A. 510(k) Number:

k113793

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative, coupled enzymatic assay; spectrophotometric assay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Homocysteine Enzymatic Assay; Homocysteine Calibrator Kit; Homocysteine Control Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.1377; urinary homocystine (nonquantitative) test system.
   21 CFR 862.1150; calibrator, multi-analyte mixture
   21 CFR 862.1660; single (specified) analyte controls (assayed and unassayed)

2. Classification:

   Class II, Class I reserved

3. Product code:

   LPS, JIX, JJX
4. **Panel:**

   Clinical Chemistry (75)

**H. Intended Use:**

1. **Intended use(s):**

   See Indications for Use below

2. **Indication(s) for use:**

   The Homocysteine Enzymatic Assay is an in vitro test for the quantitative determination of total L-homocysteine in human serum and plasma on Roche/Hitachi cobas c systems. The assay can assist in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.

   The Homocysteine Calibrator Kit is intended for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

   The Homocysteine Control Kit is intended for use in quality control by monitoring accuracy and precision for the quantitative methods as specified in the value sheets.

3. **Special conditions for use statement(s):**

   For prescription use only.

   The labeling contains a prominent black-box warning:

   Specimens from patients who are on drug therapy involving S-adenosyl-L-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:**

   Performance studies were conducted on Roche/Hitachi cobas c501 system

**I. Device Description:**

The Homocysteine Enzymatic Assay is based on an enzyme cycling assay principle that assesses the co-substrate conversion product, NAD, which is measured spectrophotometrically at 340 nm. The reagent contains three bottles
with the following ingredients:
R1 NADH reagent: S-adenosylmethionine, TCEP, 2-oxoglutarate, NADH
R2 Enzyme reagent: homocysteine S-methyltransferase, glutamate dehydrogenase, casein (bovine)
R3 Start reagent: adenosine deaminase (bovine), S-adenosyl-homocysteine hydrolase, casein (bovine).

The Homocysteine Calibrator Kit is a liquid, ready-for-use calibrator based on human serum. It is a single level calibrator with lot specific values and diluted on board the analyzer to create a 5-point calibration curve.

The Homocysteine Control Kit consists of two ready-for-use controls based on human serum. The adjusted concentrations of the control components are in the low range for Control 1 and in the elevated range for Control 2.

The labeling states that all products prepared from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HbsAg and antibodies to HCV and HIV. The testing methods were FDA approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Homocysteine Enzymatic Reagent: Diazyme Homocysteine Enzymatic Assay cleared in k061296
   Homocysteine Calibrator: Diazyme Homocysteine Calibrator cleared in k071971
   Homocysteine Controls: Diazyme Homocysteine Controls cleared in k042448

2. Predicate 510(k) number(s):
   k061296, k071971 and k042448

3. Comparison with predicate:
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Predicate k061296 Diazyme Homocysteine Enzymatic Assay</th>
<th>Proposed k113793 Roche Homocysteine Enzymatic Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended Use /Indications for Use</td>
<td>In vitro test for the quantitative determination of L-homocysteine in human serum and plasma. The assay can assist in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.</td>
<td>Same</td>
</tr>
<tr>
<td>Sample Types</td>
<td>Serum, Lithium Heparin, and EDTA</td>
<td>Same</td>
</tr>
<tr>
<td>Instrument Platform</td>
<td>COBAS INTEGRA 400</td>
<td>Cobas c 501</td>
</tr>
<tr>
<td>Reagent Active Ingredients</td>
<td>R1: S-adenosylmethionine, TCEP, 2-oxoglutarate, NADH&lt;br&gt;R2: homocysteine S-methyltransferase, glutamate dehydrogenase, casein (bovine)&lt;br&gt;R3: adenosine deaminase (bovine), S-adenosyl-homocysteine hydrolase, casein (bovine)</td>
<td>Same</td>
</tr>
<tr>
<td>Reagent Stability</td>
<td>Unopened: 2-8 °C until expiration date&lt;br&gt;On-board in use: 60 days</td>
<td>Unopened: 2-8 °C until expiration date&lt;br&gt;On-board in use: 4 weeks</td>
</tr>
<tr>
<td>Measuring Range</td>
<td>2.8 – 50 μmol/L</td>
<td>3 – 50 μmol/L</td>
</tr>
<tr>
<td>Expected Values</td>
<td>15 μmol/L is used as the cut-off value for normal levels of homocysteine in adults.</td>
<td>Same</td>
</tr>
<tr>
<td>Calibrator</td>
<td>Homocysteine Calibrator, single level, diluted to form a 5-point calibration</td>
<td>same</td>
</tr>
<tr>
<td>Calibration</td>
<td>Each lot + interval (168 hours)</td>
<td>Every 7 days,</td>
</tr>
</tbody>
</table>
Frequency: after reagent lot change, and as required following quality control procedures.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Predicate</th>
<th>Proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intended Use</strong></td>
<td>Diazyme Homocysteine Calibrator</td>
<td>Roche Homocysteine</td>
</tr>
<tr>
<td>Analyte</td>
<td>Homocysteine</td>
<td>Same</td>
</tr>
<tr>
<td>Matrix</td>
<td>Human serum</td>
<td>Same</td>
</tr>
<tr>
<td>Storage</td>
<td>2-8 °C</td>
<td>Same</td>
</tr>
</tbody>
</table>

**Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Predicate</th>
<th>Proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intended Use</strong></td>
<td>Diazyme Homocysteine Enzymatic Assay</td>
<td>Roche Homocysteine Enzymatic Assay</td>
</tr>
<tr>
<td>Analyte</td>
<td>Homocysteine</td>
<td>Same</td>
</tr>
<tr>
<td>Matrix</td>
<td>2 – level set with a normal serum homocysteine level and an abnormal homocysteine level</td>
<td>Same</td>
</tr>
<tr>
<td>Storage</td>
<td>2-8 °C</td>
<td>Same</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI EP06-A</td>
<td>Evaluation of the Linearity of Quantitative Measurement</td>
</tr>
<tr>
<td>CLSI EP17-A</td>
<td>Protocols for Determination of Limits of Detection and Limits of Quantization</td>
</tr>
<tr>
<td>CLSI EP06-A</td>
<td>Evaluation of the Linearity of Quantitative Measurement</td>
</tr>
</tbody>
</table>

**L. Test Principle:**

Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or...
Hcy conversion products of Hcy. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalyzed by a Hcy S-methyltransferase. SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. The formed Ado is immediately hydrolyzed into inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD+. The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD+ (ΔA340nm).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:
      Within run and between-run precision studies were performed following the CLSI guideline EP5-A2 for 21 days, two runs per day on the same cobas c 501 analyzer. Two aliquots per each human sera pools (HSP), (2 diluted, 1 native, and 2 spiked) samples and controls together with one reagent lot, one lot of calibrator were used in the study. The results are shown in the table below:

      | Mean Value | Within –Run CV | Between –Run CV |
      |------------|---------------|-----------------|
      | Control 1  | 12.2 µmol/L   | 1.5%            | 2.1%            |
      | Control 2  | 39.1 µmol/L   | 1.8%            | 2.0%            |
      | HSP 1      | 8.26 µmol/L   | 2.0%            | 2.3%            |
      | HSP 2      | 13.1 µmol/L   | 1.8%            | 2.1%            |
      | HSP 3      | 30.0 µmol/L   | 1.4%            | 1.8%            |
      | HSP 4      | 44.4 µmol/L   | 2.0%            | 2.2%            |

   b. Linearity/assay reportable range:
      A linearity study was performed in-house using blood samples as per CLSI EP6-A recommendations for evaluation of linearity. A dilution series consisting of 11 dilutions was prepared separately using a spiked, high analyte human serum pool and a high analyte, human plasma pool in parallel to produce samples with values ranging from 2.78 to 50.03 µmol/L. Homocysteine levels were measured in triplicate for each sample using cobas c 501 analyzer, and the recovered median values were compared to the theoretical values. The linear regression analysis of the study is shown below:
The results fulfilled the specifications for the linearity claims of 3 to 50 \( \mu \text{mol/L} \).

The extended measuring range using automated rerun with dilution was validated by performing an experiment comparing the instrument auto-rerun results with a simple manual dilution. The test results showed the difference between the automated dilution and the manual dilution is within the acceptance criteria of +/- 10% recovery.

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

**Traceability:**
This method has been standardized against NIST SRM 1955 reference material. The target values for calibrator and controls are traceable to the NIST SRM 1955 reference material.

**Stability**

i) **Reagent stability**

- Shelf (real-time) Stability: 14 months
- On-Board stability: 4 weeks after dilution

ii) **Calibrator stability**

- Closed-Vial Stability: 15 months at 2-8 \(^0\)C
- Open-Vial Stability: 2 hours at 15-25 \(^0\)C or 28 days at 2-8 \(^0\)C

iii) **Controls stability**

- Open-Vial Stability: 2 hours at 15-25 \(^0\)C or 28 days at 2-8 \(^0\)C
- Close-Vial Stability: 15 months at 2-8 \(^0\)C

d. **Detection limit:**
The study was performed in-house as per CLSI EP17-A recommendations for evaluation limits of detection and quantification.

LoB was determined by measuring an analyte-free sample with 3 reagent lots,

<table>
<thead>
<tr>
<th></th>
<th>Slope</th>
<th>Intercept</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1.0054</td>
<td>-0.2478</td>
<td>0.9995</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.0214</td>
<td>-0.7411</td>
<td>0.9995</td>
</tr>
</tbody>
</table>
5 determinations each on 2 cobas c 501 analyzers, total 6 runs (n=60) over 3 days. Data analysis was based on determination of the 95th percentile of the 60 measured values. LoD was calculated based on LoD=LoB + 1.653 X SD_{total}.

The results are LoB: 0.3 µM; LoD: 0.72 µM. The sponsor’s claimed measuring range is 3 µmol/L to 50 µmol/L.

e. Analytical specificity:
Interference testing based on CLSI “Interference Testing in Clinical Chemistry; Approved Guideline”, CLSI document EP7-A2. Significant interference was considered present by the sponsor if the % recovery exceeded ±10% of the expected 100% recovery.

i) Exogenous interferences:
Individual drug was added to two separate patient sample pools with low or high homocysteine concentrations and examined for potential effect on homocysteine determination by the Homocysteine Enzymatic test system. No significant interference was found for the following drugs:
Acetylcysteine, Ampicillin-Na, Ascorbic acid, Ca-Dobesilate, Cyclosporine, Cefoxitin, Heparin, Intralipid, Levodopa, Methyldopa, Metronidazole, Phenylbutazone, Doxycycline, Acetylsalicylic Acid, Rifampicin, Acetaminophen, Ibuprofen, Theophylline, Cystathionine, Pyruvate, Glutathione, S-adenosylmethionine, H-hydroxylamine HCl and glycerol.

Physiological concentration (Sub-uM range) of S-adenosylhomocysteine (SAH) have no significant interference on this assay, however, SAH has a significant positive interference at high pharmacological doses.

Samples containing 3-deazaadenosine should not be used since it is known to inhibit one of the key enzymes used in the assay.

ii) The effects of endogenous interference on the quantitation of homocysteine by the Homocysteine Enzymatic test system were determined on the cobas c 501 analyzer at two Homocysteine levels. The study showed:
Icterus: No significant interference up to 20 mg/dL unconjugated Bilirubin.
Hemolysis: No significant interference up to 100 mg/dl hemoglobin
Triglyceride: No significant interference up to 1790 mg/dL triglyceride

iii) Protein interference was investigated using two human serum pools at homocysteine concentration of 12.6 and 41.3 µmol/L, respectively. Each pool was used to create a dilution series of 10 test levels of protein spanning between 13 to 128.9 g/L. The results showed no interference by total protein up to 128.9 g/L.
2. Comparison studies:

a. Method comparison with predicate device:
Method comparison studies were performed using the proposed device on Cobas c 501 and with the predicate device on Cobas Integra 400 analyzer. A total of 102 native human serum samples, with concentrations between 4.76 and 46.59 µmol/L, were used in this study; the linear regression analysis of the results is shown below:

<table>
<thead>
<tr>
<th>Passing/Bablok</th>
<th>Least Square Linear regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y = 0.9986x - 0.6964$</td>
<td>$y = 0.9973x - 0.5713$</td>
</tr>
<tr>
<td>$\tau = 0.9594$</td>
<td>$r = 0.9964$</td>
</tr>
</tbody>
</table>

b. Matrix comparison:
Matrix comparison studies were performed on the Cobas c501 using paired serum and K2-EDTA, K3-EDTA and Lithium Heparin samples with values ranging from 4.63 to 48.7 µmol/L. The results are presented in the table below.

<table>
<thead>
<tr>
<th>Types of collection Tubes</th>
<th>Sample No.</th>
<th>Passing/Bablok</th>
<th>Least Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>K2- EDTA</td>
<td>37</td>
<td>$y =-0.3570 + 1.0605x$ ($\tau = 0.9285$)</td>
<td>$y = 0.1322 + 1.0234x$, $(r = 0.9975)$</td>
</tr>
<tr>
<td>K3- EDTA</td>
<td>37</td>
<td>$y =-0.1661 + 1.0399x$ ($\tau = 0.9406$)</td>
<td>$y = 0.0428 + 1.0249x$, $(r = 0.9987)$</td>
</tr>
<tr>
<td>Li-Heparin</td>
<td>37</td>
<td>$y =0.3471 + 1.0178x$ ($\tau = 0.9577$)</td>
<td>$y = 0.1828 + 1.0031x$, $(r = 0.9981)$</td>
</tr>
</tbody>
</table>

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:**

   Adult cut-off values for normal level of Hcy: 15 µmol/L

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.