A. 510(k) Number:

k103557

B. Purpose for Submission:

New device

C. Measurand:

High sensitivity C-reactive protein

D. Type of Test:

Quantitative Immunoturbidimetric assay

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme high sensitivity C-reactive protein (hsCRP) assay kit
Diazyme high sensitivity C-reactive protein (hsCRP) assay calibrator set
Diazyme high sensitivity C-reactive protein (hsCRP) assay control set

G. Regulatory Information:

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Classification</th>
<th>Regulation Section</th>
<th>Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein, Antigen, Antiserum and Control (DCK)</td>
<td>Class II</td>
<td>21 CFR 866.5270 C-reactive protein immunological test system</td>
<td>Immunology (82)</td>
</tr>
<tr>
<td>Calibrator (JIT)</td>
<td>Class II</td>
<td>21 CFR 862.1150 Calibrator</td>
<td>Clinical Chemistry (75)</td>
</tr>
<tr>
<td>Controls (JJX)</td>
<td>Class I, reserved</td>
<td>21 CFR 862.1660 Quality control material</td>
<td>Clinical Chemistry (75)</td>
</tr>
</tbody>
</table>
H. Intended Use:

1. Intended use(s):

   See indication(s) for use below.

2. Indication(s) for use:

   The Diazyme high sensitivity C-reactive protein (hsCRP) assay is for the in vitro quantitative determination of C-reactive protein (CRP) in human serum and plasma on automated clinical chemistry analyzers. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. For in vitro diagnostic use only.

   The Diazyme hsCRP assay calibrator set is intended for use of the calibration of the C-reactive protein (CRP) method. For in vitro diagnostic use only.

   The Diazyme hsCRP assay control kit is intended for use as quality controls for the Diazyme hsCRP Assay kit only. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use

4. Special instrument requirements:

   Performance characteristics were provided for the Hitachi 917 analyzer.

I. Device Description:

The device is supplied as a ready-to-use two reagent kit. Calibrators and controls are sold separately. Reagent 1 contains 100 mM Tris-buffer with 0.09% sodium azide. Reagent 2 contains a suspension of goat anti-human CRP-coated latex particles with 0.09% sodium azide.

Ready-to-use liquid calibrators at 4 levels (4 x 1 mL) plus a zero level are prepared from pooled human serum spiked with purified human C reactive protein to targeted concentrations with 0.09% sodium azide added as a preservative. Each serum donor unit used in the preparation of this product has been tested using FDA approved methods and found to be non-reactive for HBsAg, HIV and HCV.

Ready-to-use liquid controls at 3 levels (3 x 3 mL) are prepared from pooled human serum spiked with purified human C reactive protein to targeted concentrations with 0.09% sodium azide added as a preservative. Each serum donor unit used in the preparation of this product has been tested using FDA approved methods and found to be non-reactive for HBsAg, HIV and HCV.
Calibrator and control materials are provided separately from the Reagent Kit.

J. **Substantial Equivalence Information:**

1. **Predicate device name(s):**

   Roche Tina-Quant CRP  
   Roche Calibrator for Automated Systems  
   Roche CRP T Control  

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

   k042485  
   k011226  
   k982087  

3. **Comparison with predicate:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Device</th>
<th>Predicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended use – CRP assay</td>
<td>Same</td>
<td>For the in vitro quantitative determination of C-reactive protein (CRP) in human serum and plasma on automated clinical chemistry analyzers.</td>
</tr>
<tr>
<td>Principle – CRP assay</td>
<td>Same</td>
<td>Immunoturbidimetric assay</td>
</tr>
<tr>
<td>Sample type – CRP assay</td>
<td>Same</td>
<td>Serum or plasma</td>
</tr>
<tr>
<td>Intended use – CRP calibrator material</td>
<td>Same</td>
<td>is intended for use in the calibration of the C-reactive protein (CRP) assay</td>
</tr>
<tr>
<td>Intended use – CRP control material</td>
<td>Same</td>
<td>is intended for use as quality control for the C-reactive protein (CRP) assay</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Device</th>
<th>Predicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measuring range – CRP assay</td>
<td>0.2 to 20 mg/L</td>
<td>0.1 to 20 mg/L</td>
</tr>
<tr>
<td>CRP calibrator material</td>
<td>Calibrator set with 4 levels (4 x 1 mL) plus a zero level</td>
<td>1 x 1.0 mL Calibrator</td>
</tr>
<tr>
<td>CRP control material</td>
<td>3 levels (3 x 3 mL)</td>
<td>3 x 1.0 mL control</td>
</tr>
</tbody>
</table>
K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
- CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guideline
- CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- CLSI C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory

L. Test Principle:

The assay is based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP, which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change at 570 nm with the magnitude of the change being proportional to the amount of CRP in the sample. The actual concentration is then determined by the interpolation from a calibration curve prepared from calibrators of known concentration.

M. Performance Characteristics (if/when applicable):

Performance characteristics were established on the Hitachi 917 analyzer

1. Analytical performance:
   a. Precision/Reproducibility:

   The precision of the Diazyme hsCRP Assay was evaluated according to Clinical and Laboratory Standards Institute EP5-A guideline. In the study, three levels of serum based controls containing approximately 0.8, 1.7, and 8.6 mg/L of CRP, and two serum samples containing approximately 1.2 and 3.6 mg/L of CRP were tested with 2 runs per day in duplicates over 20 working days. An additional serum sample with a value of approximately 15.6 was tested over 5 days with 2 runs per day. Within-run and total precision are presented in the table below.
### Table

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean (mg/L)</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD % CV</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>80</td>
<td>0.85</td>
<td>0.03 4.0</td>
<td>0.04 4.2</td>
</tr>
<tr>
<td>Control 2</td>
<td>80</td>
<td>1.75</td>
<td>0.03 1.7</td>
<td>0.05 2.6</td>
</tr>
<tr>
<td>Control 3</td>
<td>80</td>
<td>8.62</td>
<td>0.06 0.7</td>
<td>0.12 1.4</td>
</tr>
<tr>
<td>Serum 1</td>
<td>80</td>
<td>1.18</td>
<td>0.04 3.2</td>
<td>0.09 7.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>80</td>
<td>3.62</td>
<td>0.05 1.4</td>
<td>0.09 2.4</td>
</tr>
<tr>
<td>Serum 3</td>
<td>20</td>
<td>15.56</td>
<td>0.19 1.2</td>
<td>0.24 1.6</td>
</tr>
</tbody>
</table>

#### b. Linearity/assay reportable range:

The claimed measuring range of the assay is 0.20 to 20.0 mg/L. Nine levels of samples were prepared by diluting a serum control sample with a high level of CRP with saline according to CLSI EP6-A to produce linearity samples with the following levels: 0.02, 0.4, 0.87, 1.92, 3.94, 8.50, 12.56, 16.56 and 20.31 mg/L. The linearity samples were tested in triplicate with the Diazyme hsCRP assay. A linearity analysis was performed using EP Evaluator software. Linear regression analysis results obtained were as follows: slope = 1.032, intercept = 0.0528, r² = 0.9994.

To test for High Dose “Hook Effect”, a serum control with 1000 mg/L of human CRP was diluted with CRP free serum (0 mg/L CRP) to 10 targeted concentrations. The samples were tested in triplicate. The Diazyme hsCRP assay showed no High Dose “Hook Effect” up to 160 mg/L of CRP.

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

The Diazyme hsCRP calibrator is traceable to the International Federation of Clinical Chemistry International Reference Preparation for Plasma Proteins lot CRM 472/IFCC certified by the Bureau of Reference of the European Community.

Diazyme CRP calibrators were prepared by spiking human CRP stock solution to pooled human sera to the target CRP concentrations. Value assignment of the CRP calibrators was established by transferring the target values from the ERM-DA472/IFCC CRP to the in-house calibrators.

Diazyme hsCRP reagents and ERM-DA472/IFCC CRP with proper dilutions as calibrators were used to test the Diazyme CRP calibrator in triplicate on the Hitachi 917 analyzer. The mean values were assigned as the values of Diazyme CRP calibrators. The approximate target values are 0, 1.0, 5.0, 10 and 20 mg/L.
CRP controls are prepared by spiking CRP stock solution to diluted pooled human serum to the target CRP concentrations. The Diazyme hsCRP reagents and calibrators were used in replicate analysis on the Hitachi 917 analyzer to determine the mean value of the newly prepared control materials. The target values are 0.5 to 1.0 mg/L for Level 1; 1.0 to 2.0 for Level 2; and 7.0 to 9.0 for Level 3 and are presented in the labeling.

Accelerated stability testing demonstrated that the calibrators and controls were stable for 3 months at 2-8°C with opening and closing vials. According to the Arrhenius law, the shelf-life of the closed Diazyme CRP calibrators and controls is at least 12 months at 2-8°C. Real time stability studies are ongoing.

d. Detection limit:

The LoB, LoD, and LoQ of Diazyme hsCRP Assay were determined according to CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. LoB tests were conducted using blank samples, 7.5% BSA, tested with 20 replicates daily for three days with one reagent lot and one Hitachi 917 instrument. LoB was calculated as the mean of the 57th and 58th highest values = 0.08 mg/L.

For the LoD studies, five low samples were tested with 4 replicates daily for three days with one reagent lot and one Hitachi 917 instrument. LOD = LOB + (1.645* SD of Low samples). The LoD was determined to be 0.13 mg/L.

To calculate the LoQ, five patient serum samples were diluted with 7.5% BSA to targeted concentrations of 0.1, 0.2, 0.3, 0.5, and 1.0 mg/L. The diluted serum samples were tested with the Diazyme hsCRP reagent on the Hitachi 917 with 5 runs with 4 replicates per run (20 replicates total per sample) using one reagent lot and one instrument. A curve was fit to estimate the relationship between mean and % CV. Based on the fitted model, the LoQ was determined to be 0.20 mg/L. The % CV at the level of 0.2 mg/L was determined to be 16.6 % with 95% confidence interval of 13.1% to 20.0%. At this point (0.2 mg/L), the upper 95% confidence interval has a % CV of 20 %.This is the lowest concentration for which % CV is less or equal to 20 % with 95% confidence interval.

e. Analytical specificity:

The sponsor evaluated the effect of known endogenous interferents on the Diazyme hsCRP assay following Clinical and Laboratory Standards Institute EP7-A guidelines, “Interference Testing in Clinical Chemistry.”
Two serum samples with two levels of CRP, 0.9 and 2.8 mg/L, were spiked with various concentrations of endogenous substances at five levels and the results compared to the samples without added interferent. The samples were tested using one lot of reagent and one instrument. The following substances showed no significant interference as defined by the sponsor as less than 10% deviation when tested at levels equal to the concentrations listed below:

<table>
<thead>
<tr>
<th>Interference</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>1000 mg/dL</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>176 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Bilirubin Conjugated</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>400 IU/mL</td>
</tr>
</tbody>
</table>

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method Comparison of the Diazyme hsCRP Assay versus the predicate assay was performed using CLSI EP9-A2 as a guideline. 56 unaltered individual serum samples and 1 altered serum sample were tested. The samples had values ranging from 0.2 to 18.9 mg/L. Passing-Bablok regression analysis of the Diazyme hsCRP Assay versus the predicate device, Roche Tina-Quant CRP HS Test System (k042485) produced an equation of $Y = 0.0196 + 1.0133 \times$ where $Y$ is the Diazyme method and $X$ is the predicate method. The 95% CI for slope and intercept are 0.9583 to 1.0321 and -0.1599 to -0.1017 respectively.

b. Matrix comparison:

To evaluate anticoagulant effects, EDTA plasma and Lithium Heparin plasma, the CRP levels of 46 unaltered sera / K3 EDTA / Li Heparin plasma sample sets were tested on Hitachi 917 with the Diazyme hsCRP reagents. The samples had values ranging from 0.2 to 19.42 mg/L. From the 47 serum-plasma sets tested, no significant matrix effect was observed between serum, K3 EDTA plasma, and Li Heparin Plasma. For serum vs. K3 EDTA, the linear regression equation was $y = 0.9143x + 0.0291$, $r^2 = 0.9966$. For serum vs. Lithium heparin plasma, the linear regression equation was $y = 0.9256x + 0.0548$, $r^2 = 0.997$. 
No significant difference (defined by the sponsor as less than 10% difference in hsCRP value between serum and plasma type) was seen between serum and plasma.

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:**
   
   The reference interval was determined using serum specimens from 103 apparently healthy adults from age 18 - 62 according to CLSI C28-A3. The serum samples were tested in duplicate. The results showed that < 5.0 mg/L was obtained in 95 % of the population tested.

**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.