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

A. 510(k) Number:

k121053

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative Enzymatic

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Homocysteine POC Test Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LPS	Class II	21 CFR 862.1377	Chemistry

H. Intended Use:

1. Intended use(s):

   See Indications for use below

2. Indication(s) for use:

   Diazyme Homocysteine POC Test Kit is intended to be used with the SMART analyzer in a Point-of-Care setting for the in vitro quantitative determination of total L-

homocysteine in serum or plasma. The assay can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. For *in vitro* Diagnostic Use Only

3. Special conditions for use statement(s):

   For prescription use only

   The labeling contains a prominent black-box warning:

   WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants and 6-azuridine triacetate may have elevated levels of homocysteine due to their effect on the metabolic pathway.

4. Special instrument requirements:

   For use on SMART Analyzer (k092911) only

I. Device Description:

The Diazyme HCY POC Test Kit consists of the following components (1) DRS Cuvettes pre-filled with Reagent 1, (2) DRS Caps Pre-filled with Reagent 2, and (3) one preprogrammed Radio Frequency ID (RFID) card which contains a lot specific calibration curve. Reagent 1 and 2 are comprised of S-adenosylmethionine (SAM) (0.1mM), NADH (>0.2mM), TCEP (>0.5mM), 2- oxoglutarate (5.0mM), Glutamate dehydrogenase (10 KU/L), SAH hydrolase (3.0 KU/L), Adenosine deaminase (5.0 KU/L), and Hcy methyltransferase (5.0 KU/L).

Smart Analyzer (k092911) is a compact cuvette based spectrophotometer machine for point-of-care testing designed to analyze readings from single use reagent cuvettes. The instrument only uses the Diazyme Reagent System (DRS) cuvette and caps and performs the assay with a preprogrammed Radio Frequency ID (RFID) card. The lot specific RFID card contains reagent addition time, mixing time, reading time and calibration curve for estimating Homocysteine concentration.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Diazyme Homocysteine 2 Reagent Enzymatic Assay Kit

2. Predicate 510(k) number(s):

   k071971

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device Diazyme Homocysteine POC Test Kit	Predicate k071971
Intended Use/ Indications for Use	Diazyme Homocysteine POC Test Kit is intended for *in vitro* quantitative determination of total L-homocysteine in serum or plasma. The assay can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. For *in vitro* Diagnostic Use Only	Same
Test principle	Indirect measurement of homocysteine by the measurement of the cosubstrate conversion product	Same
Type of Test	Quantitative	Same
Specimen type	20µl Human serum or plasma	13 µl Human serum or plasma
Calibration	Assay Specific Calibrators	RFID card carrying calibrator information for the SMART analyzer
Measuring Range	3 – 50 µmol/L	2.5 – 50 µmol/L

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

CLSI Guideline EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI Guideline EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: Statistical Approach

CLSI Guideline EP9-A2: Method Comparison and Bias Estimates Using Patient Samples

CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry

L. Test Principle:

Diazyme Homocysteine POC Test Kit contains reagents intended for use with the SMART analyzer for the quantitative determination of Homocysteine (HCY) in human serum or plasma. Oxidized HCY is first reduced to free HCY which then reacts with a co-substrate, S-adenosylmethionine (SAM) catalyzed by a HCY S-methyltransferase to form methionine (Met) and S-adenosylhomocysteine (SAH). SAH is assessed by coupled enzyme reactions invluding SAH hydrolase, adenosine (Ado) deaminiase and HCY by SAH hydrolase. The formed HCY that is originated from the co-substrate SAM is cycled into the HCY conversion reaction by HCY S-methyltransferase. This forms a co-substrate conversion product-based enzyme cycling reaction system with significant amplification of detection signals. The formed Ado is immediately hydrolyzed into inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversion of NADH to NAD^+ . The concentration of HCY in the sample is indirectly proportional to the amount of NADH converted to NAD^+ which is read spectrophotometrically at 340nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Internal Testing

   Precision was evaluated according to CLSI EP5-A guideline. In order to evaluate precision three levels of serum controls containing 7.3,11.87 and 29.32 µmol/L Homocysteine were tested in duplicate, two runs per day, over ten days (total measurements per sample, n=40). The results are summarized in the table below:

Sample	N	Mean (µmol/L)	Within Run		Total	
			SD	%CV	SD	%CV
Control 1	40	7.5	0.24	3.2	0.26	3.4
Control 2	40	11.8	0.22	1.8	0.42	3.5
Control 3	40	29.0	0.82	2.8	0.95	3.3

   POC Testing

   A precision study was performed at three physician office laboratories by intended users. The study was evaluated at three physician office laboratories (POL). A total of 9 serum samples with HCY levels from 10.26 µmol/L to 42.73 µmol/L were tested at each site. Total measurements per sample, n=20). The results are summarized in the table below:

Site 1

Sample	N	Mean (µmol/L)	Within Run		Total	
			SD	%CV	SD	%CV
Sample 1	20	11.05	0.73	6.7	0.77	7.0
Sample 2	20	25.82	1.60	6.2	1.38	5.3
Sample 3	20	42.73	2.39	5.6	2.73	6.4

Site 2

Sample	N	Mean (µmol/L)	Within Run		Total	
			SD	%CV	SD	%CV
Sample 4	20	10.26	0.71	6.9	0.67	6.6
Sample 5	20	25.18	1.32	5.2	1.39	5.5
Sample 6	20	41.99	1.62	3.8	1.86	4.4

Site 3

Sample	N	Mean (µmol/L)	Within Run		Total	
			SD	%CV	SD	%CV
Sample 7	20	11.63	0.49	4.2	0.69	6.0
Sample 8	20	26.34	1.98	7.5	1.79	6.8
Sample 9	20	31.63	1.83	5.8	1.73	5.5

b. *Linearity/assay reportable range:*

Percent recovery was assessed across the reportable range of the device (3-50 µmol/L). A serum sample containing 50 µmol/L homocysteine was diluted with saline to create 11 sample levels across the measuring range of the device. The samples were measured in triplicate on the SMART Analyzer. The mean of the obtained Homocysteine results (obtained on the SMART analyzer) were plotted against the expected values and an appropriate line fitted by standard linear regression resulted in the following equation:

$y=0.9749x+0.751$; $R^2=0.9992$

Based upon the linearity data and the LoQ study, the sponsor claims a measuring range of 3 to 50 µmol/L

The sponsor recommends that all samples >50µM be diluted with deionized water and a 1:1 dilution be performed. In order to support this procedure, the sponsor provided a dilution study in which 4 serum samples were spiked and then diluted 1:1 with water and analyzed on the SMART analyzer using the Homocysteine POC test. Sample range was 62.2-84.2 µmol/L and % Recovery was 99.79 – 104.68.

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

Traceability:

Homocysteine POC test calibration system is traceable to the higher order NIST SRM 1955. Homocysteine POC test utilizes a Calibration Radio Frequency Identification Card (RFID) card that is programmed with a lot specific calibration curve and is supplied in each kit. RFID cards are programmed at the manufacturer site and are subject to the same quality control checks as the reagents and controls. According to the sponsor, the calibration curve is stable until the printed expiration date which is 8 months.

To construct the Diazyme HCY SMART Assay RFID card calibration curve, 5 levels of known calibrator levels are tested with the Diazyme Homocysteine POC Test reagents on three SMART analyzers. The calibrator value and the mean absorbance change are programmed into the RFID card.

Value Assignment (controls): *previously cleared in k042448*

Detection limit:

The Limit of Blank, Limit of Detection and Limit of Quantitation of Diazyme HCY POC Test kit were determined according to *CLSI EP17-A* in the following manner:

To calculate the Limit of Blank (LoB) of the Diazyme HCY POC Test kit, the True Blank Sample (water) was tested with 20 replicates daily for three days. LOB was calculated as the mean of the 57th and 58th highest values for the true blanks. Based upon the results, the sponsor claims a LoB=0.06µmol/L.

To calculate the Limit of Detection (LoD) of the Diazyme HCY POC Test kit, five low samples were tested with 4 replicates daily for three days. LoD=LoB + (1.465* SD of Low samples). Based upon the results, the sponsor claims a LoD=0.32 µmol/L.

The lowest level of the calibrator for the HCY RFID card is between 2-3 µmol/L HCY. No values that are below this level are able to be reported by the analyzer.

To calculate the Limit of Quantitation (LoQ) of the Diazyme HCY POC Test kit, 5 serum samples were prepared with HCY values at the lower range (2.1-2.61 µmol/L). 20 replicates of the low samples were run and EP evaluator software was used to estimate the LoQ. Based upon the results and the limitations imposed by the calibrator RFID card (cannot measure samples <2 µmol/L, the sponsor claims a LoQ = 3µmol/L.

The claimed measuring range is 3 – 50 µmol/L based on linearity.

See linearity study in M.1.b of this 510(k) decision summary.

e. Analytical specificity:

The level of interference from substances normally present in serum was determined by testing two HCY serum samples (12 µM and 29 µM) spiked with various concentrations of the interferent. The following substances produced less than 10% deviation when tested at levels equal to the concentrations listed below.

Endogenous Substances	Concentration
Unconjugated Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglyceride	1000 mg/dL

Exogenous Substances	Concentration
Ascorbic Acid	10 mmol/L
Glutathione	500µM
Methionine	20 µM
Cysteine	1000 µM
Pyruvate	500 µM
Cystathionine	100 µM
Hydroxylamine	1000 µM
Carbamazepine	130 µM
Phenytoin	200 µM
6-azauridine triacetate	1000 µM
S-adenosyl-methionine	20 µM
Carbamazepine-10, 11-epoxide	60 µM
Ethosuximide	1800 µM
Primidone	200 µM
Valproic Acid	3.5 mM
Sodium Nitrate	500 µM
Methotrexate	2.0 mM

The labeling states "patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetate, may have higher levels of HCY due to metabolic interference with homocysteine metabolism."

f. Assay cut-off:

Not applicable

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:

Internal Site Testing

To assess the accuracy of the device, 74 samples (70 natural serum samples and 4 spiked samples were measured using the Diazyme Homocysteine POC test run in parallel with the Diazyme HCY Two Reagent Enzymatic Assay on the Olympus AU400 analyzer. Sample values ranged from 4.17-49.50 µmol/L. Three SMART analyzers and two lots of reagents were used and samples were assayed in singlicate.

The regression results are summarized below:

Slope	0.9612
Intercept	0.5246
Correlation Coefficient	0.9696

POC Site Testing

120 serum samples were tested at three POC sites. Each site tested 40 samples using SMART analyzers. The test results on SMART analyzers were compared to the predicate device on the Olympus AU400 analyzer.

The regression results are summarized below:

SMART HCY	Site 1	Site 2	Site 3	All sites combined
n	40	40	40	120
Slope	1.0890	1.0041	1.0600	1.0552
Intercept	-0.7438	-0.6251	-1.1564	-0.8860
R^2	0.9830	0.9645	0.9819	0.9765
Range	5.43-48.95 µmol/L	3.88-45.43 µmol/L	4.81-49.86 µmol/L	3.88-49.86 µmol/L

b. Matrix comparison

Forty paired serum and plasma matched sets (EDTAPlasma/Li-Heparin) samples were tested on the on the SMART analyzer using the Homocytsteine POC test kits. Of the 40 samples that were analyzed 32 were native samples and 8 were spiked samples. Sample range tested was 7.97 – 47.24 µmol/L.

Linear regression analysis is as follows :

EDTA plasma vs. Serum – $y=1.0197x-0.5385$ $R^2= 0.9889$

Li Heparin vs. Serum – $y=0.9632x+0.3177$ $R^2=0.99$

Based on the data, the sponsor claims EDTA plasma and Lithium Heparin are acceptable anticoagulants for this assay.

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:

   The labeling states in most of U.S. clinical laboratories, 15mmol.L is used as the cut-off value for normal level of Hcy for adults. However, each laboratory is recommended to establish a range of normal values for the population in their region.[1,2,3]

   [1] Ueland PM, Refsum H, Kvalheim G, *et al. Clinical Chem.* 39: 263-271. (1993)

   [2] Lussier-Cacan S, Xhignese M, Piolot A, *et al. Am J Clin Nutr.* 64:587-593. (1996)

   [3]Cotton F, Wautrecht J. Lechevin V,*et al. Clin Chem.* 49:315-317. (2003)

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.