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

A. 510(k) Number:
k052788

B. Purpose for Submission:
New device

C. Measurand:
Homocysteine

D. Type of Test:
Quantitative, competitive immunoassay using particle-enhanced nephelometry

E. Applicant:
Dade Behring Inc.

F. Proprietary and Established Names:
N Latex HCY,
N Protein Standard SL
N/T Protein Control SL/L, M, and H

G. Regulatory Information:

1. Regulation section:
    21 CFR §862.1377, Urinary homocystine (non-quantitative test system
    21 CFR §862.1150, Calibrator
    21 CFR §862.1660, Quality control material (assayed and unassayed)

2. Classification:
    Class II for assay reagents and calibrator material
    Class I for control material

3. Product code:
    LPS, JIX, JJY

4. Panel:
    Chemistry (75)

H. Intended Use:
1. Intended use(s):
    See item 2 below.

2. Indication(s) for use:
   N Latex HCY:
   In Vitro diagnostic reagents for the quantitative determination of total homocysteine (HCY) in human serum, heparinized plasma, and EDTA plasma by means of particle-enhanced immunonephelometry on the BN™ II and BN ProSpec® Systems. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocysteinuria.

   N Protein Standard SL:
   Establishment of reference curves for the determination of IgG $_{1-4}$, IgA, IgM, C3c, C4, transferring, albumin, alpha1-antitrypsin, alpha2-macroglobulin, haptoglobin, alpha1-acid glycoprotein, prealbumin, hemopexin, ceruloplasmin, RbP, Ig/L-chain lambda & kappa, soluble transferring receptor, ferritin, β2-microglobulin, total protein and homocysteine by immunonephelometry with BN™ Systems.

   N/T Protein Control SL/L, M, and H:
   N/T Protein Controls SL/L, M, and H are for use as accuracy and precision assayed controls in the determination of the following human serum proteins by immunonephelometry with BN™ Systems: IgG $_{1-4}$, IgA, IgM, C3c, C4, transferring, albumin, alpha1-antitrypsin, alpha2-macroglobulin, haptoglobin, alpha1-acid glycoprotein, prealbumin, hemopexin, ceruloplasmin, RbP, Ig/L-chain lambda & kappa, soluble transferring receptor, ferritin, β2-microglobulin, total protein and homocysteine

3. Special conditions for use statement(s):
   Prescription Use

   The labeling includes the following precaution: "Certain drugs such as antiepileptic, antifolates, nitrous oxide anesthesia and antagonists of vitamin B6 are known to raise the homocysteine concentration in human blood. Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine."

4. Special instrument requirements:
   Dade Behring BN™ II and BN ProSpec® Systems

I. **Device Description:**
   The reagent kit contains four different components. The N HCY Reagent consists of a suspension of polystyrene particles coated with mouse monoclonal anti-SAH antibody (S-adenosyl-homcysteine) and is supplied in three vials. Also supplied are three vials each of N HCY RA, N HCY SR A, and N HCY SR B. N HCY RA consists of a buffered, stabilized solution of the reduction reagent dithiothreitol. N HCY SR A consists of a buffered, stabilized solution of the enzyme S-adenosyl-L-homocysteine hydrolase (recombinant). N HCY SR B consists of a buffered, stabilized solution of a conjugate of S-adenosyl-cysteine with porcine thyroglobulin (PTG-SAC).

The N Protein Standard SL is a liquid, stabilized human serum that is ready to use.

The N/T Protein Controls SL (Low, Medium and High) are liquid, stabilized human sera and are ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Abbott IMx Homocysteine

2. Predicate 510(k) number(s):
   k992858

3. Comparison with predicate:

Similarities		
Item	N Latex HCY	Abbott IMx Homocysteine
Intended Use	Immunoassay for the quantitative determination of total homocysteine in human serum, heparinized plasma, and EDTA plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocysteinuria	Immunoassay for the quantitative measurement 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 homocysteinuria
Sample type	Serum, heparinized plasma, EDTA plasma	Serum, heparinized plasma (Li), EDTA plasma
Antibody	Mouse monoclonal	Mouse monoclonal

Differences		
Item	Device	Predicate
Technology	Particle enhanced immuno-nephelometry	Fluorescence Polarization immunoassay
Assay Range	2.0-64 μmol/mL Up to 256 μmol/mL (extended range with 1:20 dilution)	0.8-50 μmol/mL Up to 500 μmol/mL (extended range with 1:10 dilution)

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

Evaluation of Precision Performance of Quantitative Measurement Methods;
Approved Guideline-Second Addition (CLSI, formerly NCCLS, EP5-A2)

L. Test Principle:
Bound homocysteine in the sample is reduced to free homocysteine by the action of
dithiothreitol, and converted enzymatically to S-adenosyl-homocysteine (SAH) in the
next step. Conjugated S-adenosyl-cysteine (SAC) added at the onset of the reaction
competes with the SAH in the sample for bonding by anti-SAH antibodies bound to
polystyrene particles. In the presence of SAH there is either no aggregation or a
weaker aggregation of the polystyrene particles. In the absence of SAH in the sample
an aggregation of the polystyrene particles by conjugated SAC occurs. The higher
the SAH content of the reaction mixture, the smaller the scattered light signal. The
result is evaluated by comparison with a standard of known concentration.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*
      Within run imprecision ranged from 2.7-4.6% CV and total imprecision
      ranged from 4.6-8.5% CV.

      Precision studies were based on a modification of CLSI EP5-A2. N/T Protein
      Control SL-L/M/H and two plasma pools (~12 µmol/L and ~32 µmol/L) were
      included in the study. Four determinations (n=4) of each control or plasma
      pool were run over 10 days using the BN System (n=40 total).

   b. *Linearity/assay reportable range:*
      Samples whose concentration is greater than upper limit of the measuring
      range of the device (~64 µmol/L) may be diluted automatically using a 1:20
      dilution of the sample.

      Linearity was evaluated by diluting a serum sample spiked with homocysteine
      (~58 µmol/L) with Dade Behring N Diluent in 10% dilution steps. Each
      dilution was tested in replicates of five. The percent recovery was determined
      by comparing the mean measured concentration to the theoretical
      concentration. Additionally, the linear regression of theoretical vs. measured
      concentration was calculated. The sponsor's acceptance criteria were percent
      recovery between 80 and 120%, a slope between 0.9 and 1.1, and a correlation
      coefficient (r) ≥ 0.95. The results for the sample evaluated in the study were
      90.5 to 103.2% percent recovery, slope = 1.024, and r = 0.995.

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
      Traceability and Value Assignment: The Standard and Controls are

calibrated against an internal master calibrator, which is calibrated against Dade Behring and commercially available reference preparations. Specifically, homocysteine is calibrated against a commercially available preparation of purified S-adenosylhomocysteine.

The value assignment process for the Standard and Control is as follows. Four independent runs are performed using two different BN systems. Three vials of each new lot of Standard and Control are selected and assayed as samples, using n=4 replicates. For each of the four runs, reference standard curves are established using the internal master calibrator. The value assigned to the Standard or Control is the mean of the 144 results (4 runs x 3 reference curves x 3 vials x 4 replicates).

Stability: To support a 24 month shelf-life for unopened N Protein standard SL and N/T Protein Control stored at 2-8°C, testing is performed at Day 0 and Month 26 in duplicate. The sponsor's acceptance criterion for standard or control tested at 26 months is recovery within ±15% of assigned values.

To support an open-vial stability of 14 days at 2-8°C, testing is performed at Day 0, 14, and 15 in duplicate. The sponsor's acceptance criterion for standard or control is recovery within ±15% of assigned values.

d. *Detection limit:*
   Analytical sensitivity is determined in the following manner. Twenty replicates (n=20) of N Diluent and N Protein Standard SL at a 1:160 dilution (the lowest point on the reference curve) are analyzed on the BN II system. The mean, standard deviation, and percent CV of the 20 replicates are calculated. Finally, the sensitivity is calculated using the following equation:

   2SD Signal (N Diluent) x HCY conc. Standard (1:160)
   Signal Mean (N Diluent) – Signal Mean Standard (1:160)

   Since the analytical sensitivity of the N Latex HCY assay is based on the concentration of homocysteine in the N Protein Standard, it is stated in the labeling that the assay sensitivity is typically 2 μmol/L.

e. *Analytical specificity:*
   The cross-reactivity of seven structurally related compounds S-Adenosyl-L-Methionine (up to 0.5 mM), L-Cysteine (up to 5 mM), L-Cystathionine (up to 2 mM), L-Methionine (up to 0.4 mM), Homocysteine Thiolactone (up to 0.1 mM), Adenosine (up to 1 mM), and Glutathione (up to 50 mM) were evaluated using the N Latex HCY assay. Plasma samples with normal levels of homocysteine were spiked with each compound and these were compared to control sample spiked with an equivalent volume of saline. When control samples were compared to test samples, cross-reactivity was found to be <10% for GSH, <1% for L-Cysteine, and <5% for all other compounds tested.

Potential interference from endogenous compounds such as bilirubin, hemoglobin, lipid, and protein were evaluated. No interference (sample recovers within ±20% of the reference) was seen with compounds at the following levels: bilirubin up to 0.6 mg/mL, hemoglobin up to 10 mg/mL, protein up to 8.8 g/dL, triglycerides up to 4.21 mM. Elevated levels of rheumatoid factors also do not interfere with the assay.

    f. Assay cut-off:
    Not applicable

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

    a. Method comparison with predicate device:
    A method comparison study was performed comparing test results for EDTA plasma samples (n=87) evaluated on both the N Latex HCY assay and the Abbott IMx Homocysteine assay. Samples ranged in value from approximately 4.8-60 µmol/L. Regression analysis of the results yielded the following equation: y= 0.97x + 0.01 µmol/L (r = 0.99).

    b. Matrix comparison:
    In a study comparing homocysteine results between matched EDTA and heparin plasma samples (n=10), there was on average a -8.2% difference in HCY concentration. In a separate study comparing homocysteine results between matched EDTA and serum samples (n=10), serum samples gave on average 10% higher values than the corresponding EDTA plasma samples. The product labeling states that in patient monitoring results from different sample types (EDTA and serum) should not be interchanged.

3. <u>Clinical studies</u>:

    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. <u>Clinical cut-off</u>**:**
    Not applicable

5. <u>Expected values/Reference range:</u>
    The sponsor cites a scientific study where the reference interval for adult males and females was found to be between 5 and 15 µmol/L. The package insert states that homocysteine concentrations in plasma and serum of healthy individuals can

vary with age, gender, geographical area, nutritional status, and genetic factors. The sponsor states in the labeling that each facility should determine its own reference interval since values may vary depending on the population and specimen type studied.

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 and substantial equivalence decision.