510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k100344

B. Purpose for Submission:
New device

C. Measurand:
CA 15-3

D. Type of Test:
Quantitative, Sandwich chemiluminescent immunoassay

E. Applicant:
Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:
Dimension Vista® LOCI CA 15-3 Flex® Reagent cartridge
Dimension Vista® LOC 7 Calibrator

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.6010, Tumor-Associated antigen immunological test system
   21 CFR § 862.1150, Calibrator
2. Classification:
   Class II
3. Product code:
   MOI – System, Test, Immunological, Antigen, Tumor
   JIX – Calibrator, Multi-analyte Mixture
4. Panel:
   Immunology (82)
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The LOCI CA 15-3 method is an in vitro diagnostic test for the quantitative measurement of CA 15-3 in human serum and lithium heparin and EDTA plasma on the Dimension Vista® System. When used in conjunction with other clinical and diagnostic procedures, serial testing with the LOCI CA 15-3 assay may be used as an aid in the management of previously treated stage II and III breast cancer patients and for monitoring response to therapy in metastatic breast cancer patients.

   The LOCI 7 CAL is an in vitro diagnostic product for the calibration of the Cancer Antigen 15-3 (CA 15-3) and Cancer Antigen 19-9 (CA 19-9) methods on the Dimension Vista® System.
2. Indication(s) for use:
   Same as above
3. Special conditions for use statement(s):
   Prescription use only

4. Special instrument requirements:
   Siemens Dimension Vista® System - device performance was established on the Dimension Vista® 1500 instrument.

I. Device Description:

The LOCI CA 15-3 method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-CA 15-3 monoclonal antibody (DF3) fragment. The first bead reagent (Chemibeads) is coated with an anti-CA 15-3 monoclonal antibody (115D8) and contains a chemiluminescent dye. The second bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye.

The LOCI 7 CAL is a multi-analyte liquid, frozen bovine serum albumin, based product containing CA 15-3 from human cell culture. The kit consists of ten vials, two vials per level (A-E), 2.0 mL per vial.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ADVIA Centaur® CA 15-3 assay
   Access® BR Monitor Calibrator
2. Predicate 510(k) number(s):
   k012357 (ADVIA Centaur® CA 15-3 assay)
   k072612 (Access BR Monitor Calibrator)
3. Comparison with predicate:

Similarities and differences		
Item	**Device**	**Predicate**
Name	**LOCI CA 15-3 Flex® reagent cartridge (k100344)**	**ADVIA Centaur System CA 15-3® Assay (k012357)**
Intended Use	The LOCI CA 15-3 method is an in vitro diagnostic test for the quantitative measurement of CA 15-3 in human serum and lithium heparin and EDTA plasma on the Dimension Vista® System. When used in conjunction with other clinical and diagnostic procedures, serial testing with the LOCI CA 15-3 assay may be used as an aid in the management of previously treated stage II and III breast cancer patients and for monitoring response to therapy in metastatic breast cancer patients.	The ADVIA Centaur CA 15-3 assay is an in vitro diagnostic test for the quantitative serial determination of cancer antigen CA 15-3 in human serum using the ADVIA Centaur®System. When used in conjunction with other clinical and diagnostic procedures, serial testing with the ADVIA Centaur CA 15-3 assay is useful for monitoring the course of disease and therapy in metastatic breast cancer patients, and for detection of recurrence in previously treated patients with Stage

Similarities and differences		
Item	**Device**	**Predicate**
Name	**LOCI CA 15-3 Flex® reagent cartridge (k100344)**	**ADVIA Centaur System CA 15-3® Assay (k012357)**
		11with greater than two positive lymph nodes, or Stage III breast cancer patients. This assay is not intended for use on any other system.
Assay technology	Chemiluminescent	Same
Sample type	Serum, lithium heparin and EDTA plasma	Serum
Assay format	Sandwich bead-pair immunoassay based on LOCI® technology.	Sandwich immunoassay using direct chemiluminometric technology
Capture and Detection Antibodies	Monoclonal antibodies DF3 and 115D8	Same
Labeled Antibody Formats	DF3 is biotinylated 115D8 is coated on Chemibeads	Antibody-DF3 is acridinium ester labeled 115D8 is fluorescein labeled
Measuring Range	1 - 300 U/mL	0.5 - 200 U/mL
Sample Size	1 µL	20 µL
Instrument Platform	Siemens Dimension Vista	ADVIA Centaur automated analyzer

Similarities and differences			
Item	**Device**	**Predicate**	
Feature	**LOCI 7 Calibrator (k100344)**	**Access® BR Monitor Calibrator (k072612)**	**Dimension Vista LOCI 7 CAL (k100375)**
Calibrator Intended Use	The LOCI 7 CAL is an in vitro diagnostic product for the calibration of Cancer Antigen 15-3 (CA 15-3) and Cancer Antigen 19-9 (CA 19-9) methods on the Dimension Vista® system.	For in vitro diagnostic use for the calibration of the Access BR Monitor (CA 15-3 Antigen) Assay.	The LOCI 7 CAL is an in vitro diagnostic product for the calibration of the Cancer Antigen 19-9 (CA 19-9) method on the Dimension Vista® system.
Calibrator Levels	5 levels Target Concentrations approximately: Level 1 (Cal A): 0 U/mL Level 2 (Cal B): 20 U/mL Level 3 (Cal C): 60 U/mL Level 4 (Cal D): 150 U/mL	6 Levels Target Concentrations approximately: Level 1: 0 U/mL Level 2: 10 U/mL Level 3: 50 U/mL Level 4: 100 U/mL	None

Similarities and differences			
Item	**Device**	**Predicate**	
Feature	**LOCI 7 Calibrator (k100344)**	**Access® BR Monitor Calibrator (k072612)**	**Dimension Vista LOCI 7 CAL (k100375)**
	Level 5 (Cal E): 315 U/mL	Level 5: 500 U/mL Level 6: 1000 U/mL	
Calibrator Preparation	Liquid	Lyophilized	Liquid
Calibrator Storage	Store at -15 to -25°C.	Store at 2 to 8°C.	Store at -15 to -25 °C.
Calibrator Matrix	Bovine Serum Albumin	Same	Bovine Serum Albumin

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

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement
CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline
CLSI –EP09-A2, Method Comparison and Bias Estimation Using Patient Samples; Approved Guidelines.
CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The LOCI CA 15-3 method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-CA 15-3 monoclonal antibody (DF3) fragment. The first bead reagent (Chemibeads) is coated with an anti-CA 15-3 monoclonal antibody (115D8) and contains a chemiluminescent dye. The second bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. Sample is incubated with biotinylated antibody and Chemibeads to form bead-CA 15-3-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the CA 15-3 concentration in the sample when measured against a calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
      i) *Assay*:

      Precision testing was conducted by following CLSI EP5-A2 (Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline) over 20 days. During each day of testing, two separate runs, with two test samples (n=80), for each test material were analyzed. The duplicates were run with two separate sample cups. The studies included two

instruments (Dimension Vista® 1500 model), two flex lots, and two calibrator lots.

The test samples consisted of three levels of Bio-Rad Liquichek™ Tumor Marker control (Level-1, 2 and 3), three serum and two plasma pools. One of the serum samples was in the lower portion of the normal range, one approximately at the medical decision level and one at the upper end of the assay range. Two of the serum pools (spiked pool-B and high-spiked pool-E) and one of the plasma pools (Lithium heparin pool-C) were spiked with CA 15-3 antigen.

The range of samples tested encompassed the analytical measuring range. Analysis of variance (ANOVA) was used to evaluate the data consistent with the recommendations of EP5-A2. This experiment allowed a determination of within-run precision (repeatability), within-day, between days, between runs (within lab) precision for each site and a determination of between site precision.

Multi-Instrument/ Multi-Flex® lot study;
Between-Site plus Between-Lot

Material	Mean U/mL	Standard Deviation (%CV)
Control Level 1	23.6	1.81 (7.6)
Control Level 2	65.0	4.37 (6.7)
Control Level 3	158.1	8.29 (5.2)
Serum Pool A	13.6	1.46 (10.8)
Serum Pool B	35.5	2.80 (7.9)
Plasma Pool C (Li-Hep)	185.5	11.18 (6.0)
Plasma Pool D (EDTA)	16.96	1.88 (11.1)
Serum Pool E	279.05	6.29 (2.3)

ii) *Multicenter and multi-calibrator lot study*:
The studies were done at 3 sites in accordance with CLSI EP05-A2. The personnel at all three sites are laboratory personnel and were trained in the operation of the Dimension Vista® system. These sites and personnel are representative of the expected final users as the Dimension Vista® system is designed to be used in clinical laboratories by laboratory personnel who have been trained in the operation of the Dimension Vista® system. Study was performed over 20 days utilizing 3 calibrator lots, 1 flex lot, 3 instruments (Dimension Vista® 1500 model instruments) and 2 replicates (n=360); the duplicates were run with two separate sample cups. The three different sites provides side-by-side comparison of three different instruments using identical lots of the devices; calibrators and reagents. The test samples consisted of three levels of Bio-Rad Liquichek™ Tumor Marker control (Level-1, 2 and 3), three serum and two plasma pools. One of the serum

samples was in the lower portion of the normal range, one approximately at the medical decision level and one at the upper end of the assay range. Two of the serum pools (spiked pool-B and high-spiked pool-E) and one of the plasma pools (Lithium heparin pool-C) were spiked with CA 15-3 antigen

The range of samples tested encompassed the analytical measuring range. Analysis of variance (ANOVA) was used to evaluate the data consistent with the recommendations of EP5-A2. This experiment allowed a determination of within-run precision (repeatability), within-day, between days, between runs (within lab) precision for each site and a determination of between site precision.

Single site precision study:

Material	Mean U/mL	Standard Deviation (%CV)			
		Repeatability	Between-Run	Between-Day	Within-Lab
Control Level 1	22.3	0.48 (2.1)	0.54 (2.4)	0.00 (0.0)	0.72 (3.2)
Control Level 2	61.9	1.03 (1.7)	1.16 (1.9)	1.00 (1.6)	1.85 (3.0)
Control Level 3	152.2	2.13 (1.4)	2.90 (1.9)	0.76 (0.5)	3.68 (2.4)
Serum Pool A	12.6	0.28 (2.2)	0.30 (2.4)	0.00 (0.0)	0.41 (3.3)
Serum Pool B	33.5	0.62 (1.8)	0.82 (2.4)	0.15 (0.5)	1.04 (3.1)
Plasma Pool C (Li-Hep)	177.6	3.41 (1.9)	2.17 (1.2)	3.48 (2.0)	5.33 (3.0)
Plasma Pool D (EDTA)	15.6	0.33 (2.1)	0.21 (1.3)	0.17 (1.1)	0.43 (2.7)
Serum Pool E	274.6	7.1 (2.6)	4.1 (1.5)	5.2 (1.9)	9.7 (3.5)

Multi-Instrument/multi-calibrator lot study:

Material	Mean U/mL	Standard Deviation (%CV)				
		Repeatability	Between-Instrument	Between-Calibrator	Between-Day	Total
Control Level 1	24.2	0.5 (2.1)	0.15 (0.6)	0.10 (0.4)	0.61 (2.5)	0.81 (3.4)
Control Level 2	66	1.18 (1.8)	0.20 (0.3)	0.51 (0.8)	1.68 (2.5)	2.13 (3.2)
Control Level 3	160	3.01 (1.9)	0.00 (0.0)	1.42 (0.9)	4.22 (2.6)	5.37 (3.4)
Serum Pool	13.7	0.38 (2.8)	0.23 (1.6)	0.02 (0.2)	0.39 (2.8)	0.59 (4.3)
Serum Pool	35.7	0.70 (1.9)	0.14 (0.4)	0.19 (0.5)	1.02 (2.9)	1.26 (3.5)
Plasma Pool (Li-Hep)	184.9	3.44 (1.9)	0.00 (0.0)	1.54 (0.8)	4.97 (2.7)	6.24 (3.4)
Plasma Pool (EDTA)	17.5	0.51 (2.9)	0.04 (0.2)	0.05 (0.3)	0.47 (2.7)	0.70 (4.0)
Serum Pool	276.5	5.55 (2.0)	1.49 (0.5)	1.98 (0.7)	6.55 (2.4)	8.93 (3.2)
Data was collected for twenty days, one run per day, two replicates per run, on three separate instruments, using three different calibrator lots on each instrument						

b. Linearity/assay reportable range:

i) The linear range was determined according to CLSI EP06-A. Based on the results of linearity range testing and that from the Limit of Detection Study, the analytical measuring range of 1 – 300 U/mL was established in three matrices: serum, Lithium Heparin plasma, and EDTA plasma.

a) Serum

Linearity study of serum was completed over a range that is 20 to 30% wider than the anticipated measuring range for both the full assay range and the low end. The full assay range included serum as diluent, the low-end linearity which extends to 1 U/mL has water as diluent (as no samples were available with CA 15-3 values close to 0). The observed values in each case represent the mean of 3 replicates. Both studies indicate that the assay is linear over the assay range.

The mean concentration value was calculated for each sample. The percent recovery of observed CA 15-3 concentration relative to expected concentration was calculated. The mean recoveries for the three individual samples in the high-end range (serum diluent in serum weighted) shows acceptable linearity up to 366 U/mL using weighted linear regression model assuming constant CV. Weighted linear regression analysis was performed on the observed versus expected CA 15-3 assay results. The regression analysis yielded a slope of 1.005 and a y-intercept of -0.02 U/mL.

For the low end of the assay range, linearity testing was performed on a serum sample with dilutions ranging from 0 – 35 U/mL. All levels meet the acceptance criteria and that the method can be accepted as linear across the tested range; the analysis yielded R^2=0.999 with 95% CI for the slope of 0.98 to 0.99 and for the intercept of 0 to 0.

b) Lithium Heparin and EDTA Plasma

Linearity of Lithium Heparin plasma shows acceptable linearity up to 363 U/mL, yielding a slope of 1.00 and a y-intercept of 0.02 U/mL. EDTA plasma demonstrates acceptable linearity up to 360 U/mL using weighted data, yielding a slope of 1.00 and a y-intercept of 0.01 U/mL.

ii) *Spiking and Dilution Recovery Studies*:

a) Spiking Recovery: Known amounts of CA 15-3 at concentrations between 10.3 and 235.5 U/mL were added to human serum with baseline CA 15-3 values of 4.3 and 4.7 U/mL. The recovered CA 15-3 concentrations on these samples were then measured and the calculated percent recovery ranged from 96.3 to 105.1%.

Value Recovery Following Spiking of Samples

Serum (4.3 U/mL)			
CA 15-3 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
neat	4.3	4.3	n/a
10.3	14.4	14.7	102.1
15.4	19.4	19.4	99.8
20.6	24.5	25.2	102.9
30.8	34.6	35.1	101.5
41.1	44.7	46.9	105.0
61.7	64.9	65.0	100.2
82.2	85.1	89.4	105.1
123.3	125.5	125.0	99.6
164.4	165.8	170.3	102.7

Serum (4.7 U/mL)			
CA 15-3 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
neat	4.7	4.7	n/a
33.6	37.7	36.5	96.6
67.3	70.8	68.4	96.5
100.8	103.8	99.9	96.3
134.6	136.9	134.8	98.4
168.2	170.0	166.2	97.8
201.9	203.1	202.1	99.5
235.5	236.1	236.6	100.2

b) Dilution recovery: Serum samples were diluted with water according to the dilution levels shown in the table. The % recovery was calculated by dividing the observed result by the expected result and multiplying by 100. The percent recovery ranged from 92 - 102%.

Sample	**Dilution**	**Expected (U/mL)**	**Observed (U/mL)**	**% Recovery**
S1-1	neat	245.4	245.4	
S1-2	1:1.5	163.6	164.8	101%
S1-3	1:2	122.7	125	102%
S1-4	1:3	81.8	81.6	100%
S1-5	1:4	61.4	62.3	102%
S1-6	1:6	40.9	41.1	100%

Sample	Dilution	Expected (U/mL)	Observed (U/mL)	% Recovery
S1-7	1:8	30.7	30.9	101%
S1-8	1:12	20.5	20.5	100%
S1-9	1:16	15.3	15.2	99%
S1-10	1:24	10.2	10.2	99%
S2-1	neat	269.2	269.2	n/a
S2-2	1:1.14	235.5	233.4	99%
S2-3	1:1.33	201.9	197.9	98%
S2-4	1:1.6	168.2	160.5	95%
S2-5	1:2	134.6	126.8	94%
S2-6	1:2.67	100.9	95.8	95%
S2-7	1:4	67.3	62.1	92%
S2-8	1:8	33.6	31.1	92%

c) Auto-dilution: A study was completed to compare side-by-side auto-dilution vs. manual-dilution. Samples used were from above assay range of 300 U/mL. The results of the auto-dilution in percent of manual results for the same sample ranged from 97- 102% demonstrating that the results are accurate and precise.

Sample ID	Dilution Type	dilution (1:10)		Mean (U/mL)	SD	%CV
		run - 1	run - 2			
S31	Auto	479.7	487.5	483.6	5.52	1.14
S32	Auto	2469.0	2476.0	2472.6	4.88	0.20
S31	Manual	495.0	500.0	497.5	3.54	0.71
S32	Manual	2499.0	2327.0	2413.0	121.62	5.04

iii) *High Dose Hook-effect*

Hook effect was evaluated using samples prepared by adding commercial CA 15-3 antigen to calibrator matrix. The Dimension Vista® instrument reports an error message "Above Assay range" when the result is above the assay range of 300 U/mL. Users are instructed in the labeling (Limitations of procedure) that samples having this error code should be repeated with dilution. Based on this procedure, the data shows that CA 15-3 method shows no hook effect up to 20,000 U/mL.

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

i) *Traceability*:

There is no recognized standard or reference material for assay calibration. The LOCI 7 calibrator is a liquid, frozen, bovine serum albumin based product containing CA 15-3 from human tissue culture.

ii) *Calibrator*:
The Dimension Vista® LOCI 7 Calibrator (KC605) is an in-vitro diagnostic product intended to be used to calibrate the Vista® CA 15-3 method. It is a multi-analyte frozen liquid product consists of ten vials, packaged as two vials for each of five levels (A-E), 2.0 mL per vial. The matrix is 6% bovine albumin with buffer and preservatives; level A is zero, while levels B to E contain purified CA 15-3 antigen. Target values for CA 15-3 are 0, 20, 60, 150 and 315 U/mL. The master pool is a frozen liquid five-level material, with composition identical to the calibrator, held in reserve.

a) Value assignment:
An anchor pool of purified CA 15-3 is prepared and a value is assigned by comparing to patient samples assayed by the predicate device. Values are assigned to each lot of calibrator from the master pool using the Dimension Vista® System, through a patient sample comparison to the predicate ADVIA® Centaur CA 15-3 assay.

b) Stability:
Frozen liquid calibrator stability is 12 months from the date of manufacture when stored unopened at -20ºC. Thawed liquid calibrator stability is 30 days when stored unopened at 2-8ºC. Once the vial stopper is punctured, the assigned calibrator values are stable for 30 days on board the Dimension Vista System. LOCI 7 calibrators are not to be used on board the instrument once the cap is removed.

iii) *Kit Stability*:
Unopened flexes were stored at 4°C as control condition and on board a Dimension Vista instrument for 15 and 33 days. After 15 days and 33 days, the instrument was calibrated and the recovery of calibrators and QC materials with an on-board stored flex was compared to recovery with a control flex. The losses as a result of storage were within acceptable limits of ±_5%, indicating Sealed wells on the instrument are stable for 30 days.

Open vial stability: CA 15-3 flexes were tested for open well stability by using a linear depletion approach. The lot was calibrated on Day 0 and then tested on Day 0, 3, 4, 5, 6 and 7. Two flex well sets were used per sample. All wells were opened on day 0. For each well set, 5 tests were run on Day 0, and 3 on each of the other test days, for a total of 20 tests. Samples were calibrator level A and E, and a serum pool between 30 and 40 U/mL. The testing time for day 7 was at least 2 hours later than on day 0, to ensure the tested interval was wider than the claim of 7 days open-well stability. The losses as a result of storage were within acceptable limits of ± 5%, indicating Open wells are stable for 7 days.

d. Detection limit:
CLSI/NCCLS EP 17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation, were followed to determine the lowest concentration of CA 15-3 that can be detected with at least 95% probability; that is, with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 120

determinations, with 5 blank and 5 low level samples. Five CA 15-3 free test samples and five non-zero samples were tested for three days, one run per day, 2 replicates per run, with two reagent (Flex®) lots, on two instruments. Testing was performed using the same lot of calibrator, by the same operator, instrument and operator.

LOB – Highest measurement result which has a 95% probability to be observed for a blank sample. It is the 95th percentile of a blank distribution. CA 15-3 negative sera were not available. Therefore, five lots of LOCI-7 calibrator A (0 level calibrator) were tested; the calibrator matrix has a 6% BSA base. The Limit of Blank (LoB) calculated was 0.31 U/mL (claimed 0.3 U/mL).

LOD – No serum samples in the concentration range of the expected LoD were available. Five serum samples with concentrations in the range of 3-6 U/mL were diluted between 4-fold and 10-fold with calibrator A (0 level calibrator) to concentrations covering the range where LoD was expected to fall. The Limit of Detection (LoD) for CA 15-3 calculated was 1.0 U/mL.

LOQ – Lowest actual amount of analyte that can be reliably detected and at which total error meets lab requirements for accuracy. LOQ was not claimed for the Dimension Vista CA 15-3 assay.

The results are noted to support a claim for LOB of 0.3 U/mL and of LOD of 1.0 U/mL. Therefore, the lowest value for the range of the assay is 1.0 U/mL.

e. Analytical specificity:

Interference testing was performed according to CLSI/NCCLS EP7-A2 to determine the effect of various endogenous and exogenous substances on the Dimension Vista® CA 15-3 assays. For all interferents the percent bias was determined by testing a control sample without the interferent and comparing it to the value obtained from a test sample to which the potential interferent had been added.

i) *Endogenous Substance Interference*:

Testing was performed at two CA 15-3 concentrations: 30 ± 6 U/mL and 130 ± 25 U/mL using two lots of Flex® reagents. Test samples were prepared by spiking the potential interferent into serum. Bias exceeding 10% was considered interference. Results for hemoglobin, bilirubin and intralipid are summarized below:

Substance tested	**Substance concentration**	**LOCI CA 15-3 U/mL**	**Bias %**
Hemoglobin (hemolysate)	Hemoglobin (monomer) 1000 mg/dL [0.62 mmol/L]	33	<10
		141	<10
Bilirubin (unconjugated)	60 mg/dL [1026 µmol/L]	28	<10
		132	<10
Bilirubin (conjugated)	20 mg/dL [342 µmol/L]	28	<10
		132	<10
Lipemia (Intralipid®)	3000 mg/dL [33.9 mmol/L]	30	<10
		123	<10

Seven additional endogenous substances, including Uric acid, Urea, Creatinine, Cholesterol, IgG and serum proteins, were also tested with bias <10%.

ii) *Exogenous Substance Interference*:
Fifty two exogenous substances were tested for interference including common over-the-counter drugs and cancer drugs. The substances did not interfere with the LOCI CA 15-3 method when present in serum and plasma (lithium heparin, EDTA). Inaccuracies (biases) due to these substances were less than 10% at CA 15-3 concentrations of 30 ± 6 U/mL and 130±25 U/mL.

iii) *HAMA interference*:
A number of optimized concentrations of HAMA blockers were employed in the reaction, as well as reagents designed to minimize non specific binding (NSB) interference. Interference from HAMA was evaluated by testing commercially available and in-house sera containing HAMA or heterophilic antibodies. Results from samples to which 10% of a blocker cocktail was added served as controls and were compared to the same samples without blocker cocktail. The blocker cocktail consisted of several commercially available HAMA/ heterophilic blocking substances from multiple vendors. Thirty-six in-house HAMA/NSB sera and 19 commercial HAMA sera were tested by processing two replicates for each sample and control. All samples recovered within ± 10% of their controls.

iv) *Method Cross-Reactivity*:
Interference/ cross-reactivity of cancer marker antigens in the LOCI CA 15-3 assay was tested by spiking the tumor marker antigens into two sera and comparing the recovery of the spiked sample to control samples spiked with Phosphate Buffered Saline. No cross-reactivity was observed for the tested cancer marker antigens.

Spiked Antigen	**Testing concentration**	**Serum Sample U/mL**	**Bias (%)**
CA19-9	11,608 U/mL	28.8 U/mL	-4.0%
		123.0 U/mL	-2.0%
CA125	10,466 U/mL	32.0 U/mL	-3.0%
		135.3 U/mL	-2.0%
AFP	500 ng/L	33.3 U/mL	-2.0%
		140.2 U/mL	1.0%
CEA	5,000 ng/L	32.9 U/mL	-1.0%
		143.6 U/mL	-2.0%
PSA	361 ng/L	33.8 U/mL	-4.0%
		140.4 U/mL	1.0%

f. Assay cut-off:

For the Dimension Vista® LOCI CA 15-3 assay, the reference change value (RCV) was used to determine if a significant change occurred. The RCV is a percentage change in measured CA 15-3 values when compared to a baseline value. The RCV takes into account the maximum imprecision of the assay within the reportable range, the intra-individual biological variation, and the 95% confidence interval (z value for $p<0.05$). The RCV was derived by taking into account the published biological variation; within-subject biological variation (6.2%) was obtained from the literature. In determining the RCV, the analytical variation used was 7.9% (which is the reported multi-instrument/ multi-Flex®-lot variability (Multi-Instrument/ Multi-Flex® lot study) at 35.5 U/mL. The RCV for the Dimension Vista® LOCI CA 15-3 method was calculated to be 27.9%.

2. Comparison studies:

a. Method comparison with predicate device:

The predicate device CA 15-3 Assay for the ADVIA Centaur® System was used to demonstrate substantial equivalence to the LOCI CA 15-3 Flex® Reagent Cartridge according to EP9-A2. One serum sample randomly chosen from each of the 75 female patients who were tested for the monitoring of their disease status were combined with 248 excess, de-identified serum samples containing measurable amounts of CA 15-3. Each sample was assayed for the observed values (323 samples) using the Dimension Vista® CA 15-3 (Y) assay and compared with the expected values using the Advia Centaur® CA 15-3 (X).

There were no missing responses or invalid results. Samples above the assay range were re-tested to confirm the results; the repeat tests were not included in any analysis. Using least squares regression, four (4) statistical outliers were identified. These 4 samples and 18 samples that were outside the range of the assay were excluded from the data analysis. Using a Passing Bablok regression, all results were included in the analysis. CA 15-3 values on the Dimension Vista® spanned 4.7 - 4814.1 U/mL across the samples tested; values from 4.7 - 264.4 U/mL (301 samples) were included in the analysis.

Comparative Method	Slope (95% CI)	Intercept U/mL (95% CI)	Correlation Coefficient	n
Dimension Vista® CA 15 vs. ADVIA Centaur® CA 15-3	0.91 (0.89 to 0.92)	2.43 (1.53 to 3.33)	0.988	301

b. Matrix comparison:

The Dimension Vista Flex Assay for CA 15-3 was evaluated in serum, LiHeparin plasma, and EDTA plasma.

i) Serum samples were evaluated with matched samples of Lithium Heparin plasma. The two matrices were evaluated using 68 samples over the measuring range of the device (linear regression line $y=0.98x + 0.4$ and $R^2=1.000$).

ii) Serum samples were evaluated with matched samples of EDTA plasma. The two matrices were evaluated with 82 samples over the measuring range of the device (linear regression line y=0.97x + 0.5 and R^2=0.999).

Sample comparison	Slope (95% CI)	Intercept U/mL (95% CI)	Correlation Coefficient	n
Lithium heparin versus serum	0.98 (0.97 to 0.99)	0.4 (0 to 0.9)	1.0	68
EDTA versus serum	0.97 (0.96 to 0.98)	0.5 (0 to 1.0)	0.999	82

3. Clinical studies:
Serial samples from 75 female patients were obtained from a supplier of stored retrospective samples to the medical device industry. Samples were selected for age (range 21.8 years old to 75.9 years old), ethnicity and stage of disease (stage 1 through IV). A minimum of 3 serial samples were obtained for each of the 75 patients. Clinical information that details the status of the subject's disease was required for inclusion of samples in the study. Changes in CA 15-3 concentrations and in disease status were analyzed on a per visit basis. Patients were categorized as Active/Progressive, Responding, Stable, or No Evidence of Disease (NED) by the attending physician based on the clinical information (medical imaging, physical examination, and other clinical investigations). All 75 patient sets were analyzed to determine the change in disease status per sequential pair (n=258). Table below shows the distribution of results when compared to the disease status

Disease State Frequency using the Dimension Vista® LOCI CA 15-3 Assay

	Change in Disease State				
Change in CA 15-3	Responding n (%)	Stable n (%)	No Evidence of Disease n (%)	Progression n (%)	Total
>27.9% increase	4 (1.5%)	27 (10.5%)	3 (1.2%)	64 (26.8%)	98 (38%)
No significant Change	10 (3.9%)	42 (16.3%)	37 (14.3%)	17 (6.6%)	106 (41%)
>27.9% decrease	8 (3.1%)	28 (10.9%)	3 (1.2%)	15 (5.8%)	54 (21%)
Total	22 (8.5%)	97 (37.7%)	43 (16.7%)	96 (37.2%)	258 (100%)

For the Dimension Vista® LOCI CA 15-3 assay, the reference change value (RCV) was used to determine if a significant change occurred. A change of 21% was considered to be significant for the predicate method (AVDIA Centaur® CA 15-3 assay). This value was obtained from the manufacturer's published insert sheet. Per patient visit clinical performance results for the Dimension Vista® LOCI CA 15-3 test and predicate devices are given in following two tables. In this evaluation, disease status was classified as "Progression" and "No Progression" with "No Progression" consisting of responding, stable, and no evidence of disease. Using a cut-off of >27.9% rise in CA 15-3 value, 66.7% of subject visits (95% confidence interval 56.3 % - 76.0 %) had a rise in CA 15-3 value when the patient's disease

status was classified as progression. This value represents the positive percent agreement of significant CA 15-3 rise with a clinical disease status classified as progression. Using a cut-off of a >27.9% rise in CA 15-3 value, 79.0% of subject visits (95% confidence interval 71.9 % - 85.0 %) had no rise in CA 15-3 value when the patient's disease status was classified as no progression. This value represents the negative percent agreement of CA 15-3 rises with a progressive disease status.

Dimension Vista® LOCI CA 15-3 Value vs. Disease Progression

	Progression	**No-Progression**	**Total**
>27.9% increase	64	34	98
≤27.9% increase	32	128	160
Total	96	162	258
		Estimate	***Exact 95% Confidence Interval***
Total Concordance		74.40%	(68.6 % - 79.6 %)
Positive Concordance		66.70%	(56.3 % - 76.0 %)
Negative Concordance		79.00%	(71.9 % - 85.0 %)

The positive and negative agreement for the FLEX CA 15-3 method, when taken together, show similar performance to the predicate method (below)

Predicate CA 15-3 Value vs. Disease Progression

	Progression	**No-Progression**	**Total**
>21% increase	67	47	116
≤21% increase	29	115	142
Total	96	162	258
		Estimate	***Exact 95% Confidence Interval***
Total Concordance		70.50%	(64.6% - 76.0%)
Positive Concordance		69.80%	(59.6 % - 78.8%)
Negative Concordance		71.00%	(63.4% - 77.8 %)

All specimens were analyzed for agreement between the two assays using the Reference Change Value for the Dimension Vista® LOCI CA 15-3 assay and the predicate assay when used according to the manufacturer's instructions; these results are shown in the table below.

Dimension Vista® LOCI CA 15-3 Concordance to Comparative Method (on a per visit basis)

Dimension Vista® LOCI CA 15-3	Comparative Method		Total
	>21% increase	≤21% increase	
>27.9% increase	94	4	110
≤27.9 % increase	20	140	148
Total	114	144	258
		Estimate	***Exact 95% Confidence Limits***
Total Concordance		90.70%	(86.5% - 94.0%)
Positive Concordance		82.50%	(74.2% - 88.9%)
Negative Concordance		97.20%	(93.0% - 99.2%)

4. Clinical cut-off:
   Clinical Cut-off is based upon a 27.9% reference change value (RCV). When the measurement of CA 15-3 varies by >27.9% of the baseline measurement (either positive or negative) the change is considered significant. The RCV was used to ensure that the change in CA 15-3 value is not attributed to assay variation or biological variation. This percent variation represents values within the 95% confidence interval for real alteration in CA 15-3 values above system noise.
5. Expected values/Reference range:
   The distribution of CA 15-3 values determined in specimens from patients with nonmalignant or malignant disease are shown in the two tables below. The distribution of CA 15-3 values was determined in specimens from healthy individuals (n = 350; 150 males and 200 females) and from patients with nonmalignant or malignant diseases. From apparently healthy adult females (age 18-64 years), 99.0 % of 200 samples had CA 15-3 levels of less than or equal to 35 U/mL. Each laboratory should establish its own expected values for CA 15-3 as performed on the Dimension Vista® System. Expected values of CA 15-3 measurements are dependent upon the individual patient's baseline reading for CA 15-3. Decreases from baseline value of 27.9% or more in subsequent repeated measurements are indicative of response to therapy or remission. Increases of 27.9% or more suggest no response to therapy and are weakly correlated with progression of disease. Estimation and empirical distributions of CA 15-3 values in various populations of subjects was performed. The distribution of CA 15-3 values in 350 apparently healthy individuals, 200 patients with nonmalignant and 398 patients with malignant disease is shown in the tables below.

Non-malignant Disease and Healthy Normals:
# of Patients (%Patients) with CA 15-3 level U/mL

Sample Category	n	0-35 (%)	35.1–60 (%)	60.1-120 (%)	120.1-300 (%)	>300 (%)
Healthy Normal Females	200	198 (99.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Females < 50 yrs age	151	150 (99.3)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Females ≥ 50 yrs age	49	48 (98.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)
Healthy Males	150	148 (98.7)	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Benign Diseases						
Breast	30	27 (90)	2 (6.7)	1 (3.3)	0 (0.0)	0 (0.0)
Ovarian Cyst*	30	29 (96.7)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cervix / Uterus	33	33 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
GI Tract	20	20 (100.))	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
UTI	30	28 (93.3)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
Pancreas	27	22 (81.5)	5 (18.5)	0 (0.0)	0 (0.0)	0 (0.0)
Colon	10	7 (70.0)	3 (30)	0 (0.0)	0 (0.0)	0 (0.0)
Congestive Heart Failure	20	16 (80.0)	3 (15.0)	1 (5.0)	0 (0.0)	0 (0.0)
Malignant Diseases:						
Breast: stage I	4	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
Breast: stage II	30	22 (73.3)	7 (23.3)	0 (0.0)	0 (0.0)	1 (3.3)
Breast: stage III	12	11 (91.7)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0)
Breast: stage IV	40	14 (35.0)	8 (20.0)	3 (7.5)	8 (20.0)	7 (17.5)
Breast: (stage unknown)	20	16 (80.0)	1 (5.0)	0 (0)	2 (10.0)	1 (5.0)
Ovarian	54	27 (50.0)	14 (25.9)	8 (14.8)	3 (5.6)	2 (3.7)
Cervix / Uterus	41	33 (80.5)	7 (17.1)	0 (0.0)	0 (0.0)	1 (2.4)
Liver*	23	21 (91.3)	1 (4.3)	0 (0.0)	0 (0.0)	1 (4.3)
Colorectal	41	38 (92.7)	3 (7.3)	0 (0.0)	0 (0.0)	0 (0.0)
Pancreatic	30	23 (76.7)	6 (20.0)	1 (3.3)	0 (0.0)	0 (0.0)
Lung	30	22 (73.3)	6 (20.0)	1 (3.3)	0 (0.0)	1 (3.3)
Lymphoma	30	25 (83.3)	5 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Prostate/Testicle	28	26 (92.9)	2 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Upper GI*	13	12 (92.3)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)
Renal	2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)

Liver* = Gall Bladder, Bile Duct
Upper GI* = Oral, Larynx, Esophagus, Stomach
Ovarian Cyst* = Polycystic Ovaries/Ovarian Cyst

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.