

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

A. 510(k) Number:

k133512

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:

LOCI® Vitamin B12 Flex® reagent cartridge
LOCI® Anemia Calibrator

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® EXL™ with LM integrated chemistry system. Measurements of vitamin B12 may be used in the diagnosis of B12 deficiency.

The LOCI® ANEMIA CAL is an *in vitro* diagnostic product for the calibration of LOCI® FOLA, and LOCI® VB 12 assays on the Dimension® EXL™ with LM integrated chemistry system.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Dimension® EXL™ with LM integrated chemistry system

I. Device Description:

LOCI® Vitamin B12 Flex® reagent cartridge contains 8 wells per cartridge and the contents are listed below:

Well #	Form	Ingredient	Concentration
1	Liquid	VB12 Sensibead reagent	360 µg/mL
2	Liquid	VB12 Chemibead reagent	200 µg/mL
3	Empty	N/A	N/A
4	Liquid	Biotinylated IF	3 ng/mL
		Dicyanocobinimide	60 ng/mL
5	Tablet	Dithioerythritol (DTE)	12.5 mg/mL
6	Liquid	Sodium Hydroxide (NaOH)	0.75 M
		Potassium Cyanide (KCN)	3 mM
7, 8	Empty	N/A	N/A

LOCI® Anemia Calibrator (LOCI ANEMIA CALIBRATOR)

The LOCI® Anemia Calibrator is a liquid frozen product containing Folate and Vitamin B12 in bovine serum albumin matrix. Each LOCI® Anemia Calibrator kit consists of ten vials (2.0 mL each, two vials per each of five levels). Calibrators have the following approximate target concentrations:

Calibrator	Vit B12 (pg/mL)	Folate (ng/mL)
1	45	0
2	200	2.5
3	500	5.0
4	1000	10.0
5	2200	21.0

The LOCI® Anemia Calibrator contains human source materials, which were tested with FDA approved assays and found to be negative for HBsAg, HIV-1, HIV-2 and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Dimension Vista® Vitamin B12 Flex® reagent cartridge
Dimension Vista® LOCI® 4 Calibrator

2. Predicate k number(s):

k121994

3. Comparison with predicate:

Reagent

Similarities and Differences		
Item	Candidate Device LOCI® Vitamin B12 (k133512)	Predicate Device Dimension Vista® Vitamin B12 (k121994)
Intended Use	<i>In vitro</i> diagnostic test for the quantitative measurement of vitamin B12 in human serum and plasma	Same
Methodology	Chemiluminescent, homogenous sandwich immunoassay based on LOCI® technology	Same
Sample Matrix	Serum, EDTA plasma, lithium heparin plasma and sodium heparin plasma	Same
Sample Size	12 µL	Same
Measuring range	80 – 2000 pg/mL	60 – 2000 pg/mL
Cartridge Components	8 wells	12 wells
Instrument	Dimension EXL with LM integrated Chemistry system	Dimension Vista

Calibrator:

Similarities and Differences		
Item	Candidate Device LOCI® Anemia Calibrator (k133512)	Predicate Device LOCI® 4 Calibrator (k121994)
Intended use	The LOCI ANEMIA CAL is an <i>in vitro</i> diagnostic product for the calibration of the LOCI Folate, and LOCI Vitamin B12 assays on the Dimension EXL with LM integrated chemistry system	The LOCI 4 CAL is an <i>in vitro</i> diagnostic product for the calibration of the LOCI Ferritin, LOCI Folate, and LOCI Vitamin B12 methods on the Dimension Vista System
Constituents	Folate and Vitamin B12	Folate, Vitamin B12 and Ferritin
Traceability	Folate: United States Pharmacopeia Grade Folic Acid Vitamin B12: United States Pharmacopeia Grade Vitamin B12	Same

Form	Frozen liquid	Same
Matrix	Level 1-5: bovine serum base (BSA) based matrix	Level A: HEPES Buffer Level B-E: bovine serum base (BSA) based matrix
Target Concentrations	Vitamin B12: Level 1: 45 pg/mL Level 2: 200 pg/mL Level 3: 500 pg/mL Level 4: 1000 pg/mL Level 5: 2200 pg/mL Folate: Level 1: 0 ng/mL Level 2: 2.5 ng/mL Level 3: 5.0 ng/mL Level 4: 10.0 ng/mL Level 5: 21.0 ng /mL	Vitamin B12: Level A: 45 pg/mL Level B: 200 pg/mL Level C: 500 pg/mL Level D: 1000 pg/mL Level E: 2200 pg/mL Folate: Level A: 0 ng/mL Level B: 2.5 ng/mL Level C: 5.0 ng/mL Level D: 10.0 ng/mL Level E: 21.0 ng /mL

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-A2: *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*
- 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 EP14-A2: *Evaluation of Matrix Effects*
- CLSI Guideline C28-A3: *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*

L. Test Principle:

The 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. Commercially available control materials, patient serum and lithium heparin plasma pools were used for the precision study. One lot of reagent and one instrument was used (Dimension® EXL™ with LM integrated chemistry system). For each sample, a single test from two independent cups was analyzed twice per day for 20 days. The repeatability and within-lab standard deviations were calculated by the analysis of variance method.

The results are summarized below.

Samples	N	Mean (pg/mL)	Repeatability		Within-Lab	
			SD	%CV	SD	%CV
Serum pool 1	80	180	10.1	5.6	11.6	6.5
Plasma pool 1	80	467	13.1	2.8	15.8	3.4
Serum Pool 2	80	978	24.9	2.5	27.7	2.8
Serum Pool 3	80	1733	27.4	1.6	35.2	2.0
Low control	80	290	13.2	4.6	15.0	5.2
Medium control	80	498	11.7	2.3	18.2	3.7
High control	80	645	15.9	2.5	21.5	3.3

b. *Linearity/assay reportable range:*

Linearity:

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 10 dilution pools covering the intended assay range. All 10 pools were measured 5 times on a single Dimension® EXL integrated chemistry analyzer. The range of tested sample was from 66 to 2066 pg/mL. The weighted linear fitting was determined by plotting the observed values against the expected values and generated the following equation:

$$y = 1.00x - 1.56$$

The linearity study supports the sponsor’s claim that the Vitamin B12 assay has a reportable range of 80 to 2000 pg/mL.

Dilution recovery:

Dilution recovery was done to evaluate the dilution accuracy of Vitamin B12 values measured for results >2,000 pg/mL, using water as a sample diluent. Six native serum samples, with Vitamin B12 results ranged from 2412 pg/mL to 5907 pg/ml (value assigned by the predicate device, Vista LOCI VB12 assay) were tested after

manual dilution (1:3) or with the auto dilution feature (1:3) on the Dimension® EXL with LM integrated chemistry system. The observed % recoveries ranged from 100% to 108%, vs. the expected value and support the sponsor's claim that the sample could be diluted either manually or automatically by the instrument.

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

Traceability:

The Vitamin B12 in LOCI® Anemia calibrator is traceable to USP grade vitamin B12 by gravimetric method. Folate in LOCI® Anemia calibrator is traceable to the US Pharmacopeia grade folic acid.

Value assignment:

Vitamin B12: The anchor pool consists of 6 levels of Vitamin B12 in human serum based material. The assigned value of the anchor pool is based on the Dimension Vista® VB12 method. To provide stability, a large reserve of anchor pool is stored frozen at -70 ± 20 °C. The master pool is a frozen liquid, five-level material. The assigned value of the master pool is obtained while tested against anchor pool on the Dimension EXL™ with LM System. The master pool, which is held in reserve, has the same composition as the calibrator. Values are assigned to each lot of calibrator from the master pool using the Dimension EXL™ with LM System. The value assignment is derived from 9 runs (n=5 replicates) on 3 instruments using 3 reagents lots.

Folate: The anchor pool consists of 5 levels of Folate in human serum base material. The assigned value of the anchor pool is based on the Dimension Vista® folate method. To provide stability, a large reserve of anchor pool is stored frozen at -70 ± 20 °C. The master pool is a frozen liquid, five-level material. The assigned value of the master pool is obtained while tested against anchor pool on the Dimension EXL™ with LM System. The master pool, which is held in reserve, has the same composition as the calibrator. Values are assigned to each lot of calibrator from the master pool using the Dimension EXL™ with LM System. The value assignment is derived from 9 runs (n=5 replicates) on 3 instruments using 3 reagents lots.

Stability of calibrators:

Real-time stability protocols and acceptance criteria for calibrator stability (shelf-life and in-use) were reviewed and found to be acceptable. Unopened thawed calibrator material is stable for 30 days at 2-8°C. Once thawed and 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-A2. Blank samples (N=5) were prepared using five lots of HEPES buffer to determine the LoB. Low analyte samples (N=9) were serum samples with low endogenous B12 ranging from 27 to 94 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 for LoB study and total N=108 for LoD study).

The limit of quantification (LoQ) is defined as the lowest analyte concentration that can be reproducibly measured with a total %CV of $\leq 20\%$. Seven serum pools with low endogenous B12 levels ranging from 39 to 158 pg/mL were analyzed in duplicate, once a day for twenty days for a total of 40 results per sample on each of two lots.

The results are summarized in the table below.

	LoB	LoD	LoQ
Serum	30 pg/mL	50 pg/mL	80 pg/mL

The VB12 assay has a measuring range of 80- 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 625 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 when compared against the unspiked sample. Sponsor defined non-significant interference as $\leq 10\%$ difference between the spiked and the unspiked sample. All interferents tested were non-significant 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 labeling that Dextran 40 at 6 g/dL decreases Vitamin B12 results by -14.9% at 200 pg/mL and by -11.8% at 1000 pg/mL. In addition, hemoglobin at 500 mg/dL increases Vitamin B12 results by 15.2% at 200 pg/mL and by $<10\%$ at 1000 pg/mL.

The hemolysis, icterus, and lipemia limits where the non-significant interference were determined to be are summarized in the table below:

Bilirubin (unconjugated)	≤ 60 mg/dL
Bilirubin (conjugated)	≤ 20 mg/dL
Hemoglobin	≤ 300 mg/dL
Triglycerides	≤ 3000 mg/dL

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

For common exogenous and endogenous interference substance, results of the concentration tested that did not show significant interference are summarized in the table below.

Substance tested	Concentration tested that showed non-significant interference
Acetaminophen	20 mg/dL
Amikacin	8 mg/dL
Ampicillin	5.3 mg/dL
Ascorbic Acid	6 mg/dL
Biotin	100 ng/mL
Caffeine	6 mg/dL
Carbamazepine	3 mg/dL
Chloramphenicol	5 mg/dL
Chlordiazepoxide	1 mg/dL
Chlorpromazine	0.2 mg/dL
Cholesterol	503 mg/dL
Cimetidine	2 mg/dL
Creatinine	30 mg/dL
Diazepam	0.51 mg/dL
Digoxin	6.1 ng/mL
Erythromycin	6 mg/dL
Ethanol	400 mg/dL
Ethosuximide	25 mg/dL
Furosemide	6 mg/dL
Gentamicin	1 mg/dL
Heparin	3 U/mL
Ibuprofen	50 mg/dL
Immunoglobulin G	5 g/dL
Lidocaine	1.2 mg/dL
Lithium	2.2 mg/dL
Nicotine	0.1 mg/dL
Penicillin G	25 U/mL
Pentobarbital	8 mg/dL
Phenobarbital	10 mg/dL
Phenytoin	5 mg/dL
Primidone	4 mg/dL
Propoxyphene	0.16 mg/dL
Protein: Albumin	6 g/dL
Protein: Total	12 g/dL
Rheumatoid Factors	500 IU/mL
Salicylic Acid	60 mg/dL
Theophylline	4 mg/dL
Triglycerides	3000 mg/dL
Urea	500 mg/dL
Uric Acid	20 mg/dL
Vancomycin	10 mg/dL

Valproic Acid	50 mg/dL
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Cross reactivity:

Cross reactivity of cobinamide with the LOCI® 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 and to an aliquot of LOCI® Anemia Calibrator level 1. The cross-reactivity was determined by testing a control sample (n=10) without cobinamide and comparing it to the value obtained from a test sample (n=10) 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 ($\leq 1\%$) with cobinamide.

Intrinsic Factor Blocking Antibody (IFBA) interference:

An IFBA interference study was performed to assess the interference effects of IFBA on Vitamin B12 measurements. A total of two hundred thirteen (213) samples were tested. One hundred sixty-eight (168) were from the general population (presumed IFBA negative) samples that spanned the range of the assay. Forty-five (45) samples were known to be positive for IFBA with titers ranging from 2 to 173 IU/mL. Patient samples with both positive and negative IFBA were assayed by both the candidate and the predicate device. The sponsor followed CLSI Guideline EP14-A2: Evaluation of Matrix Effects to evaluate the results and concluded that the LOCI Vitamin B12 assay on Dimension EXL™ with LM System effectively mitigates the interference of IFBA.

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.

Patient samples may contain heterophilic antibodies that could react with immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The Dimension LOCI® Vitamin B12 assay on the Dimension EXL™ with LM integrated chemistry system was compared to the Vitamin B12 assay on the Dimension Vista System by evaluating one hundred sixty-six native serum samples across the claimed measuring range. The samples ranged from 60 to 1966 pg/mL on the Dimension Vista, and 86 to 1901 pg/mL on the Dimension EXL™ with LM

integrated chemistry system. The sample set included 35 samples with known Intrinsic Factor Binding Antibody (IFBA) titers ranging from 15 to 164 IU/mL.

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

Regression	Bias	95% CI
Constant (Y-intercept)	-6.37	-12.9 to 0.36
Proportional (X-slope)	1.03	1.02 to 1.04

The correlation coefficient for this data set is 0.997.

b. Matrix comparison:

Serum (plain and serum separator (SST)), EDTA plasma, lithium heparin plasma and sodium heparin plasma are the recommended specimen types for the Dimension LOCI® VB12 assay. Seventy-one native matched sets of serum (plain and serum separator (SST), lithium heparin, sodium heparin, and EDTA plasma freshly drawn from healthy donors were tested on the Dimension LOCI® VB12 assay covering the range of 169 to 1227 pg/mL. In addition, six spiked and one diluted samples were prepared to all sample types to cover a sample range up to 1798 pg/mL and down to 107 pg/mL.

Passing- Bablok regression was used to fit the vitamin B12 results of each of the plasma types and SST against plain serum. The data and regression analysis are summarized below:

Passing-Bablok Regression Analysis Results

	Li-Heparin Plasma	Na-Heparin Plasma	EDTA Plasma	SST serum
Slope	1.00	1.02	1.00	1.01
Y-Intercept	-4.33	-10.73	-7.26	-2.31
Correlation Coefficient	0.998	0.998	0.998	0.998

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 range:

United States Population Reference Range: 193-986 pg/mL

European Union Population Reference Range: 182-625 pg/mL

The reference range was established according to the CLSI C28-A3 guideline *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*; based on the capability of transferring the predicate device's reference range to the candidate device's reference range because of the comparability of the two testing systems. Sponsor followed the transferability guidance and verification procedure of the CLSI and has confirmed that the established reference range from the predicate device are transferable to the candidate device using 20 apparently healthy subjects in the US and European Union, respectively. All 20 samples were within the established reference range. The reference range was previously established in k121994 for the predicate device by conducting a reference range study using 399 samples.

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.