

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

k080851

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative

E. Applicant:

AntiCancer, Inc.

F. Proprietary and Established Names:

A/C Portable Enzymatic Homocysteine Assay on the A/C Diagnostics Reader

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1377, Urinary Homocysteine Test System

2. Classification:

Class II

3. Product code:

LPS

4. Panel:

75, Chemistry

H. Intended Use:

1. Intended use(s):

See Indications for Use below

2. Indication(s) for use:

The A/C Portable Enzymatic Homocysteine Assay on the A/C Diagnostics Reader (HyTek-205) is intended for the quantitative determination of total homocysteine (tHCY) in human plasma or serum. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia. The A/C Enzymatic Homocysteine Assay is for *in vitro* diagnostic use.

3. Special conditions for use statement(s):

Prescription Use only

This assay is for central laboratory use.

4. Special instrument requirements:

A/C Diagnostics Reader

I. Device Description:

The kit consists of the following:

Kit Contents for 100 samples

(1) Reagents (Store at 2-8°)

Reagent	Compound	Components	Quantity
Reagent I	Reducing Reagent	Dithiothreitol (lyophilized powder)	10 mg
Reagent II	Enzyme	Homocysteinase (lyophilized powder)	0.5 mg
Reagent III	Chromophore	N,N-dibutyl phenylene diamine chloride)	10 mL
Reagent IV	Oxidant	Potassium ferricyanide in buffer	6 mL
Buffer I	Assay Buffer	Sodium phosphate buffer	50 mL
Buffer II	Enzyme Buffer	Pyridoxal 5-phosphate buffer	1 mL
Surfactant	Surfactant	Triton X-100	1 mL

- (2) Calibrators and controls (Store at -20°C) supplied with the kit

Controls: Low and High Controls each 0.3 ml

Controls are prepared from a human plasma matrix spiked with L-homocysteine

Calibrators: Low, Medium and High Calibrators each 0.3 ml

Calibrators are prepared from a human plasma matrix spiked with L-homocysteine

- (3) Thermowell TM tubes (120)

0.5 mL Costar* PCR Tubes - Thin-walled polypropylene PCR/centrifuge tubes. Tubes are plain polypropylene with no coating.

Human source material was tested by an FDA approved method for the presence of antibody to HIV-1/2, antibody to HCV, as well as for the Hepatitis B Surface Antigen (HbsAg), and found to be negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

A/C Enzymatic HCY Assay on Hitachi 912

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

k030754

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Principle	Enzymatic	Enzymatic
Indications for Use	Intended for the quantitative determination of total homocysteine (tHCY) in human plasma or serum. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia.	Intended for the quantitative determination of total homocysteine (tHCY) in human plasma or serum. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia.
Calibrator levels	Low, medium and high	Low, medium and high

Differences		
Item	Device	Predicate
Performance	Manual	Fully automatic
Instrumentation	A/C Diagnostics Reader	Hitachi 912 Analyzer
Throughput	100 tests/hr	360 tests/hr

Differences		
Item	Device	Predicate
Reportable ranges	4.1 to 43.8 µmol/L	2.5 - 80 µmol/L

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

CLSI EP5-T2, Evaluation of Precision Performance of Clinical Chemistry Devices;

L. Test Principle:

The A/C Portable Enzymatic Homocysteine (HCY) Assay measures total homocysteine (tHCY) in plasma and serum. The principle of the assay is that tHCY is degraded by Homocysteinase (rHCYase) and produces hydrogen sulfide (H₂S), which is measured using N,N-dibutyl phenylene diamine (DBPDA), the combination of which forms a chromophore. After oxidation, the A/C Diagnostics Reader, a fluorescence reader is used to obtain the tHCY values.

The A/C Portable Enzymatic Homocysteine Assay is a manual assay with the results read by the A/C Diagnostics Reader. Five µL of EDTA plasma is used in a dithiothreitol reduction reaction for 60 minutes to release bound homocysteine. The rHCYase reaction is then run for 5 minutes. The DBPDA chromophore is then added and one minute later an oxidant, potassium ferricyanide, is added. Ten minutes later, the end-points are read at Em_{710 nm}/ Ex 660 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the assay on the A/C Diagnostics Reader was evaluated in a study based on CLSI EP5-T2 guidelines “Evaluation of Precision Performance of Clinical Chemistry Devices.” In this study, precision was determined after analyzing three plasma samples containing low, medium and high tHCY in parallel assays. The within-assay precision was determined from results from 10 parallel analytical set-ups. The between-assay precision was calculated in 10 successive assays carried out over 10 days. The CVs, shown in the table below, for within-assay and between-assay were less than 5% and 10%, respectively.

Within-Assay Precision

Assay	Sample 1	Sample 2	Sample 3
Average tHCY (μmol/L)	8.1	14.9	35.2
SD	0.31	0.71	1.64
CV (%)	3.8	4.8	4.7

Between-Assay Precision

Assay	Sample 1	Sample 2	Sample 3
Average tHCY (μmol/L)	7.4	15.1	35.3
SD	0.5	0.9	1.8
CV (%)	7.4	5.9	5.0

b. Linearity/assay reportable range:

Five plasma samples containing different levels of homocysteine (3.2-44.6 μmol/L) were measured by the A/C Portable Enzymatic HCY Assay on the A/C Diagnostics Reader. The data demonstrated linearity of the assay up to 43.8 μmol/L with the regression equation of $y = 0.9536x + 0.5963$, $R^2 = 0.9969$. The studies support the stated measuring range of the assay of 4.1 to 43.8 μmol/L.

A recovery study was performed in which one sample with tHCY a concentration of 45.0 μmol/L was serially diluted 2, 4 and 8 times. The samples were analyzed by the Portable A/C Enzymatic HCY Assay in 10 replicates in a single run. The average % recoveries ranged from 91 to 104 %.

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

Calibrators and Controls were prepared by spiking homocysteine into a plasma matrix. The plasma matrix was manufactured from material such that each donor unit was tested by an FDA approved method for the presence of antibody to HIV-1/2, antibody to HCV, as well as for the Hepatitis B Surface Antigen (HbsAg), and found to be negative. An HPLC method was used for the value determination of Calibrators and Controls for the A/C Enzymatic Homocysteine Assay. Calibrators and controls are stated to be stable for 24 hours at 25 °C, 2 months at 4 °C and 2 years at -20 °C. The stability was determined by testing the vials stored at different temperatures for certain periods compared with the standard in the Portable A/C Enzymatic HCY Assay. Calibrators and controls are supplied with the kit.

c. Detection limit:

The limit of quantitation (LoQ) is used as the low detection limit of the assay. The limit of quantitation was defined as the lowest concentration having a CV <20%.

The results demonstrate that the LoQ of the A/C Portable Enzymatic HCY Assay on the A/C Diagnostics Reader is 3.7 $\mu\text{mol/L}$. The sponsor has established the measuring range of the assay as from 4.1 to 43.8 $\mu\text{mol/L}$ (see Section b. Linearity).

d. Analytical specificity:

To study the effect of bilirubin, plasma samples with tHCY levels of 5.7 and 14.8 $\mu\text{mol/L}$ were spiked with bilirubin to achieve bilirubin levels of 12.5, 25 and 50 mg/dL. The samples were analyzed in quadruplicate by the assay. The results demonstrate that bilirubin concentrations of up to 50 mg/dL show < 10% interference and therefore, do not interfere with the measurement of tHCY by the assay. Interference from glycerol was studied using plasma samples with tHCY levels of 6.8 and 11.6 $\mu\text{mol/L}$ which were spiked with glycerol to achieve levels of 1,250, 2,500 and 5,000 mg/dL. The results demonstrated that glycerol concentrations of up to 5000 mg/dL show < 10% interference and therefore, do not interfere with the measurement of tHCY by the assay. In addition, plasma samples with tHCY levels of 6.8 and 11.6 $\mu\text{mol/L}$ spiked with a commercially available lipid mixture containing 450 mg/dL showed < 10% interference. To study the effect of hemoglobin plasma samples with 5.5 and 14.6 $\mu\text{mol/L}$ tHCY were spiked with hemoglobin to achieve hemoglobin levels of 0.25 g/L, 0.5 g/L and 1.0 g/L. The results demonstrate that hemoglobin concentrations of up to 1.0 mg/mL (1.0 g/L) show < 10% interference and therefore, do not interfere with the measurement of tHCY by the assay.

Cross reactivity was tested and is < 10% for cystathione (up to 500 $\mu\text{mol/L}$), L-cysteine (up to 200 $\mu\text{mol/L}$), methionine (up to 0.2 mol/L) and glutathione (up to 0.5 $\mu\text{mol/L}$).

Other Limitations

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

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Fifty EDTA plasma samples with values ranging from 5.0 to 37.6 $\mu\text{mol/L}$ were measured both by the A/C Portable Enzymatic HCY Assay on the A/C Diagnostics

Reader and the A/C Automatic Enzymatic HCY Assay on the Hitachi 912 Automatic Analyzer (predicate device). The comparison yielded a regression equation of $y = 1.0098x + 0.9124$, $r^2 = 0.946$

b. Matrix comparison:

Samples collected from 10 donors were immediately separated into three different vacutainers, one without additive, one containing K_2 –EDTA and one containing sodium heparin, respectively. Plasma and serum were separated from whole blood within one hour by centrifugation. High HCY samples were prepared by spiking L-homocysteine into 6 samples to prepare a total of 16 samples. Total HCY values of the samples were measured by the A/C Portable Enzymatic HCY Assay on the A/C Diagnostics Reader. The results are shown in the table below.

Sample type (x vs. y)	n	Sample range ($\mu\text{mol/L}$)	Regression equation	R^2 value
EDTA Plasma vs. Heparin plasma	16	5.6 – 34.8	$y = 0.990x + 0.11$	0.996
Serum vs. EDTA plasma	16	5.4 – 36.1	$y = 0.970x + 0.33$	0.995
Serum vs. Heparin plasma	16	6.2 – 35.0	$y = 0.964x + 0.4$	0.997

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):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Reference ranges are based on literature references provided in the labeling and referenced below. Total HCY values in plasma/serum from 5 to 15 $\mu\text{mol/L}$ are considered as normal. However, total HCY values in plasma/serum differ with age,

gender, stress and other physical conditions. Total HCY values in plasma/serum > 15 $\mu\text{mol/L}$ are considered abnormal.

1. Ueland PM, Refsum H, Stabler SP, Malinow R, Anderson A, Allen RH. Total homocysteine in plasma or serum: Methods and clinical applications. Clin. Chem. 39, 1764-1779, 1993.
2. Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ. The effect of blood sample aging and food consumption on plasma total homocysteine levels. Clinica Chimica Acta 207, 119-128, 1992.
3. Moller J, Rasmussen K. Homocysteine in plasma: Stabilization of blood samples with fluoride. Clin. Chem. 41, 758-759, 1995.

N. Instrument Name:

A/C Diagnostics Reader

O. System Descriptions:

1. Modes of Operation:

The modes of operation are Setup, which includes setting up the number and value of the standards; Testing, which includes calculation of the standard curve and testing samples; and Test Reports, which includes looking up and/or printing the results.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ☒X_ or No _____

The software documentation for this device follows our software guidance for a moderate level of concern.

3. Specimen Identification:

Sample tubes (Thermowell TM tubes) are labeled with the ID number of the sample. During the test, the operator can choose the number of the sample using the keys on the instrument panel. After testing the sample, the instrument screen displays the result. Data can be sent out to a computer using the RS-232 serial port.

4. Specimen Sampling and Handling:

Manual assay – samples are put one by one into the test well and the lid closed to start testing.

5. Calibration:

Calibrators are supplied with the kit. Calibrator values are entered into the instrument in Setup mode. After the calibrators are tested, the instrument calculates the calibration curve. The labeling recommends to run a calibration curve each day.

6. Quality Control:

Controls are supplied with the kit. The labeling recommends to run a set of controls with patient samples in every batch.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.