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

A. 510(k) Number:

k121994

B. Purpose for Submission:

New device

C. Measurand:

Vitamin B12

D. Type of Test:

Quantitative chemiluminescent immunoassay based on LOCI® technology

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

Dimension Vista® LOCI Vitamin B12 Flex® reagent cartridge and Dimension Vista® LOCI 4 Calibrator (LOCI 4 CAL)

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDD	II	21 CFR 862.1810, Vitamin B12 Test System	Chemistry (75)
JIX	II	21 CFR 862.1150, Calibrator	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   The VB12 method is an *in vitro* diagnostic test for the quantitative measurement of vitamin B12 in human serum and plasma on the Dimension Vista® System. Measurements of vitamin B12 are used in the diagnosis and treatment of anemias of gastrointestinal malabsorption.

The LOCI 4 CAL is an *in vitro* diagnostic product for the calibration of LOCI Ferritin (FERR), LOCI Folate (FOL), and LOCI Vitamin B12 (VB 12) methods on the Dimension Vista® System.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Dimension Vista® 1500 System

I. Device Description:

Dimension Vista® LOCI Vitamin B12 Flex® reagent cartridge:

- Wells 1-2: 360 µg/mL Liquid VB12 Sensibead reagent
- Wells 3-4: 0.75N Sodium Hydrocide and 3mM Potassium Cyanide
- Wells 5-6: 10 mg/mL Dithioerythriol tablet
- Wells 9-10: 200 µg/mL VB12 Chemibead reagent
- Wells 11-12: 3 ng/mL Biotinylated IF and 60 ng/mL Dicyanocobinamide

Dimension Vista® LOCI 4 Calibrator:

10 Vials (2.0 mL each) of Dimension Vista® LOCI 4 Calibrator. The LOCI 4 Calibrator is a liquid multi-analyte product containing Ferritin from human liver, Folate, and Vitamin B12 in HEP ES buffer matrix (CAL A) or a bovine serum albumin Matrix (CAL B-E). Calibrators have the following approximate target concentrations:

Calibrator	Vit B12(pg/mL)	Folate (ng/mL)	Ferritin (ng/mL)
A	45	0	0
B	200	2.5	25
C	500	5.0	210
D	1000	10.0	1050
E	2200	21.0	2200

The human albumin donor units used in the Dimension Vista® LOCI 4 Calibrator were tested with FDA approved assays and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Elecsys Vitamin B12 Immunoassay

2. Predicate K number(s):

   k060755

3. Comparison with predicate:

Reagent

Similarities		
Item	Proposed Device Dimension Vista VB12	Predicate Device Roche Elecsys VB12 (k060755)
Intended Use	*In vitro* diagnostic test for the quantitative measurement of vitamin B12 in human serum and plasma	Same

Differences		
Item	Proposed Device	Predicate Device (k060755)
Platform	The Dimension Vista Vitamin B12 method (V12) is for use on the Dimension Vista System	The Roche Vitamin B12 Assay is for use on Elecsys 2010 and cobas e immunoassay analyzers
Methodology	Chemiluminescence	Electrochemiluminescence
Measuring range	60 – 2000 pg/mL	30 - 2000 pg/mL
Sample Size	12 µL	15 µL

Calibrator:

Similarities		
Item	Proposed Device Modified LOCI 4 CAL	Predicate Device LOCI 4 CAL (k071224)
Intended Use/Indications For use	*The* LOCI 4 CAL is an in vitro diagnostic product for the calibration of the LOCI Ferritin, LOCI Folate, and LOCI Vitamin B12 methods on the Dimension Vista System	Same
Traceability	Ferritin: WHO Standard for Ferritin, 3rd IS 94/572	Same

Similarities		
Item	Proposed Device Modified LOCI 4 CAL	Predicate Device LOCI 4 CAL (k071224)
	Folate: United States Pharmacopeia Grade Folic Acid VB12: United States Pharmacopeia Grade VB12	

Differences		
Item	Proposed Device Modified LOCI 4 CAL	Predicate Device LOCI 4 CAL (k071224)
Matrix	HEPES Buffer Level A 2% BSA based matrix Level B-E	6% BSA based matrix Level A-E
Target Concentrations Vitamin B12 (pg/mL)	Level A: 45 Level B: 200 Level C: 500 Level D: 1000 Level E: 2200	Level A: 0 Level B: 200 Level C: 500 Level D: 1000 Level E: 2200

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

- Clinical and Laboratory Standards Institute (CLSI) Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods*
- CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Guideline EP9-A2*: Method Comparison and Bias Estimation Using Patient Samples*
- CLSI Guideline EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Guideline C28-A2*: How to Define and Determine Reference Intervals in the Clinical Laboratory*

L. Test Principle:

The LOCI Vitamin B12 method is a homogeneous, competitive chemiluminescent immunoassay based on LOCI technology. LOCI reagents include two synthetic bead reagents and biotinylated intrinsic factor (IF). The first bead reagent (Chemibead) is coated with a B12 derivative and contains a chemiluminescent dye. The second bead reagent (Sensibead) is coated with streptavidin and contains photosensitive dye. The patient sample is pretreated with sodium hydroxide (NaOH) and dithioerythritol (DTE) to release the serum B12 from its carrier proteins. Potassium cyanide (KCN) is added to convert all the forms of B12 into a single, cyanocobalamin form, and dicyanocobinamide is added to keep the B12 from rebinding with the carrier proteins. After the sample pretreatment, the biotinylated IF and chemibead reagents are added sequentially to the reaction vessel. Vitamin B12 from the sample competes with the B12-chemibead for a limited amount of biotinylated IF. Sensibead reagent is then added and binds to the biotin to form bead pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from the Sensibeads which diffuses to the Chemibeads triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is an inverse function of the concentration of vitamin B12 in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   The precision study was designed based on the CLSI document EP5-A2. Bio-Rad Liquichek Control materials, patient serum and lithium heparin plasma pools were used for the precision study. One lot of reagent and one instrument was used (Vista 1500 analyzer). For each sample, a single test from two independent cups was analyzed twice per day for 20 days. The within-run and within-lab standard deviations were calculated by the analysis of variance method.

   The results are summarized below.

Samples	N	Mean (pg/mL)	Within-Run		Within-Lab	
			SD	%CV	SD	%CV
Serum pool 1	80	238	8.4	3.5	18.0	7.6
Plasma pool 1	80	365	8.9	2.4	12.3	3.4
Serum Pool 2	80	1007	14.4	1.4	21.0	2.1
Serum Pool 3	80	1716	20.6	1.2	32.7	1.9
Low control	80	275	11.0	4.0	19.2	7.0
Medium control	80	518	8.2	1.6	20.3	3.9
High control	80	682	16.3	2.4	20.1	3.0

   b. *Linearity/assay reportable range:*

   A linearity study was performed to support the measuring range claim. A native high and a native low serum pool were combined in different ratios to produce 9 dilution

pools covering the intended assay range. All 9 pools were measured 5 times on a single instrument (Vista 1500 analyzer). Sample ranges tested were between 22 to 2109 pg/mL. The linear regression was determined by plotting the observed values against the expected values and generated the following equation: y= 0.99x + 0.22. A summary of the linearity results are listed in the table below.

Sample	% High Sample	Expected pg/mL	Mean Observed value, n=5 pg/mL	Linear Fit	Difference vs. Linear fit %
LV1	100.0%	2337	2109	2276	-7.3%
LV2	82.5%	1898	1958	1881	4.1%
LV3	75.0%	1727	1695	1712	-1.0%
LV4	62.5%	1443	1436	1431	0.4%
LV5	50.0%	1159	1169	1149	1.7%
LV6	37.5%	875	875	867	0.9%
LV7	25.0%	591	586	586	0.1%
LV8	12.5%	306	308	304	1.3%
LV9	0.0%	22	22	22	-0.1%

The linearity study supports the sponsor's claim that the B12 assay has a reportable range of 60 to 2000 pg/mL.

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

Traceability: The Dimension Vista B12 calibrators are traceable to USP grade vitamin B12 by gravimetric method. Ferritin is traceable to the WHO Standard for ferritin, 3rd IS 94/572. Folate is traceable to the US Pharmacopeia Grade folic acid.

Value assignment:

Vitamin B12: The anchor pool consists of 5 levels of VB12 in human serum based material. The assigned value of the anchor pool is based on the Roche Elecsys. To provide stability, a large reserve of anchor pool is stored frozen at 70 ±20 ˚C. Aliquots of the anchor pool are used to produce the master pools. Values are assigned to each lot of calibrator from the master pool using the Dimension Vista System. The master pool is a frozen liquid, five-level material. The master pool has the same composition as the calibrator which is held in reserve. The value assignment is derived from 9 runs (n=5 replicates) on 3 instruments using 3 reagents lots.

Folate: The anchor pool consists of 6 levels of Folate in human serum base material. The assigned value of the anchor pool is based on the ADVIA Centaur folate method at 3 external sites. To provide stability, a large reserve of anchor pool is stored frozen at -70 ±20 ˚C. Aliquots of the anchor pool are used to produce the master pools. Values are assigned to each lot of calibrator from the master pool using the Dimension Vista System. The master pool is a frozen liquid, five-level material. The

master pool has the same composition as the calibrator which is held in reserve. The value assignment is derived from 9 runs (n=5 replicates) on 3 instruments using 3 reagents lots.

Ferritin: The anchor pool is based on WHO Standard for Ferritin 3rd IS 94/572. Nine levels of ferritin are gravimetrically prepared. To provide stability, a large reserve of anchor pool is stored frozen at -70 ±20 ˚C. Aliquots of the anchor pool are used to produce the master pools. Values are assigned to each lot of calibrator from the master pool using the Dimension Vista System. The master pool is a frozen liquid, five-level material. The master pool has the same composition as the calibrator which is held in reserve. The value assignment is derived from 9 runs (n=5 replicates) on 3 instruments using 3 reagents lots.

Stability of calibrators (folate, ferritin, and vitamin B12): Real–time stability protocols and acceptance criteria for shelf-life and in-use (open vial) stability were reviewed and found to be acceptable. Unopened thawed calibrator material is stable for 30 days at 2-8 ˚C. Once the stopper of the vial is punctured, assigned values are stable for 7 days when stored aboard the instrument. Once the cap is removed, assigned values are stable for 30 days when recapped immediately and stored at 2-8 ˚C.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) for the VB12 method was determined based on the CLSI document EP17-A. Blank samples (N=5) were prepared using five lots of HEPES buffer to determine the LoB. Low analyte samples (N=5) were serum samples with low endogenous B12 ranging from 32 to 62 pg/mL to determine the LoD. Each sample was tested in duplicate, using two lots of reagents once a day over 3 days for a total of 12 results per sample (total N=60).

The limit of quantification (LoQ) is defined as the lowest analyte concentration that can be reproducibly measured with a total CV of ≤20%. Seven serum pools with low endogenous B12 levels ranging from 40 to 180 pg/mLwere analyzed in duplicate, once a day for twenty days for a total of 40 results per sample.

The results are summarized in the below table.

	LoB	**LoD**	**LoQ**
Serum	18.0 pg/mL	28.0 pg/mL	52 pg/mL

The B12 assay has a measuring range of 60- 2000 pg/mL.

e. Analytical specificity:

Interference: Interference testing was performed according to CLSI EP7-A2 guideline to determine the effect of various endogenous and exogenous substances on the Dimension Vista VB12 assay. For all interferents, except rheumatoid factors (RF), 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. For RF interference, a human serum sample with a RF level of 500 IU/mL and a normal human serum sample were spiked with the same level of vitamin B12. Interferents were tested at two levels of vitamin B12, approximately 200 pg/mL and 1000pg/mL. For each spiked sample, the % recovery was determined. The acceptance criteria of ≤ 10% relative deviation from the control were met for all interferents tested except Dextran 40 and Hemoglobin. Dextran 40 and Hemoglobin with a >10% bias were repeated at lower concentrations to determine a level where the bias became less than 10%. The sponsor states in the Instructions for Use labeling that Dextran 40 at 6 g/dL decreases VB12 by -11.9% at 200 pg/dL and that hemoglobin at 500 mg/dL increases VB12 results by 19.8% at 200 pg/dL.

The hemolysis, icterus, and lipemia (HIL) limits where the interference bias does not exceed 10% were determined to be as follows:

- Bilirubin ≤ 60 mg/dL
- Hemoglobin ≤ 300 mg/dL
- Triglycerides ≤ 3000 mg/dL

The sponsor states in the Instructions for Use labeling to avoid using hemolyzed samples.

Cross reactivity: Cross reactivity of cobinamide with the Dimension Vista VB12 antibody was challenged at a concentration of 200 ng/mL. Cobinamide was added to a serum pool with a vitamin B12 level of 200 pg/mL. The cross-reactivity was determined by testing a control sample (n=5) without cobinamide and comparing it to the value obtained from a test sample (n=5) to which cobinamide (200 ng/mL) had been added. The percent cross-reactivity was calculated and the sponsor concluded that there is no cross-reactivity (≤ 1%) with cobinamide.

Intrinsic Factor Blocking Antibody (IFBA) interference:

An IFBA interference study was performed to assess the interference effects of IFBA on VB12 measurements. Patient samples with both positive and negative IFBA were assayed by both the candidate and the predicate device (Roche Elecsys). The VB12 concentration of the samples ranged from 60-2000 pg/mL. The sponsor concludes that test performed demonstrates that the Dimension Vista Vitamin B12 assay effectively mitigates the interference of IFBA. Results from the candidate device (Y) were plot against the predicate device (X) and a linear regression analysis was performed. The table below summarizes the IFBA interference study results:

	IFBA negative samples	IFBA positive samples
r^2	0.971	0.990
slope	1.00	1.01
intercept	-2.0	-2.0
N	78	79

The sponsor included the following limitation in their labeling:

Intrinsic blocking antibodies are present in approximately half of pernicious anemia patients. There is a low frequency possibility that these antibodies may not be completely inactivated during the reaction pretreatment step. If test results are in conflict with the clinical diagnosis, the sample can be tested for intrinsic factor blocking antibodies.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The Dimension Vista VB12 assay was compared to the Vitamin B12 assay on the Roche Elecsys System by evaluating one hundred sixty-two native serum samples across the claimed measuring range. The samples ranged from 69 to 1829 pg/mL on the Roche Elecsys, and 68 to 1963 pg/mL on the Dimension Vista. The sample set included 32 samples with known Intrinsic Factor Binding Antibody (IFBA) titers ranging from 7 to 133.85 U/mL.

Passing-Bablok regression analysis of the results yielded the following:

Regression	Bias	95% CI
Constant (Y-intercept)	-7.64	-17 to -0.48
Proportional (X-slope)	0.985	0.965 to 1.004

The correlation coefficient for this data set (R) is 0.993

b. Matrix comparison:
Serum, EDTA plasma, lithium heparin plasma and sodium heparin plasma are the recommended specimen types for the Dimension Vista VB12 assay. Seventy-one matched sets of serum, lithium heparin, sodium heparin, and EDTA plasma freshly drawn from healthy donors were tested on the Dimension Vista VB12 assay. Sixty-four sets were native samples with serum B12 levels ranging from 125 to 1174 pg/mL. Seven sets were spiked with B12 in equal amounts to all sample types to cover ranges from 1116 to 1821 pg/mL. All samples were tested (n=2) with the VB12 assay on the Dimension Vista 1500 System. The first replicate of the results were used for analyzing the linear regression analysis.

Ordinary least squares linear regression was used to fit the mean B12 results of each

of the plasma types against serum. The data and regression analysis is summarized below:

Sample Description

Vitamin B12	EDTA Plasma	Li-Hep Plasma	Na-Hep Plasma	Serum
n	71	71	71	71
Min (pg/mL)	108	116	126	125
Max (pg/mL)	1836	1853	1788	1821

Least Squares Regression Analysis (Plasma vs. Serum)

	EDTA Plasma	Li-Hep Plasma	Na-Hep Plasma
Slope	0.991	0.998	0.991
Y-Intercept	-11.2	-4.9	-2.7
R	0.998	0.999	0.999

3. Clinical studies:
   a. *Clinical Sensitivity:*

      Not applicable.

   b. *Clinical specificity:*

      Not applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):

      Not applicable.

4. Clinical cut-off:

   Not applicable.

5. Expected values/Reference range:

   Reference intervals were established for the Dimension Vista VB12 assay according to C28-A3. Samples from apparently healthy adults (age 20-64, 60 % male and 40% female) were collected in the US (N=200) and in the European Union (N=199). The results were analyzed non-parametrically to determine the central 95% region for each

population and a reference interval for each population was determined. The results are summarized as follows:

United States Population Reference Range: 193-986 pg/mL (n=200)

European Union Population Reference Range: 182-625 pg/mL (n=199)

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.