

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

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

k100321

**B. Purpose for Submission:**

New device.

**C. Measurand:**

CA 125

**D. Type of Test:**

Quantitative, Sandwich chemiluminescent immunoassay

**E. Applicant:**

Siemens Healthcare Diagnostics Inc.

**F. Proprietary and Established Names:**

Dimension Vista® LOCI CA 125 Flex® reagent cartridge

Dimension Vista® LOCI 6 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:

LTK-Test, Epithelial Ovarian Tumor-associated Antigen (CA 125)

JIX-Calibrator, Multi-analyte Mixture

4. Panel:

Immunology (82)

Clinical Chemistry (75)

**H. Intended Use:**

1. Intended use(s):

The LOCI CA 125 II™ method is an *in vitro* diagnostic test for the quantitative measurement of CA 125 antigen in human serum and lithium heparin and EDTA plasma on the Dimension Vista® System. Measurements of CA 125 are used as an aid in monitoring disease progress or response to therapy or for the recurrent or residual disease for patients with epithelial ovarian cancer. Serial testing for patient CA 125 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer. It is recommended that the LOCI CA 125 II method be used in conjunction with signs and symptoms of a clinical evaluation by a physician trained and experienced in the management of gynecological cancers. This assay is not intended for screening or diagnosis of ovarian cancer or for use on any other system.

The LOCI 6 CAL is an *in vitro* diagnostic product for the calibration of Alpha-Fetoprotein (AFP), Carcinoembryonic Antigen (CEA) and the CA 125 II™ methods on the Dimension Vista® System.

2. Indication(s) for use:  
Same as above.
3. Special conditions for use statement(s):  
For 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 125 II™ Flex® method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-CA 125 monoclonal antibody (M11) fragment. The first bead reagent (Chemibeads) is coated with an anti-CA 125 monoclonal antibody (OC 125) and contains a chemiluminescent dye. The use of the M11 antibody in combination with OC 125 defines this method as a second generation CA 125 assay. The second bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye.

The LOCI 6 CAL is a multi-analyte liquid, frozen bovine serum albumin based product containing Alpha-Fetoprotein from human cord blood, Carcinoembryonic Antigen from human cell culture and CA 125 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) and 510(k) numbers
  - a. CA 125II Assay for the ADVIA Centaur System (k020828)
  - b. LOCI 5 Calibrator (k071597 and k071603)

Comparison with predicate:

Similarities and Differences		
Item	Device	Predicate
	Dimension Vista® LOCI CA 125 Flex® reagent cartridge	CA 125II Assay for ADVIA Centaur System (k020828)
Intended Use	An <i>in vitro</i> diagnostic test for the quantitative measurement of CA 125 antigen in human serum and lithium heparin and EDTA plasma on the Dimension Vista® System. Measurements of CA 125 are used as an aid in monitoring disease progress or response to therapy or for the early detection of recurrent or residual disease for	For <i>in vitro</i> diagnostic use in the quantitative, serial determination in human serum and to aid in the management of patients with ovarian carcinoma using the ADVIA Centaur and ADVIA Centaur XP systems. The test is intended for use as an aid in monitoring patients who are clinically free of disease and should be

<b>Similarities and Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	<p>patients with epithelial ovarian cancer. Serial testing for patient CA 125 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.</p> <p>It is recommended that the LOCI CA 125 assay be used under the order of a physician trained and experienced in the management of gynecological cancers.</p> <p>This assay is not intended for screening or diagnosis of ovarian cancer or for use on any other system.</p>	<p>used in conjunction with other clinical methods used for monitoring ovarian cancer. Serial testing for CA 125 in the serum of patients who are clinically free of disease should be used in conjunction with other clinical methods used for early detection of cancer recurrence. The test is also intended for use as an aid in the management of ovarian cancer patients with metastatic disease by monitoring the progression or regression of disease in response to treatment. It is recommended that the ADVIA Centaur CA 125II assay be used under the order of a physician trained and experienced in the management of gynecological cancers.</p> <p>This assay is not intended for screening or diagnosis of ovarian cancer or for use on any other system.</p>
Sample Type	Serum, lithium heparin plasma and EDTA plasma	Serum
Measuring Range	1.5-1000 U/mL	2-600 U/mL
Sample Size	5 µL	50 µL
Measurement	Chemiluminescent: Homogeneous sandwich immunoassay based on LOCI® technology	Chemiluminescent: Two site sandwich immunoassay using direct chemiluminometric technology
Instrument Platform	Siemens Dimension Vista	ADVIA Centaur and Centaur XP
Capture and Detection Antibody	Monoclonal antibodies M11 and OC 125	Same

Similarities and Differences		
Item	Device	Predicate
	LOCI 6 Calibrator	LOCI 5 Calibrator (k071597 and k071603)
Intended Use	An <i>in vitro</i> diagnostic product for the calibration of Alpha-Fetoprotein (AFP), Carcinoembryonic Antigen (CEA) and CA 125 methods on the Dimension Vista® system.	An <i>in vitro</i> diagnostic product for the calibration of Alpha-Fetoprotein (AFP), Carcinoembryonic Antigen (CEA) methods on the Dimension Vista® system.
Matrix	BSA-based matrix	Same
Preparation	Frozen	Liquid - Ready to use
Target CA 125 Concentrations	Level 1 (CAL A): 0 U/mL Level 2 (CAL B): 25 U/mL Level 3 (CAL C): 100 U/mL Level 4 (CAL D): 525 U/mL Level 5 (CAL E): 1050 U/mL	None
Storage	Store at -15 to -25 °C	Store at 2 to 10 °C

**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 version 1 Protocols for Determination of Limits of Detection and Limits of Quantitation published 10/31/2004

**L. Test Principle:**

The LOCI CA 125 II™ method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-CA 125 monoclonal antibody (M11) fragment. The first bead reagent (Chemibeads) is coated with an anti-CA 125 monoclonal antibody (OC 125) and contains a chemiluminescent dye. The use of the M11 antibody in combination with OC 125 defines this method as a second generation CA 125 assay. 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 125-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 125 concentration in the sample when measured against a calibration curve.

The Dimension Vista® LOCI 6 Calibrator (KC604) is a multi-analyte frozen liquid product packaged as two vials for each of five levels. The matrix is 6% bovine albumin with buffer and preservatives. The calibrator level A is zero, while levels B to E contain purified CA 125 antigen. Measuring these values on the Dimension Vista® allows for a calibration curve to be established. Patients CA 125 measurements are then compared to the calibration curve to determine serum or plasma CA 125 concentrations.

**M. Performance Characteristics (if/when applicable):**

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

Precision testing for the LOCI CA 125 method was performed over twenty days according to CLSI/NCCLS EP5-A2. Data from 2 Flex® lots, 2 calibrator lots and 2 Dimension Vista® 1500 model instruments are included. The test samples consisted of three levels of Bio-Rad Liquichek Tumor Marker control, three serum pools and one to two plasma pools depending on the study. Two of the serum samples and one of the plasma pools (Lithium heparin) were spiked with CA 125 antigen. On each day of testing, each sample was run in duplicate, in two separate runs (N=80). The duplicates were run with two separate sample cups. One site used instrument 238, reagent lot09268AE, calibrator lot 9ED083, and QC lot 19830. The second site used instrument 190, reagent lot 09196AD, calibrator lot 9DD099, and QC lot 19830.

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.

		Liquichek® Control Level 1 (25.0-26.0 U/mL)	Liquichek® Control Level 2 (61.4-65.6 U/mL)	Liquichek® Control Level 3 (164.4- 177.8 U/mL)	SA (serum) (10.4- 11.2 U/mL)	SB (serum) (37.3- 37.5 U/mL)	SC (Li- Hep plasma) (146.9- 149.6 U/mL)	SD (EDTA plasma) (15.0 U/mL)	SE (serum high spike) (978.5 (U/mL)	SF (serum high spike) (766.6 U/mL)
Source	N	CV%	CV%	CV%	CV%	CV%	CV%	CV%	CV%	CV%
Between-Site plus Between-Lot Reproducibility	160	3.0	4.7	5.6	1.6	1.2	1.2	N/A	N/A	N/A
Within Lab (Site 1)	80	3.0	2.1	2.3	3.2	2.7	2.4	3.8	2.5	N/D
Within Lab (Site 2)	80	2.6	2.4	2.4	3.7	2.2	2.2	N/D	N/D	1.9
Between-day (Site 1)	80	1.0	0.3	0.9	1.6	1.0	0.8	0.0	1.2	N/D
Between-day (Site 2)	80	1.2	0.7	1.4	1.1	1.2	1.0	N/D	N/D	0.6

		Liquichek® Control Level 1 (25.0-26.0 U/mL)	Liquichek® Control Level 2 (61.4-65.6 U/mL)	Liquichek® Control Level 3 (164.4- 177.8 U/mL)	SA (serum) (10.4- 11.2 U/mL)	SB (serum) (37.3- 37.5 U/mL)	SC (Li- Hep plasma) (146.9- 149.6 U/mL)	SD (EDTA plasma) (15.0 U/mL)	SE (serum high spike) (978.5 (U/mL)	SF (serum high spike) (766.6 U/mL)
Between-run (Site 1)	80	0.6	1.2	0.8	0.8	1.4	1.3	2.8	1.2	N/D
Between-run (Site 2)	80	0.6	1.3	1.2	0.8	1.0	1.0	N/D	N/D	1.3
Repeatability (Site 1)	80	2.7	1.7	1.9	2.6	2.1	1.8	2.5	1.7	N/D
Repeatability (Site 2)	80	2.2	1.9	1.4	3.4	1.6	1.6	N/D	N/D	1.2

ii) *Calibrator:*

Precision testing for the CA 125 calibrators was performed over twenty days according to CLSI/NCCLS EP5-A2. Data from 3 calibrator lots, 2 replicate samples, over 20 days, on 3 Dimension Vista® 1500 model instruments. The test samples consisted of three levels of Bio-Rad Liquichek Tumor Marker control, three serum pools and two plasma pools depending on the study. One of the serum samples and one of the plasma pools (Lithium heparin) were spiked with CA 125 antigen. On each day of testing, each sample was run in duplicate, on three instruments, calibrated from 3 different calibrator lots (N=360). The duplicates were run with two separate sample cups. This experiment used instruments Vista 145, 190, and 238; calibrator lots 9HD098, 9DD099, 9ED083; and reagent lot09196AD.

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**

<b>Material</b>	<b>Mean</b>	<b>Standard Deviation (%CV)</b>			
	<b>U/mL</b>	<b>Repeatability</b>	<b>Between-Run</b>	<b>Between-Day</b>	<b>Within-Lab</b>
Control Level 1	25.0	0.68 (2.7)	0.16 (0.6)	0.24 (1.0)	0.74 (3.0)
Control Level 2	61.4	1.07 (1.8)	0.75 (1.2)	0.17 (0.3)	1.32 (2.2)
Control Level 3	164.4	3.17 (1.9)	1.33 (0.8)	1.51 (0.9)	3.75 (2.3)
Sample A	11.2	0.30 (2.6)	0.09 (0.8)	0.17 (1.6)	0.36 (3.2)
Sample B	37.5	0.79 (2.1)	0.52 (1.4)	0.39 (1.0)	1.02 (2.7)
Li-Hep Spike	146.9	2.62 (1.8)	1.89 (1.3)	1.24 (0.8)	3.46 (2.4)
EDTA	15.0	0.37 (2.5)	0.43 (2.8)	0.00 (0.0)	0.57 (3.8)
High Serum Spike	978.5	17.0 (1.7)	11.8 (1.2)	12.2 (1.2)	24.0 (2.5)

**Multi-Instrument/ multi-calibrator lot study**

Material	Mean		Standard Deviation (%CV)			
	U/mL	Repeatability	Between-Instrument	Between-Calibrator	Between-Day	Total
Control Level 1	25.1	0.52 (2.1)	0.21 (0.8)	0.51 (2.0)	0.63 (2.5)	0.98 (3.9)
Control Level 2	60.7	0.97 (1.6)	0.68 (1.1)	1.64 (2.7)	1.50 (2.5)	2.51 (4.1)
Control Level 3	160.4	2.35 (1.5)	1.47 (0.9)	5.45 (3.4)	3.67 (2.3)	7.13 (4.4)
Sample A	11.2	0.29 (2.6)	0.19 (1.7)	0.20 (1.8)	0.43 (3.8)	0.59 (5.2)
Sample B	38.6	0.66 (1.7)	0.56 (1.5)	0.91 (2.4)	0.88 (2.3)	1.54 (4.0)
Li-Hep Spike	150.4	1.87 (1.2)	1.99 (1.3)	5.04 (3.3)	3.22 (2.1)	6.57 (4.4)
EDTA	15.6	0.40 (2.6)	0.24 (1.5)	0.27 (1.7)	0.48 (3.1)	0.72 (4.6)
High Serum Spike	814.7	9.71 (1.2)	13.48 (1.7)	18.37 (2.3)	13.41(1.6)	28.16(3.5)

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.

The data was merged to form one data set for analysis and a variance component analysis using the REML method was performed.

*b. Linearity/assay reportable range:*

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

a. Serum

A study covering the whole assay range was performed using two natural serum samples. One sample with a low CA 125 concentration (3.3 U/mL) and one with a high CA 125 concentration (1376.2 U/mL), were mixed in varying proportions distributed over the measurement range. Each dilution was tested five times. Dimension Vista Flex assay data correlated with expected sample concentration according to the weighted linear regression formula ( $y=0.99x + 0.07$ ,  $R^2=1.00$ ).

A low-end linearity studies were performed by diluting mid-level patient samples with water in varying proportions covering the range from 1-280.8 U/mL. Dimension Vista Flex assay data correlated with expected sample concentration according to the linear regression formula ( $y=0.9494x + 1.5508$ ,  $R^2=0.9991$ ).

b. Lithium Heparin Plasma

A study covering the whole assay range was performed using two natural serum samples. One sample with a low CA 125 concentration (3.2 U/mL) and one with a high CA 125 concentration (1578.5 U/mL), were mixed in varying proportions distributed over the measurement range. Samples were measured in triplicate. When data was limited to plus or minus 30% of the measuring range, as per CLSI guidelines, Dimension Vista Flex assay data correlated with expected sample

concentration according to the weighted linear regression formula ( $y=1.00x - 0.1, R^2=1.00$ ).

c. EDTA Plasma

A study covering the whole assay range was performed using two natural EDTA plasma pools. One sample with a low CA 125 concentration (7.1 U/mL) and a separate sample spike with CA 125 antigen to a high concentration (1562.8 U/mL), were mixed in varying proportions distributed over the measurement range. Samples were measured in triplicate. When data was limited to plus or minus 30% of the measuring range, as per CLSI guidelines, Dimension Vista Flex assay data correlated with expected sample concentration according to the weighted linear regression formula ( $y=1.00x - 0.1, R^2=0.999$ ).

ii) Spiking and Dilutional Recovery was evaluated.

a. Spiking Recovery

Known amounts of CA 125, approximately 35, 70, 140, 560 and 840 U/mL, were added to serum samples with baseline CA 125 values of 5.4 U/mL or 44.4 U/mL) and to plasma samples with a baseline CA 125 value of 16.7 U/mL. The CA 125 concentrations were measured and the percent recovery ranged from 77.9 - 104% for serum and 89.2- 100.7% for EDTA plasma.

**Serum (44.4 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	40.0	39.5	98.9
42.8	82.8	77.8	93.9
85.7	125.7	113.7	90.5
175.8	215.8	193.3	89.6
704.5	744.4	651.1	87.5
1019.9	1059.8	973.7	91.9

**EDTA (16.7 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	15.0	15.1	100.7
42.8	57.8	54.9	94.9
85.7	100.7	98.2	97.5
175.8	190.8	185.2	97.0
704.5	719.5	700.8	97.4
1019.9	1034.9	998.6	96.5

**Serum (5.4 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	4.8	4.6	94.5
42.8	47.6	46.3	97.2
85.7	90.5	94.5	104.4
175.8	180.6	173.2	95.9
704.5	709.3	682.4	96.2
1019.9	1024.7	1050.4	102.5

**Seum (3.7 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	3.3	3.6	108.7
42.8	46.2	42.2	91.3
85.7	89.1	86.7	97.4
175.8	179.2	171.8	95.9
704.5	707.8	651.7	92.1
1019.9	1023.2	986.3	96.4

**Lithium Heparin (8.5 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	7.7	7.7	100.7
42.8	50.5	43.4	86.0
85.7	93.4	85.2	91.2
175.8	183.5	153.7	83.8
704.5	712.1	654.3	91.9
1019.9	1027.6	1015.3	98.8

**Lithium Heparin (9.5 U/mL)**

CA125 Spiked In U/mL	Expected U/mL	Recovered U/mL	% Recovery
0.0	8.5	8.5	99.4
42.8	51.3	46.6	90.8
85.7	94.2	84.4	89.6
175.8	184.4	172.6	93.6
704.5	713.0	644.9	90.5
1019.9	1028.4	973.4	94.7

**b. Dilutional recovery**

Three serum and two plasma samples with CA 125 values from 1321.1 to 1846.7 U/mL were diluted 1:2, 1:4, 1:8 or 1:16 with reagent grade water and assayed for recovery. The recoveries ranged from 90-108.7% for serum, 89.2%-97.1 for Lithium Heparin plasma, and 92.9-102.6% for EDTA plasma.

Sample	Dilution	Expected (U/mL)	Observed (U/mL)	% Recovery
S1-1	neat	1846.7	1846.7	
S1-2	1:2	923.4	874.9	94.8%
S1-3	1:4	461.7	471.4	102.1%
S1-4	1:8	230.8	237.6	102.9%
S1-5	1:16	115.4	124.5	107.8%
S2-1	neat	1251.4	1251.4	
S2-2	1:2	625.7	566.3	90.5%

Sample	Dilution	Expected (U/mL)	Observed (U/mL)	% Recovery
S2-3	1:4	312.9	296.6	94.8%
S2-4	1:8	156.4	159.8	102.2%
S2-5	1:16	78.2	78.8	100.7%
S3-1	neat	1561.9	1561.9	
S3-2	1:2	781.0	703.1	90.0%
S3-3	1:4	390.5	383.3	98.2%
S3-4	1:8	195.2	194.9	99.8%
S3-5	1:16	97.6	104.8	107.4%
S4-1	neat	1321.1	1321.1	
S4-2	1:2	660.6	615.5	93.2%
S4-3	1:4	330.3	337.1	102.1%
S4-4	1:8	165.1	173.7	105.2%
S4-5	1:16	82.6	89.7	108.7%
P1-1	neat	1425.9	1425.9	
P1-2	1:2	713.0	636.3	89.2%
P1-3	1:4	356.5	330.5	92.7%
P1-4	1:8	178.2	173.0	97.1%
P1-5	1:16	89.1	82.7	92.8%
P2-1	neat	1481.0	1481.0	
P2-2	1:2	740.5	687.7	92.9%
P2-3	1:4	370.3	362.9	98.0%
P2-4	1:8	182.1	186.8	102.6%
P2-5	1:16	92.6	91.4	98.7%

iii) High Dose Hook effect

Specimens were prepared by spiking normal human serum with CA 125 antigen from 1233 to 665,600 U/mL. The samples represented a large measurement range and all were above the claimed measurement range. The specimens were tested with one lot each of Flex reagents on two different Vista instruments. No hook effect was observed for CA 125 concentrations up to 656,600 U/mL.

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

i) Traceability

There is no recognized standard for CA 125

ii) Calibrator Manufacturing Description

The Dimension Vista® LOCI 6 Calibrator (KC604) is a multi-analyte frozen liquid product packaged as two vials for each of five levels. The matrix is 6% bovine albumin with buffer and preservatives. There are five levels (A-E) with target values for CA 125 of 0, 25, 100, 500 and 1050 U/mL.

a. Value Assignment

An anchor pool of CA 125 is made from highly purified, cell culture derived CA 125 material. Value is assigned to the Anchor pool by comparing it to patient samples assayed by the predicate device. The anchor pool is then diluted into a series of 5 Master pools whose values are determined on the Dimension® Vista System. The Master pool materials are then aliquoted into the commercial product whose values are confirmed on the Dimension® Vista System. The five-level calibrators are then used to calibrate the CA 125II method.

b. Stability

Frozen liquid calibrator stability is 12 months from the date of manufacture when stored unopened at -20°C. Thawed liquid calibrator stability is 30 days when stored unopened at 2-8°C. Once the vial stopper is punctured, the assigned calibrator values are stable for 30 days on board the Dimension Vista System. LOCI 7 calibrators should not be used on board the instrument once the cap is removed. One lot of control calibrator (reference material stored at -70°C) and three production lots of calibrator were tested. The acceptance criterion was <5% deviation from reference concentration for levels B-E

iii) Kit Stability

Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days. Once opened, reagents are stable for 7 days on-board the instrument.

Shelf-life stability (expiration) dating assignment at commercialization reflects the real time stability data on file at Siemens Healthcare Diagnostics. LOCI CA125II assay cartridges are stable for 12 months from the date of manufacture when stored at 2-8°C.

d. *Detection limit:*

Limits of blank (LoB) and limit of detection (LoD) were determined using 2 kit lots on 2 instruments (one per lot) using CLSI protocol EP17-A.

i) Serum: Five low CA 125 serum samples were tested using two lots of LOCI CA 125 reagents on two Vista instruments. Tested CA 125 values in Blank serum samples ranged from approximately -1.260 to -0.365U/mL with standard deviation (SD) of 239. Samples ranging from 0.710 to 4.507 U/mL were used to estimate LoD.

ii) Plasma: Three low CA 125 Lithium Heparin Plasma samples or three EDTA plasma samples were tested using two lots of LOCI CA 125 reagents on two Vista instruments. Tested CA 125 values in Blank plasma samples ranged from approximately -1.3 to -0.0 U/mL with SD of 0.302. Lithium Heparin plasma samples ranging from -0.9 to 2.0 U/mL with SD=0.590 or EDTA plasma samples ranging from -0.6 to 3.6 U/mL with SD=0.578 were used to estimate LoD.

iii) Acceptance criteria for the LoB, LoD, and Limit of quantitation (LoQ), were described as follows:

*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. Where Blank values are negative, this is estimated as

1.645 x SD of blank values. The data generated here give the highest value of  $LoB=1.645 \times 0.302=0.497$  which was rounded to  $LoB=0.5$  U/mL.

*LoD* – lowest amount of analyte that can be detected with 95% probability, though not quantified at an exact value.  $LoD=LoB+ SD \times C_{\beta}$  or  $0.5 + 0.590 \times 1.653=1.475$  which was rounded to  $LoD=1.5$  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 125 assay.

The results are noted to support a claim for *LoB* and *LoD* of less than 1.5 U/mL. Therefore, the lowest value for the range of the assay is 1.5 U/mL, based upon Lithium Heparin data.

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 125 assays. For all interferences the percent bias was determined by testing a control serum sample without the interferent and comparing it to the value obtained from a test sample to which the potential interferent had been added.

Testing was performed with one lot of Flex® reagents. Hemolytic and icteric interference was tested with two lots of Flex® reagents.

i) Endogenous Substance Interference

Testing was performed at two CA 125 concentrations:  $45 \pm 5$  U/mL and  $970 \pm 50$  U/mL ( $45 \pm 5$  and 500-1000 U/mL for Lipemia, Hemoglobin and Bilirubin testing).

Hemoglobin, triglyceride, bilirubin: The CA 125 method was evaluated for interference according to CLSI/NCCLS EP7-A2.25. Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Bias exceeding 10% is considered interference.

Substance tested	Substance concentration	LOCI CA 125 U/mL	Bias %
Hemoglobin (hemolysate)	Hemoglobin (monomer) 1000 mg/dL [0.62 mmol/L]	44	<10
		873	<10
Bilirubin (unconjugated)	60 mg/dL [1026 µmol/L]	45	<10
		888	<10
Bilirubin (conjugated)	20 mg/dL [342 µmol/L]	45	<10
		888	<10
Lipemia (Intralipid)	3000 mg/dL [33.9 mmol/L]	43	<10
		521	<10

Nine additional endogenous substances include serum proteins were also

tested with bias <10%.

ii) Exogenous Substance Interference

61 additional endogenous and exogenous substances were tested for interference including common over-the-counter drugs and cancer drugs. All recorded biases were less than the 10% acceptance criteria.

iii) HAMA interference

A number of optimized concentrations of HAMA blockers are 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 sera (three replicates) containing up to 327.1 mg/mL HAMA. All of the CA 125 results from the 10 samples evaluated were within 10% of control values or showed <1 U/mL difference from control.

f. *Assay cut-off:*

The sponsor defined a  $\geq 70.1\%$  percentage change in CA 125 values as significant. This significant change is the calculated Reference Change Value for the assay. This is a statistical calculation that considers the analytical imprecision of the CA 125 assay on the Dimension Vista instrument as well as the intra-individual biological variation noted for CA 125 levels in a study of healthy females determined by reference. The sponsor chose this value to ensure that the change in CA 125 value is not attributed to assay variation or normal variation. The RCV value also considers a z value for significant change (p,0.05), so that assay results that change by RCV should be within the 95% confidence interval.

The RCV was derived by taking into account the published biological variation and total imprecision for the Dimension Vista® LOCI CA 125 assay. In determining the RCV, the analytical variation used was 5.2% (which is the reported total variability (Within laboratory Total %CV at 11.2 U/mL). The within-subject biological variation (24.7%) was obtained from the literature. The RCV for the Dimension Vista® LOCI CA 125 method was calculated to be 70.1%.

2. Comparison studies:

a. *Method comparison with predicate device:*

One serum sample randomly chosen from each of the 75 women with cervical cancer who were tested for the monitoring of their disease status were combined with 77 excess de-identified serum samples containing measureable CA 125. Each sample was assayed for the observed values (154 samples) using the Dimension Vista® CA 125 (Y) assay and compared with the expected values using the ADVIA Centaur® CA 125 (X). Because the Advia device has a different measuring range than the Vista, the results limited to the measuring range of both devices (139 samples) are presented below (Passing & Bablock). The equation represents the relationship between the two techniques.

n = 139

$Y = 1.09X + 2.15$   $R^2 = 0.94478$

95% Confidence interval for the intercept: -0.62 to 2.89

95% Confidence interval for the slope: 1.06 to 1.13

Range of samples: 5.2 – 770.2 U/mL (DimensionVista);  
3.1 – 1099 U/mL (Advia Centaur assay).

<b>Comparative Method</b>	<b>Slope (95% CI)</b>	<b>Intercept (95% CI)</b>	<b>Correlation Coefficient</b>	<b>n</b>
Dimension Vista® CA 125 To ADVIA Centaur® CA 125	1.09 (1.06 to 1.13)	2.15 (-0.62 to 2.89)	0.972	139

*b. Matrix comparison:*

The Dimension Vista Flex Assay for CA 125 was evaluated in Serum, LiHeparin Plasma, and EDTA plasma.

- i.* Serum samples (1.8-1268.3 U/mL) were evaluated with matched samples of Lithium Heparin plasma (2.4-1264.9 U/mL). The two methods were evaluated with 90 samples over the measuring range of the device (linear regression line  $y=0.93x - 0.93$  and  $R=0.999$ ).
- ii.* Serum samples were evaluated with matched samples of EDTA plasma. The two methods were evaluated with 68 samples over the measuring range of the device (linear regression line  $y=1.00x - 0.29$  and  $R=0.999$ ).

<b>Sample comparison</b>	<b>Slope</b>	<b>Intercept U/mL</b>	<b>Correlation Coefficient</b>	<b>N</b>
Lithium heparin versus serum	0.96	-0.93	0.999	90
EDTA versus serum	1.00	-0.29	0.999	68

**3. Clinical studies:**

In a study of frozen specimens from 75 subjects with at least 3 serum samples collected during the course of follow-up surveillance for ovarian cancer progression, 255 visits for each of these 75 patients, following their initial evaluation (330 total CA 125 determinations) were cross-tabulated with a change in Dimension Vista CA 125 concentration of  $\geq 70.1\%$  at each surveillance visit. Clinical information that details the status of the subject's disease was required for inclusion of samples in the study.

Patient CA 125 values were compared to disease state 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 ( $n=255$ ). Table below shows the distribution of results when compared to the disease status.

A  $\geq 70.1\%$  rise in CA 125 value correlated with clinical progression in 62.12% of cases. No significant change in CA 125 values correlated with stable disease or no evidence of disease in 66% of cases. A  $\geq 70.1\%$  decrease in CA 125 value correlated with clinical response or no evidence of disease in 58.9% of cases. Overall expected changes in clinical status corresponded with CA 125 changes in

64.3% of cases.

**Disease State Frequency using the  
Dimension Vista® LOCI CA 125II Method**

Change in CA125	Change in Disease State				Total
	Responding n (%)	Stable n (%)	No Evidence of Disease n (%)	Progression n (%)	
>70.1% increase	3 (1.18)	17 (6.67)	4 (1.57)	42 (16.47)	66 (25.89)
No significant Change	22 (8.63)	45 (17.65)	54 (21.18)	29 (11.37)	150 (58.83)
>70.1% decrease	11 (4.31)	13 (5.10)	12 (4.71)	3 (1.18)	39 (15.3)
<b>Total</b>	36 (14.1)	75 (29.4)	70 (27.5)	74 (29.0)	255 (100)

Per patient visit clinical performance results for the Dimension Vista® LOCI CA 125 II™ test and predicate devices are given in the 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  $\geq 70.1\%$  rise in CA 125 value, 56.8% of subject visits (95% confidence interval 44.7% to 68.2%) had a rise in CA 125 value when the patient’s disease status was classified as progression. This value represents the positive percent agreement of significant CA 125 rise with a progression disease status.

**Dimension Vista® LOCI CA 125 II™ Value vs. Disease Progression**

	Progression	No- Progression	Total
>70.1% increase	<b>42</b>	24	66
$\leq 70.1\%$ increase	32	<b>157</b>	189
Total	74	181	255

***Exact 95% Confidence***

	<b><i>Estimate</i></b>	<b><i>Interval</i></b>
Total Concordance	78.0%	72.5 - 83.0%
Positive Concordance	56.8%	44.7 - 68.2%
Negative Concordance	86.7%	89.9 - 91.3%

Using a cut-off of a  $\geq 70.1\%$  rise in CA 125 value, 86.7% of subject visits (95% confidence interval 89.9% to 91.3%) had no rise in CA 125 value when the patient’s disease status was classified as no progression. This value represents the negative percent agreement of CA 125 rises with a progressive disease status.

The positive and negative agreement for the LOCI CA125II method, when taken together, show similar performance to the predicate method (below):

**Predicate CA 125 II™ Value vs. Disease Progression**

	<b>Progression</b>	<b>No-Progression</b>	<b>Total</b>
>30% increase & >35 U/mL	<b>47</b>	24	71
≤30% increase	27	<b>157</b>	184
<b>Total</b>	74	181	255

	<i>Estimate</i>	<i>Exact 95% Confidence Interval</i>
Total Concordance	80.0%	74.6 - 84.7%
Positive Concordance	63.5%	51.5 - 74.4%
Negative Concordance	86.7%	80.9 - 91.3%

4. Clinical cut-off:

Clinical Cut-off is based upon a 70.1% reference change value (RCV). When the measurement of CA 125 varies by 70.1% of the baseline (either positive or negative) the change is considered significant. The RCV was used to ensure that the change in CA 125 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 125 values above system noise.

5. Expected values/Reference range:

The distribution of CA 125 values was determined in specimens from healthy individuals (n= 198; females) and from patients with nonmalignant or malignant diseases. 96.5% of 198 samples from apparently healthy adult females (age 18-64 years) had CA 125 levels less than or equal to 35 U/mL.

Each laboratory should establish its own reference values for healthy patients' CA 125 levels as performed on the Dimension Vista® System.

Expected values of CA 125 measurements are dependent upon the individual patient's baseline reading for CA 125. Decreases from baseline value of 70.1% or more in any subsequent repeated measurements are indicative of response to therapy or remission. Increases of 70.1% or more suggest no response to therapy and correlate with progression of disease.

Estimation and empirical distributions of CA 125 II values in various populations of subjects was performed. The distribution of CA 125 values in 198 healthy individuals, 198 patients with nonmalignant and 370 patients with malignant disease is shown in the attached tables.

<b>Non-malignant Disease and Healthy Normal: # of Patients (%Patients) with CA 125 level U/mL</b>							
<b>Sample Category</b>	<b>N</b>	<b>0-35 (%)</b>	<b>35.1-60(%)</b>	<b>60.1-100 (%)</b>	<b>100.1-500 (%)</b>	<b>500.1-1000 (%)</b>	<b>&gt;1000 (%)</b>
Healthy Normal Female	198	191(96.5)	5(2.5)	2(1.0)	0(0.0)	0(0.0)	0(0.0)
Females < 50 years of age	147	140(95.2)	5(3.4)	2(1.4)	0(0.0)	0(0.0)	0(0.0)

<b>Non-malignant Disease and Healthy Normal: # of Patients (%Patients) with CA 125 level U/mL</b>							
<b>Sample Category</b>	<b>N</b>	<b>0-35 (%)</b>	<b>35.1-60(%)</b>	<b>60.1-100 (%)</b>	<b>100.1-500 (%)</b>	<b>500.1-1000 (%)</b>	<b>&gt;1000 (%)</b>
Females ≥ 50 years of age	51	51(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Breast	30	27(90)	1(3.3)	0(0.0)	1(3.3)	0(0.0)	
Polycystic Ovaries	15	14 (93.3)	0(0.0)	1(6.7)	0(0.0)	0(0.0)	0(0.0)
Cervix / Uterus	31	27(87.1)	4(12.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GI Tract	20	17(85)	1(5.0)	1(5.0)	1(5.0)	0(0.0)	0(0.0)
Ovarian Cyst	15	13(86.7)	1(6.7)	0(0.0)	0(0.0)	1(6.7)	0(0.0)
UTI	30	22(73.3)	5(16.7)	3(10.0)	0(0.0)	0(0.0)	0(0.0)
Pancreas	27	23(85.2)	2(7.4)	0(0.0)	2(7.4)	0(0.0)	0(0.0)
Colon	10	10(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Congestive Heart Failure	20	17(85.0)	1(5.0)	2(10.0)	0(0.0)	0(0.0)	0(0.0)

<b>Malignant Diseases</b>							
<b>Sample Category</b>	<b>N</b>	<b>0-35 (%)</b>	<b>35.1 - 60 (%)</b>	<b>60.1-100 (%)</b>	<b>100.1-500 (%)</b>	<b>500.1-1000 (%)</b>	<b>&gt;1000 (%)</b>
Breast:*	31	23 (74.2)	4 (12.9)	2 (6.5)	1 (3.2)	1 (3.2)	0 (0.0)
Ovarian	129	45(34.9)	9 (7.0)	16 (12.4)	27 (20.9)	14 (10.9)	18 (14.0)
Cervix/Uterus	41	32 (78.0)	5 (12.2))	1 (2.4)	3 (7.3)	0 (0.0)	0 (0.0)
Liver (Gall Bladder, Bile Duct)	23	19 (82.6)	2 (8.7)	0 (0.0)	1 (4.3)	1 (4.3)	0 (0.0)
Upper GI (Oral, Larynx, Esophagus, Stomach)	13	7 (53.8)	4 (30.8)	1 (7.7)	1 (7.7)	0 (0.0)	0 (0.0)
Colorectal	41	27 (65.9)	7 (17.1)	0 (0.0)	7 (17.1)	0 (0.0)	0 (0.0)
Pancreatic	30	12 (40.0)	4 (13.3)	3 (10.0)	10 (33.3)	1 (3.3)	0 (0.0)
Lung	30	22 (73.3)	1 (3.3)	2 (6.7)	4 (13.3)	0 (0.0)	1 (3.3)
Renal	2	2(100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lymphoma	30	23 (76.7)	4 (13.3)	2 (6.7)	1 (3.3)	0 (0.0)	0 (0.0)

**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.