

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

A. 510(k) Number:

k032012

B. Analyte:

Homocysteine

C. Type of Test:

Quantitative

D. Applicant:

Diazyme Laboratories

E. Proprietary and Established Names:

Homocysteine Microplate HPB Assay; Homocysteine Controls (Lyophilized Form)

F. Regulatory Information:

1. Regulation section:
21 CFR 862.1377; 21 CFR 862.1660
2. Classification:
Class II; Class I
3. Product Code:
LPS; JJX
4. Panel:
75

G. Intended Use

1. Intended use(s):
See indication for use below.
2. Indication(s) for use:
The Homocysteine Microplate HPB Assay is intended for the quantitative determination of total L-homocysteine in human serum or plasma.

The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

The Homocysteine Controls (Lyophilized Form) are intended for use as an assayed quality control serum to monitor the precision of the laboratory testing procedures for homocysteine.

3. Special condition for use statement(s):
NA

4. Special instrument Requirements:
Microtiter plate reader capable of reading at 450nm

H. Device Description:

The *in vitro diagnostic* reagent kit contains avidin coated microtiter stripwells (96), buffers, hydrolase, substrate, conjugate, enzyme inhibitor, adenosine in tris buffer, adenosine deaminase, stopping solution, Hcy-binding protein, and calibration materials (6). The two control materials containing lyophilized human plasma are sold separately.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Axis Homocysteine EIA
2. Predicate K number(s):
k980907
3. Comparison with predicate:

Item	Homocysteine Microplate HPB	Axis Homocysteine EIA
Intended use	For the quantitative determination of total L-homocysteine in human serum or plasma.	Same
Type of assay	enzyme immunoassay-like assay	antibody based enzyme immunoassay
Calibrator and control materials	6 liquid calibrator materials included with kit; 2 lyophilized control materials sold separately	6 liquid calibrator materials included with kit; 3 liquid control materials sold separately
Analytical range	1.5 to 60 $\mu\text{mol/L}$	1.0 to 50 $\mu\text{mol/L}$
Type of samples to be assayed	serum or EDTA plasma	Same
Pretreatment of samples	Add 20 μL of sample and 150 μL of pretreatment reagent	Add 25 μL of sample and 500 μL of pretreatment reagent
Calculation of results and detection instrument	Four parameter logistic curve using a microplate reader at 450 nm	Same

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

NCCLS Guideline EP5-A, (Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1999))

K. Test Principle:

This is an EIA-like assay using a genetically engineered homocysteine binding protein as the capture reagent. After pretreatment of samples with a reducing agent, a

competition between free homocysteine from the samples and the tracer conjugate for binding sites on the binding protein occurs.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Three specimens with homocysteine concentrations of 7.0 $\mu\text{mol/L}$, 10.5 $\mu\text{mol/L}$ and 22.0 $\mu\text{mol/L}$ were assayed in quadruplicate, for twenty days. The intra-assay %CVs were 4.1%, 4.6%, 3.8%, respectively and the inter-assay %CVs were 9.0%, 6.7% and 9.2%, respectively.

b. *Linearity/assay reportable range:*

The linearity was evaluated by diluting four samples, homocysteine concentrations between 15 and 60 $\mu\text{mol/L}$, with assay buffer. The assayed results were plotted on the Y axis and the theoretical results were plotted on the X axis. The resulting regression equation was $Y = 0.98X - 0.57 \text{ umol/L}$, $r^2 = 0.99$. The dynamic range of the assay is stated as 1.5 – 60 $\mu\text{mol/L}$.

c. *Traceability (controls, calibrators, or method):*

The traceability of the control and calibrator materials is stated as using a know reference standard that is characterized by an HPLC reference method.

d. *Detection limit (analytical sensitivity):*

The 4 $\mu\text{mol/L}$ SAH-calibrator was diluted with solution A to obtain sample concentrations of 3, 2.5, 2.0, 1.5 and 1.0 $\mu\text{mol/L}$. The samples were analyzed in replicates of six. The limit of quantification is defined as the lowest concentration having a CV <20%. The value obtained is 1.5 $\mu\text{mol/L}$.

e. *Analytical specificity:*

The interference was determined for bilirubin, hemoglobin, lipids (triglycerides), red blood cells, protein and sodium fluoride by spiking them into plasma samples. The analysis showed that all interference values were less than 10% except for the following: bilirubin (0.2 mg/mL) showed 12.6% interference for homocysteine at 6.2 $\mu\text{mol/L}$; protein (60 mg/mL) showed -13.3% interference for homocysteine at 21 $\mu\text{mol/L}$; and sodium fluoride (5 mg/mL) showed -11.3% interference for homocysteine at 6.2 $\mu\text{mol/L}$.

The cross-reactivity was determined for adenosine (5 mmol/L), adenosyl-L-methionine (0.5 mmol/L), cystathionine (0.5 mmol/L), L-cysteine (100 mmol/L), glutathione (5 mmol/L), thiolactone (0.5 mmol/L) by spiking them into plasma samples. The analysis showed that all cross-reactivity values were less than 2.5 %, except, L-cysteine which showed 2.7% cross-reactivity for homocysteine at 19.7 $\mu\text{mol/L}$.

f. *Assay cut-off:*

Not determined

2. Comparison studies:a. *Method comparison with predicate device:*

A study using 101 patient samples, ranging in concentration from 6.41 to 19.19 $\mu\text{mol/L}$ and 6 spiked samples, ranging in concentration from 7.49 to 45.53 $\mu\text{mol/L}$, was performed. The samples were assayed by this device (Y) and the predicate device (X). The results of the linear regression analysis yielded the equation, $Y = 0.95X + 0.72$, $r = 0.96$ for the 107 samples tested.

b. *Matrix comparison:*

No separate matrix comparison studies were performed. Published literature is provided in the labeling as a reference for the use of both serum and plasma samples.

3. Clinical studies:

None performed

4. Clinical cut-off:

Not determined

5. Expected values/Reference range:

Studies published in the literature are provided in the labeling for expected values for males and females. In particular, the U.S. National Health and Nutrition Examination Survey (NHANES), published in *Annals of Internal Medicine* (1999) 131, 331-339, is cited as representing the US population. A chart is provided in the labeling as follows:

	12-19 years	≥ 60 years of age	Cut-off for "high" levels
Male	4.3-9.9 $\mu\text{mol/L}$	5.9 -15.3 $\mu\text{mol/L}$	≥ 11.4 $\mu\text{mol/L}$
Female	3.3-7.2 $\mu\text{mol/L}$	4.7-11.6 $\mu\text{mol/L}$	≥ 10.4 $\mu\text{mol/L}$

Clinical limitations:

The labeling states that persons taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants or 6-azauridine triacetate may have higher homocysteine values due to metabolic interference with homocysteine metabolism.

M. Conclusion:

Based upon the information provided for the file, I recommend that this device is substantially equivalent to the predicate device, regulated by 21 CFR 862.1377.