EPO [Erythropoietin]  
ELISA [Enzyme-Linked ImmunoSorbent Assay]  
Catalog # 7025  
Specific quantitative assay for the determination of erythropoietin in serum  
Jan 2005

I. INTENDED USE

The Biomerica EPO ELISA is intended for the quantitative determination of Erythropoietin (EPO) in human serum. This assay is intended for in vitro diagnostic use, as an aid in the diagnosis of anemias and polycythemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment in persons with anemias.

II. SUMMARY AND EXPLANATION

Erythropoietin (EPO) is a heavily glycosylated protein with a molecular weight of about 30,000 - 34,000 Daltons. Human EPO is a polypeptide consisting of 165 amino acids, containing one O-linked and three N-linked carbohydrate chains. The recombinant EPO is a good substitute for the native protein for use in an immunoassay. Serum EPO levels are dependent on the rate of production and the rate of clearance of the protein. Ninety percent of EPO is produced in the peritubular cells of the adult kidney in response to a decrease in tissue oxygenation. There is evidence indicating that the protein on these cells which detects oxygen saturation of the blood is a heme-containing moiety. As the pO2 of the plasma, a function of the hematocrit decreases, EPO concentration will increase. There are also observations suggesting that normally there is an inverse correlation between serum EPO levels and red blood cell mass.

Quantitation of serum erythropoietin concentration serves as a diagnostic adjunct in determining the cause of anemia or erythrocytosis. Aplastic anemia, hemolytic anemia and anemia due to iron deficiency all result in serum EPO elevation. Whereas, EPO levels in patients with secondary anemia due to renal failure and anemia due to iron deficiency all result in serum EPO elevation. Quantitation of serum erythropoietin concentration serves as a diagnostic adjunct in determining the cause of anemia or erythrocytosis. Aplastic anemia, hemolytic anemia and anemia due to iron deficiency all result in serum EPO elevation. Whereas, EPO levels in patients with secondary anemia due to renal failure and anemia due to iron deficiency all result in serum EPO elevation.

III. PRINCIPLE OF THE TEST

The Biomerica EPO Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay] for the measurement of the biologically active 165 amino acid chain of EPO. It utilizes two different mouse monoclonal antibodies to human EPO specific for well-defined regions on the EPO molecule. One mouse monoclonal antibody to human EPO, is biotinylated and the other mouse monoclonal antibody to human EPO is labeled with horseradish peroxidase [HRP] for detection.

In this assay, calibrators, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidin-coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of EPO in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of EPO present in the controls and patient samples are determined directly from this curve. The Biomerica, Inc. standards have been calibrated against the World Health Organization (WHO) erythropoietin international standard that consists of recombinant DNA derived EPO. The WHO reference standard used was erythropoietin 1st international standard (87/684).

IV. KIT COMPONENTS

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGT 1 = Reagent 1</td>
<td>Biotinylated EPO Antibody [mouse monoclonal anti human EPO] containing ProClin 300 as preservative</td>
<td>1 x 3.5 mL</td>
</tr>
<tr>
<td>RGT 2 = Reagent 2</td>
<td>Peroxidase (Enzyme) labeled EPO Antibody [mouse monoclonal anti human EPO]</td>
<td>1 x 3.5 mL</td>
</tr>
<tr>
<td>RGT A = Reagent A</td>
<td>ELISA Wash Concentrate [saline with surfactant with the preservative ciprofloxacin hydrochloride]</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>RGT B = Reagent B</td>
<td>TMB Substrate [tetramethylbenzidine]</td>
<td>1 x 20 mL</td>
</tr>
<tr>
<td>SOLN = Stopping Solution</td>
<td>ELISA Stop Solution [1 N sulfuric acid]</td>
<td>1 x 20 mL</td>
</tr>
<tr>
<td>PLA = Microplate</td>
<td>One holder with Streptavidin Coated Strips</td>
<td>12 x 8-well strips</td>
</tr>
<tr>
<td>CAL = Calibrators</td>
<td>A: 0 mU/mL, B: Refer to vial labels, C: Refer to vial labels for exact concentrations, D: for exact</td>
<td>1 x 4 mL for the zero calibrator, 1 x 2 mL for all other calibrators</td>
</tr>
<tr>
<td>CTRL = Controls</td>
<td>1 &amp; 2: Refer to vial labels for exact ranges, 2: Levels, Synthetic h-EPO (1-165) in a buffered protein solution</td>
<td>1 x 2 mL per level</td>
</tr>
</tbody>
</table>

V. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450nm and 405nm.
- Microplate washer [if washer is unavailable, manual washing is acceptable].
- Precision Pipettors to deliver 25, 200, 100 and 150 µL.
- [Optional]: A multi-channel dispenser or a repeating dispenser for 25, 100 and 150 µL.
- Timer capable of ± 2 minute accuracy.
- Distilled or Deionized water.
- Orbital rotator or shaker.

VI. SAMPLE COLLECTION AND STORAGE

The determination of EPO should be performed on human serum. To assay the specimen in duplicate, 400 µL of human serum is required. It is highly recommended that the specimen be collected between 7:30 a.m. to 12:00 noon, because diurnal variation of erythropoietin has been reported in literature.

Collect whole blood without anticoagulant and allow blood to clot between 2-8°C, if possible. It has been reported that serum samples clotted at room temperature (22°C to 28°C) caused a decrease in EPO value as assessed by radiommunoassay of about 30% over clotting on ice. Then, the serum should be promptly separated, and stored at -20°C if possible. It has been reported that serum samples clotted at room temperature (22°C to 28°C) caused a decrease in EPO value as assessed by radiommunoassay of about 30% over clotting on ice.
preferably in a refrigerated centrifuge, and stored at -15°C or lower. Serum samples may be stored up to 24 hours at 2-8°C. Serum samples frozen at -15°C are stable for up to 12 months. Do not store samples in self-defrosting freezers. Avoid repeated freezing and thawing of samples. For long term storage of samples, it is recommended that samples should be aliquoted into sample vials or priors to freezing. Prior to use, allow all specimens to come to room temperature (22°C to 28°C) and mix by gentle inversion or swirling. Avoid grossly hemolyzed or grossly lipemic samples.

VII. REAGENT PREPARATION AND STORAGE

Store all kit components at 2-8 °C except the Wash Concentrate

1. All reagents except the calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8 °C except the Wash Concentrate, which should be kept at room temperature (22°C to 28°C) until dilution to avoid precipitation.

2. For Zero Calibrator (Calibrator A) reconstitute vial with 4 mL of distilled or deionized water and mix. For each of the non-zero calibrators (Calibrator B through F) and kit controls 1 and 2, reconstitute each vial with 2 mL of distilled or deionized water and mix. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution.

3. ELISA Reagent A: Wash Concentrate: Mix contents of wash concentrate in a holder to run all six (6) calibrators, A - F of the EPO CALIBRATORS [Exact concentration is stated on the vial label]. Controls and patient samples.

4. Pipet 200 µL of calibrators, controls and samples into the designated or mapped well. Freeze (-15°C) the remaining calibrators and controls as soon as possible after use. Standards and controls are stable at -15 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in “Procedural Notes” section.

5. ELISA Reagent A: Wash Concentrate: Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4°C, dissolve by placing the vial in a 37°C water bath or oven with swirling or stirring. Add wash concentrate (30 mL) to 570 mL of distilled or deionized water and mix. The diluted working wash solution is stable for 90 days when stored at room temperature.

VIII. ASSAY PROCEDURE

1. Place sufficient Streptavidin Coated Strips in a holder to run all six (6) calibrators, A - F of the EPO CALIBRATORS [Exact concentration is stated on the vial label]. Controls and patient samples.

2. Pipet 200 µL of calibrators, controls and samples into the designated or mapped well. Freeze (-15°C) the remaining calibrators and controls as soon as possible after use. Standards and controls are stable at -15 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in “Procedural Notes” section.

3. Add or dispense 25 µL of Reagent 1 (Biotinylated Antibody) into each of the wells which already contain the calibrators, controls and samples.

4. Add or dispense 25 µL of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells. Tap the microplate firmly against a rigid object, such as a pen, to achieve thorough mixing of the sample with Reagents. For complete assurance of mixing, repeat the tapping for a minimum of 5 times for each of the remaining three of the four sides of the plate. Be careful to avoid spillage.

5. Add or dispense 25 µL of Reagent 2 (Enzyme Labeled Antibody) into each of the wells which already contain the calibrators, controls and samples.

6. Add or dispense 150 µL of the ELISA Reagent B (TMB Substrate) into each of the wells. Tap the microplate as described in Step #4.

7. With appropriate cover to avoid light exposure, place the microplate(s) on an orbital shaker or rotator set at 170 ± 10 rpm for 20 minutes ± 5 minutes at room temperature (22-28°C).

8. Add or dispense 100 µL of the Stopping Solution into each of the wells. Tap the microplate as described in Step #4. Be careful to avoid spillage.

9. Add or dispense 150 µL of the ELISA Reagent B (TMB Substrate) into each of the wells. Tap the microplate as described in Step #4.

10. By using the final absorbance values obtained in the previous step, construct two calibration curves using 405 nm reading and 450 nm reading via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of EPO.

PROCEDURAL NOTES

- Samples that have values below the limit of detection (1.2 mU/mL) should be reported as “< 1.2 mU/mL”.

- It is recommended that all calibrators, controls, and patient samples are assayed in duplicate, until the analyst or technician has gained sufficient experience (as evidenced by the coefficient of variation duplicate being less than 10% [except for the values below the 2nd non-zero lowest standard] and the ability to obtain results for the kit controls within the suggested acceptable ranges).

- The samples should be pipetted into the well with minimum amount of air bubble.

- Patient samples with values greater than the highest calibrator (Calibrator F), which is approximately 450 mU/mL (see exact concentration on vial label, because it can vary from one lot to another), must be diluted with Calibrator A (Zero Calibrator) and re-assayed. Multiply the result by the dilution factor. Alternatively, the result may be reported as greater than the highest calibrator concentration (Calibrator F). For example, if the Calibrator F has an assigned EPO value of 494 mU/mL, the report should be “> 494 mU/mL”.

- Reagents from different lot numbers must not be interchanged.

- If preferred, mix in equal volumes, in sufficient quantities for the assay. Reagent 1 (Biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) in a clean amber bottle. The combined reagent is stable for seven (7) days when stored at 4°C. Then use 50 µL of the mixed antibody into each well. This alternative method should replace Step (3) and (4), to be followed with the incubation.

- When mixing avoid splashing of reagents from wells. This will affect assay precision and accuracy.

IX. CALCULATION OF RESULTS

Manual Method

1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using Calibrators A, D, E and F.

2. Assign the concentration for each calibrator stated on the vial in mU/mL. Plot the data from the calibration curve on linear graph paper with the concentration on the X-axis and the corresponding A.U. on the Y-axis.

3. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation. Obtain the concentration of the sample by locating the absorbance unit on the Y-axis and finding the corresponding concentration value on the X-axis. Patient and control samples should be read using the 450 nm for EPO concentrations up to the penultimate (2nd to the highest) calibrator, i.e. Calibrator E. EPO concentrations above the concentration of the penultimate calibrator (the example shown below as 120 mU/mL) should be interpolated using the 405 nm reading.

Automated Method:

4. Computer programs using cubic spline or 4 PL [4 Parameter Logistics] or Point-To-Point can generally give a good fit. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using Calibrators A, D, E and F.

Sample Data at 450 nm [raw A.U. readout against distilled or deionized water]

<table>
<thead>
<tr>
<th>Microplate Well</th>
<th>1st Reading Absorbance Unit</th>
<th>2nd Reading Absorbance Unit</th>
<th>Average Absorbance Unit</th>
<th>EPO mU/mL</th>
<th>EPO mU/mL – Result to report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0.019</td>
<td>0.021</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator B</td>
<td>0.122</td>
<td>0.114</td>
<td>0.118</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Calibrator C</td>
<td>0.200</td>
<td>0.207</td>
<td>0.204</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>Calibrator D</td>
<td>0.411</td>
<td>0.423</td>
<td>0.417</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Calibrator E</td>
<td>1.308</td>
<td>1.263</td>
<td>1.286</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>0.176</td>
<td>0.178</td>
<td>0.177</td>
<td>15.7</td>
<td>15.7</td>
</tr>
<tr>
<td>Control 2</td>
<td>1.395</td>
<td>1.493</td>
<td>1.444</td>
<td>&gt;120</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Patient Sample 1</td>
<td>0.115</td>
<td>0.125</td>
<td>0.120</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Patient Sample 2</td>
<td>0.414</td>
<td>0.420</td>
<td>0.417</td>
<td>40.4</td>
<td>40.4</td>
</tr>
<tr>
<td>Patient Sample 3</td>
<td>3.314</td>
<td>...</td>
<td>3.314</td>
<td>&gt;120</td>
<td>&gt;120</td>
</tr>
</tbody>
</table>
in the table above. This practice should give the results with parallelism compared to reference standards.

Dilutions of any elevated and/or suspect positive results be assayed to detect non-cancer, and ulcerative colitis17, sickle cell disease, and in premature neonates.18 Lower EPO levels than expected have been seen with anemias associated with the discrimination abnormally low EPO values from normal levels of EPO. Waldenstrom’s disease have impaired production of erythropoietin in relation to the reference standards obtained from the female and male population of data. This finding, that there is no gender difference, is consistent with the literature 21. Further, the EPO values do not appear to have significant age dependence, except higher values were obtained in samples from early phases of adulthood, i.e. approximately 22 to 42 years of age. Using the nonparametric method for the analysis of reference values outlined in the NCCLS publication “How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory” (NCCLS Document C28-A, Vol. 15 No. 4) the reference ranges (2.5 – 97.5 percentile) were 4.3 – 32.9 mU/mL for EPO in serum. Each laboratory should establish their own range of expected normal values.

“In patients with erythrocytosis due to uncompensated hypoxia, serum immunoreactive EPO is elevated; in those with compensated hypoxia, the serum immunoreactive EPO level is usually within the range of normal, and in patients with polycythemia vera, serum immunoreactive EPO is either normal or low. Thus, while an elevated serum EPO level suggests that erythrocytosis is a secondary phenomenon and a low EPO level supports the possibility of autonomous erythropoiesis, a normal serum EPO level excludes neither hypoxia nor autonomous EPO production as the cause of erythrocytosis.” 20

---

**X. QUALITY CONTROL**

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. When the laboratory first introduces this EPO assay, the release of patient sample results should be based on whether the kit Control results fall within the suggested acceptable ranges. If one or more of the quality control sample values lie outside the acceptable limits, the assay should be repeated. Once the laboratory has generated data of its own, the quality control parameters should be based on the statistical data by the laboratory, using either kit Control and/or serum pools made by the laboratory. Levy-Jennings plots on control results should be used. If the results for all the control samples are within mean ± 2 standard deviations, with no definitive trend or bias of the quality control data, the assay should be deemed acceptable. The Westgard rule should be followed to be compliant with CLIA 88 regulations. If the control results do not fall within the stated parameters as described, assay results are invalid.

---

**XI. LIMITATIONS OF THE PROCEDURE**

Like any analyte used as a diagnostic adjunct, EPO results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests. Purified IgG proteins of the same species as the ones for which the capture and the label antibodies, were derived, in addition to one commercial heterophile antibody blocker, have been incorporated in the reagents to minimize the heterophile antibodies.14 Nonetheless, there can be no assurance that the heterophile interference has been completely eliminated. Therefore, it is recommended that at least three dilutions of any elevated and/or suspect positive results be assayed to detect non-parallelism compared to reference standards.18

Because results obtained with one commercial EPO assay may differ significantly from those obtained with any other, it is recommended that any serial testing performed on the same patient over time should be performed with the same commercial EPO test.18 This test may not be sufficiently sensitive to consistently discriminate abnormally low EPO values from normal levels of EPO.

Lower EPO levels than expected have been seen with anemias associated with the following conditions: rheumatoid arthritis, acquired immunodeficiency syndrome, cancer, and ulcerative colitis17, sickle cell disease, and in premature neonates.18 After allogeneic bone marrow transplant, impaired erythropoietin response may delay erythropoietin recovery.17

Patients with hypermagnaglobulinemia associated with multiple myeloma or Waldenstrom’s disease have impaired production of erythropoietin in relation to hemoglobin concentration. This has been linked to increased plasma viscosity.17

---

### Sample Data at 405 nm [raw A.U. readout against distilled or deionized water]

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st Reading</th>
<th>2nd Reading</th>
<th>Average Absorbance</th>
<th>EPO mU/mL</th>
<th>EPO mU/mL – Result to report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0.003</td>
<td>0.007</td>
<td>0.005</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Calibrator B</td>
<td>0.122</td>
<td>0.127</td>
<td>0.125</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Calibrator E</td>
<td>0.405</td>
<td>0.389</td>
<td>0.397</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Calibrator F</td>
<td>1.331</td>
<td>1.257</td>
<td>1.294</td>
<td>494</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>0.053</td>
<td>0.056</td>
<td>0.054</td>
<td>&lt; 120</td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>0.427</td>
<td>0.460</td>
<td>0.444</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Patient Sample 1</td>
<td>0.036</td>
<td>0.038</td>
<td>0.037</td>
<td>&lt; 120</td>
<td></td>
</tr>
<tr>
<td>Patient Sample 2</td>
<td>0.125</td>
<td>0.128</td>
<td>0.127</td>
<td>&lt; 120</td>
<td></td>
</tr>
<tr>
<td>Patient Sample 3</td>
<td>1.035</td>
<td></td>
<td>1.035</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

* For samples with concentrations < the concentration of Calibrator E, e.g. 120 mU/mL, it is recommended to use the data obtained at 450 nm as shown in Sample Data at 450 nm in the table above. This practice should give the results with optimum sensitivity of the assay.

NOTE: The data presented are for illustration purposes only and must not be used in place of data generated at the time of the assay.

---

### XII. EXPECTED VALUES

EPO levels were measured in 120 apparently normal individuals in the U.S. with the Biomerica EPO ELISA. The samples consist of 61 males and 59 females, ranging from 18 to 96 years of age. There is no significant statistical difference on the reference ranges obtained from the female and male population of data. This finding, that there is no gender difference, is consistent with the literature 21. Further, the EPO values do not appear to have significant age dependence, except higher values were obtained in samples from early phases of adulthood, i.e. approximately 22 to 42 years of age. Using the nonparametric method for the analysis of reference values outlined in the NCCLS publication “How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory” (NCCLS Document C28-A, Vol. 15 No. 4) the reference ranges (2.5 – 97.5 percentile) were 4.3 – 32.9 mU/mL for EPO in serum. Each laboratory should establish their own range of expected normal values.

---

### XIII. PERFORMANCE CHARACTERISTICS

#### Accuracy

Eighty five (85) patient samples, with EPO values ranging from 3.8 to 304 mU/mL, were assayed by the Biomerica ELISA procedure and an ELISA kit. Linear regression analysis gives the following statistics:

**Biomerca ELISA = 0.94 ELISA Kit – 0.41 mU/mL**

\[ r = 0.989 \quad N = 85 \]

#### Sensitivity

The sensitivity, or minimum detection limit, of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit. The Biomerica EPO ELISA has a calculated sensitivity of 1.2 mU/mL. Hence, patient sample results below 1.2 mU/mL should be reported as “Less than 1.2 mU/mL.”

#### Precision and Reproducibility

The Intra-assay precision of the Biomerica EPO ELISA Test was calculated from 22 replicate determinations on each of the two samples.

### Intra-Assay Variation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (mU/mL)</th>
<th>N</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.4</td>
<td>22</td>
<td>8.4</td>
</tr>
<tr>
<td>B</td>
<td>189</td>
<td>22</td>
<td>4.8</td>
</tr>
</tbody>
</table>

The inter-assay precision of the Biomerica EPO ELISA Test was calculated from data on two samples obtained in 22 different assays.

### Inter-Assay Variation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (mU/mL)</th>
<th>N</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20.4</td>
<td>22</td>
<td>8.8</td>
</tr>
<tr>
<td>B</td>
<td>183</td>
<td>22</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Specifity and Cross-Reactivity

Cross-reactivity in the EPO was studied by the addition of various substances to the diluted and re-assayed for correct values. Samples with EPO levels greater than the highest calibrator, however, should be diluted with Calibrator A (Zero Calibrator).

Various amounts of EPO were added to four different patient sera to determine the recovery within the statistical limits of intraassay variation.

None of the cross reactants interferes with this EPO ELISA in the concentrations studied. The very small changes in EPO seen for some cross reactants were well within the statistical limits of intraassay variation.

Recovery

No cross-reactivity in the EPO was studied by the addition of various substances to the
Specificity and Cross-Reactivity

diluted and re-assayed for correct values. Samples with EPO levels greater than the highest calibrator, however, should be diluted with Calibrator A (Zero Calibrator).

Crossreactant | Amount of Crossreactant Added
--- | ---
Human Transferrin | 400 µg/mL
Human Bilirubin (unconjugated) | 200 µg/mL
Human Hemoglobin | 5 mg/mL
Human Alpha-1-Globulin | 60 mg/mL
Human Alpha1-Macroglobulin | 500 µg/mL
Human α1-Acid Glycoprotein | 800 µg/mL
Human α1-Antitrypsin | 500 µg/mL
Triglycerides | 30 mg/mL
Human Albumin | 60 mg/mL
Human Gamma Globulin | 60 mg/mL
ACTH (intact molecule: amino acid sequence 1-39) | 5,000 pg/mL
TSH | 100 µU/mL

None of the cross reactants interferes with this EPO ELISA in the concentrations studied. The very small changes in EPO seen for some cross reactants were well within the statistical limits of intraassay variation.

Recovery

Various amounts of EPO were added to four different patient sera to determine the recovery. The results are described in the following table:

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Endogenous EPO (mU/mL)</th>
<th>EPO added (mU/mL)</th>
<th>Expected Value (mU/mL)</th>
<th>Measured Value (mU/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>50.0</td>
<td>57.1</td>
<td>52.8</td>
<td>92.5%</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>150.0</td>
<td>155.5</td>
<td>150.0</td>
<td>96.5%</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>50.0</td>
<td>55.4</td>
<td>57.2</td>
<td>103.2%</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>150.0</td>
<td>154.2</td>
<td>168.0</td>
<td>108.9%</td>
</tr>
<tr>
<td>C</td>
<td>53.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>50.0</td>
<td>98.2</td>
<td>105.0</td>
<td>106.9%</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>150.0</td>
<td>187.5</td>
<td>202.0</td>
<td>107.7%</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.2</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>150.0</td>
<td>150.0</td>
<td>145.0</td>
<td>96.7%</td>
</tr>
</tbody>
</table>

Linearity of Patient Sample Dilutions: Parallelism

Three patient serum samples were diluted with Calibrator A (Zero Calibrator). Results in mU/mL are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Expected</th>
<th>Observed</th>
<th>% Observed – Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Undiluted</td>
<td></td>
<td>247.0</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>123.5</td>
<td>119.0</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>61.8</td>
<td>58.5</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>30.9</td>
<td>28.8</td>
<td>93%</td>
</tr>
<tr>
<td>B</td>
<td>Undiluted</td>
<td></td>
<td>139.0</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>69.5</td>
<td>74.0</td>
<td>106%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>34.8</td>
<td>39.9</td>
<td>114%</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>17.4</td>
<td>19.8</td>
<td>114%</td>
</tr>
<tr>
<td>C</td>
<td>Undiluted</td>
<td></td>
<td>&lt;500.0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>126.5</td>
<td>116.0</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>63.3</td>
<td>57.0</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

High Dose Hook Effect

The Biomerica EPO ELISA kit has exhibited no “high dose hook effect” in standard diluent spinked with 200,000 mU/mL of EPO. Additionally, three samples with known high EPO values (1,920 mU/mL, 1,520 mU/mL, and 966 mU/mL) were tested without dilution and their results read much greater than the highest standard. Samples with EPO levels greater than the highest calibrator, however, should be diluted and re-assayed for correct values.

XIV. REFERENCES:


XV. SYMBOLS

- Storage Temperature
- Lot Code
- Expiration
- Authorized Representative
- Manufacturer
- Caution, see instructions
- For in vitro diagnostic use
- Catalog No.

XVI. ORDERING INFORMATION

ORDERING: Send purchase order to:
BIOMERICA, INC.
1533 Monrovia Avenue
Newport Beach, CA 92663
U.S.A.

Phone: (949) 645-2111
FAX: (949) 722-6674
Website: www.biomerica.com
e-mail: bmra@biomerica.com
67025-03_eng.doc Jan 2005
according to IVDD 98/79/ EC
MDSS
Burckhardtstrasse 1
30163 Hannover, Germany