagnosis and prior to death, loss to follow-up or remission of disease. The sponsor statistically defines  $w_{ij}$  as a variable representing disease progression and has 2 values as follows:

1 if there is disease progression from visit  $i$  to visit  $j$

0 if no progression (stable disease, response to therapy) from visit  $i$  to visit  $j$

Disease progression is determined by the subject's physician and is based on any or a composite of physical signs/symptoms, results of lab tests for colorectal cancer, radiographic findings (CAT scans, PET scans, MRI, x-ray, or ultrasound), or patient reported symptoms.

The sponsor defined the variable  $v_{ij}$  as 1 if the difference in value of the test assay at visit  $i$  ( $x_i$ ) and value of the test assay at a later visit  $j$  ( $x_j$ ) is greater than or equal to 12.0% (i.e.  $v_{ij} = 1$  if  $(x_j - x_i) \geq 12.0\%$ ). The variable  $v_{ij}$  is 0 if the difference is otherwise (i.e.  $v_{ij} = 0$  if  $(x_j - x_i) < 12.0\%$ ). The sponsor chose this value to ensure that the change in CA 15-3 value is not attributed to assay variation and is statistically significant. According to the predicate's package insert, a positive (significant change) is defined as an increase of more than 10%. To determine an association between the variables  $w$  (disease progression) and  $v$  (change in CA 15-3 value) a 2 x 2 contingency table can be constructed to find an association between variables. Items in each of the 4 cells represent pairs of  $v$  and  $w$  (1 and/or 0) for visits for all subjects (or for subjects only). Concordance between the significance of the change in CA 15-3 values between visits and the recorded change in the patient's disease state was reported as percent agreement.

|                              | Change in Disease State |                |       |
|------------------------------|-------------------------|----------------|-------|
| Change in Test Device        | Progression             | No Progression | Total |
| $\geq 12.0\%$ (significant)  | 78                      | 57             | 135   |
| $< 12.0\%$ (not significant) | 28                      | 110            | 138   |
| Total                        | 106                     | 167            | 273   |

Positive percent agreement: 73.6% (78/106) (CI<sub>95%</sub>: 65.4 - 81.5%)

Negative percent agreement: 65.9% (110/167) (CI<sub>95%</sub>: 59.0 - 72.5%)

Overall percent agreement: 68.9% (188/273) (CI<sub>95%</sub>: 63.4 - 74.2%)

A per subject analysis was provided for the 80 subjects for the change in CA 15-3 values between visits and change disease state.

|                              | Change in Disease State |                |       |
|------------------------------|-------------------------|----------------|-------|
| Change in Test Device        | Progression             | No Progression | Total |
| $\geq 12.0\%$ (significant)  | 53                      | 15             | 68    |
| $< 12.0\%$ (not significant) | 3                       | 9              | 12    |
| Total                        | 56                      | 24             | 80    |

Positive percent agreement: 94.6% (53/56) (CI<sub>95%</sub>: 88.1 - 100%)

Negative percent agreement: 37.5% (9/24) (CI<sub>95%</sub>: 18.5 - 58.3%)

Overall percent agreement: 77.5% (62/80) (CI<sub>95%</sub>: 67.5 - 88.3%)

A second analysis of the same study data was performed, in which the subjects diagnosed as Stage I who never increased in stage were omitted from the analysis. From the Stage II – IV patients, there were a total of 316 evaluable observations yielding 246 observation pairs. The breakdown of the serial sets is presented in Table 1 in the submission. The average number of observation pairs per subject is 3.5 and the median number of observation pairs per subject was 3.

| Change in Test Device     | Change in Disease State |                | Total |
|---------------------------|-------------------------|----------------|-------|
|                           | Progression             | No Progression |       |
| ≥12.0% (significant)      | 78                      | 50             | 128   |
| <12.0 % (not significant) | 28                      | 90             | 118   |
| Total                     | 106                     | 140            | 246   |

Positive percent agreement: 73.6% (78/106) (CI<sub>95%</sub>: 65.6 – 81.6%)  
 Negative percent agreement: 64.3% (90/140) (CI<sub>95%</sub>: 56.8 – 71.6%)  
 Overall percent agreement: 68.3% (168/246) (CI<sub>95%</sub>: 62.6 – 74.1%)

4. Clinical cut-off:  
See assay cut-off above.
5. Expected values/Reference range:  
The reference values were determined from a healthy population of 202 ambulatory women from 18 – 80 years old. The group was made of 130 pre-menopausal (≤ 50 yrs) and 72 post-menopausal women (> 50 yrs). The results for these normal healthy subjects were as follows:

| Menopausal status | N = | Percentage (%) of the population according to the range of values in U/mL |               |                |          | 95 <sup>th</sup> percentile (U/mL) | 95% CI        |
|-------------------|-----|---|---------------|----------------|----------|------------------------------------|---------------|
|                   |     | < 30.00   | 30.01 – 60.00 | 60.01 – 120.00 | > 120.00 |                                    |               |
| Pre               | 130 | 99.23   | 0.77          | 0.00           | 0.00     | 23.16                              | 22.07 – 24.09 |
| Post              | 72  | 87.50   | 12.50         | 0.00           | 0.00     | 33.79                              | 29.62 – 36.95 |
| Total             | 202 | 95.05   | 4.95          | 0.00           | 0.00     | 29.10                              | 26.99 – 32.07 |

#### Benign Disease Cohort

Prospectively collected serum samples from a total of 433 subjects with diagnosed benign diseases were tested using the VIDAS<sup>®</sup> CA 15-3 assay.

| Benign disease                                      | N=  | Percentage (%) of the population according to the range of values in U/mL |               |                |          | 95 <sup>th</sup> percentile (U/mL) | 95% CI        |
|---|-----|---|---------------|----------------|----------|------------------------------------|---------------|
|   |     | < 30.00   | 30.01 – 60.00 | 60.01 – 120.00 | > 120.00 |                                    |               |
| Gastro-intestinal/Lung                              | 59  | 98.31   | 1.69          | 0.00           | 0.00     | 26.42                              | 24.84 – 28.56 |
| Urogenital disease                                  | 96  | 90.63   | 9.37          | 0.00           | 0.00     | 32.20                              | 29.34 – 38.14 |
| Chronic heart disease/<br>Hypertension/Benign liver | 116 | 92.24   | 7.76          | 0.00           | 0.00     | 32.64                              | 29.05 – 36.99 |
| Benign breast                                       | 55  | 98.18   | 1.82          | 0.00           | 0.00     | 24.33                              | 22.17 – 30.80 |
| Diabetes  | 107 | 86.92   | 12.15         | 0.93           | 0.00     | 39.06                              | 33.72 – 48.54 |
| Total   | 433 | 92.15   | 7.62          | 0.23           | 0.00     | 34.97                              | 30.46 – 37.13 |

These figures are provided as a guide. It is recommended that each laboratory establishes its own reference values from a rigorously selected population.

### **Malignant Disease Cohort**

Using banked serum samples from a total of 406 subjects with a diagnosed malignant carcinoma, the following results were observed using the VIDAS<sup>®</sup> CA 15-3 assay.

| Malignant disease                                    | N=  | Percentage (%) of the population according to the range of values in U/mL |               |                |          | 95 <sup>th</sup> percentile (U/mL) | 95% CI          |
|--|-----|---|---------------|----------------|----------|------------------------------------|-----------------|
|  |     | < 30.00   | 30.01 – 60.00 | 60.01 – 120.00 | > 120.00 |                                    |                 |
| Lung/liver cancer                                    | 53  | 58.49   | 33.96         | 3.77           | 3.77     | 65.60                              | 46.60 – 225.57  |
| Uterine/cervical cancer                              | 40  | 80.00   | 15.00         | 2.50           | 2.50     | 47.10                              | 33.35 – 155.74  |
| Ovarian cancer                                       | 55  | 65.45   | 21.82         | 9.09           | 3.64     | 71.82                              | 45.19 – 210.25  |
| Colorectal cancer                                    | 101 | 78.23   | 20.79         | 0.99           | 0.00     | 48.36                              | 37.71 – 57.40   |
| Breast   | 105 | 52.38   | 26.67         | 7.62           | 13.33    | 272.16                             | 184.61 – 663.20 |
| Other cancers (Gall bladder/ gastric/ pancreatic...) | 52  | 76.92   | 23.08         | 0.00           | 0.00     | 40.98                              | 34.00 – 44.93   |
| Total  | 406 | 67.24   | 23.89         | 4.19           | 4.68     | 94.29                              | 66.53 – 209.27  |

### **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.