6. A calibrator curve must be established for every run.

5. All kit reagents and specimens should be brought to room temperature before use. Avoid multiple freezing and thawing cycles.

4. In order to reduce exposure to potentially harmful substances, wash and dry all vessels thoroughly (example: To a tube containing 2 mL of assay buffer add 20 µL of estrone-biotin and 20 µL of avidin-HRP conjugate working solution and mix thoroughly). Avoid handling any liquid that is left on labware.

3. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.

2. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

CLINICAL APPLICATIONS
Estrone is a steroid like estriol and estradiol, belonging to the class of estrogens. The estrogens are involved in the growth and function of the reproductive system. Before the ovum is fertilized the main action of the estrogens is on the growth of the endometrial lining. After fertilization does not occur, then the production of estrone decreases. A level shows a slight increase. The production of estrone then increases and peaks around day 14 in the cycle. At this time, the estrogen level is on the growth and function of the reproductive system. As the endometrium thickens, it is responsive to the hormones of the corpus luteum. When the endometrium is ready for implantation, it begins to form the decidua basalis. If fertilization occurs and a blastocyst implants in the endometrium, then the production of estradiol continues to increase. If fertilization does not occur, then the production of estrone decreases.

SAFETY CAUTIONS AND WARNINGS
1. CLINICAL APPLICATIONS
2. PROCEDURAL CAUTIONS AND WARNINGS
3. CHEMICAL HAZARDS
4. SPECIMEN COLLECTION AND STORAGE
5. SPECIMEN PRETREATMENT

1. All the reagents within the kit are calibrated for the determination of estrone in human serum. The kit is not calibrated for the determination of estrone in saliva, plasma or other specimens of human or animal origin.

2. Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation

3. Avidin-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation

4. Estrone Calibrators — Ready To Use


Stability: 12 months or as indicated on label.

5. Controls — Ready To Use


Stability: 12 months or as indicated on label.

6. Wash Buffer Concentrate — Requires Preparation

Contents: One bottle containing a 1:100 dilution of a non-detergent and a non-mercury preservative.

Stability: 12 months or as indicated on label.

7. Assay Buffer — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Stability: 12 months or as indicated on label.

8. LIA Substrate Reagent A — Requires Preparation

Contents: One vial containing luminol enhancer.

Stability: 12 months or as indicated on label.

9. LIA Substrate Reagent B — Requires Preparation

Contents: One bottle containing peroxidase substrate solution.

Stability: 12 months or as indicated on label.

10. LIA Substrate Reagent C — Requires Preparation

Contents: One bottle containing a proteinase K and butanone preservative.

Stability: 12 months or as indicated on label.

INTENDED USE
For the direct quantitative determination of estrone in human serum by a chemiluminescence immunoassay (LIA).

PRINCIPLE OF THE TEST
The principle of the following chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. In this test, estrone is labeled with a radioactive isotope and is mixed with a non-radioactive antibody. The antibody and antigen are then incubated in the presence of a labeled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound antibody, leaving the labeled antigen to react with the microplate. The luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of estrone in the sample. A kit of calibrators is used to plot a standard curve from which the amount of estrone in patient samples and controls can be directly read.

LIMITATIONS
1. All the reagents within the kit are calibrated for the determination of estrone in human serum. The kit is not calibrated for the determination of estrone in saliva, plasma or other specimens of human or animal origin.

2. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.

3. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Pipette tips to dispense 50, 100, 150 and 300 µL.
2. Disposable pipette tips
3. Distilled or deionized water
4. Centrifuge and carefully remove supernatant layer. Store at 4°C to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

5. All kit reagents and specimens should be brought to room temperature and mixed and allowed to stand for at least 20 minutes before use. Avoid multiple freezing and thawing cycles.

6. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any human specimen.

7. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any human specimen.

8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.

9. The luminiscence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.

10. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.

11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample or patient specimen.

12. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any human specimen.

13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

Contents: One bottle containing a non-mercury preservative.

Stability: 12 months or as indicated on label.
**PREPARATION OF LIA WORKING SUBSTRATE SOLUTION**

In a clean plastic container (glass is not suitable) mix 1 part of LIA substrate reagent A with 1 part of LIA substrate reagent B and 10 parts of LIA substrate reagent C. This gives the ready to use substrate solution. If the whole plate is to be used prepare working substrate solution as follows: Combine 1.4 mL of LIA substrate reagent A with 1 part of LIA substrate reagent B and 15 parts of LIA substrate reagent C. It is suggested to wait at least 2 minutes prior to use after preparation of the working substrate solution. The working substrate solution is stable for up to 2 hours at room temperature. Discard the leftovers.

**ASSAY PROCEDURE**

Important Notes:
1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.
4. Incubation on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
5. Draw a calibrator curve on semi-log paper with the mean RLU of each unknown duplicate.

**CALCULATIONS**

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibration curve on semi-log paper with the mean RLU of each calibrator duplicate on the Y-axis and the concentration of the calibrator on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibration curve.
5. If a sample reads more than 2000 pg/mL then dilute it with LIA substrate reagent as for the working substrate solution. The working substrate solution is stable for up to 2 hours at room temperature. Discard the leftovers.

**TYPICAL CURVE**

Sample curve only. Do not use to calculate results.

**TYPICAL TABLED DATA**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean RLU</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>4.1</td>
<td>8.2</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>9.9</td>
<td>6.6</td>
</tr>
</tbody>
</table>

**PERFORMANCE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Estrone (pg/mL)</th>
<th>Estrone-3-Glucuronide</th>
<th>Estrone-3-Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**SPECFICITY (CROSS-REACTIVITY)**

The following compounds were tested for cross-reactivity with the DBC estrone LIA kit with estrone cross-reacting at 100%:


**COMPARATIVE STUDIES**

The DBC estrone LIA kit was tested with the DBC estrone ELISA kit. The comparison of 50 serum samples yielded the following linear regression results:

$$ y = 0.8872x - 2.39, \quad r^2 = 0.899 $$

**EXPECTED NORMAL VALUES**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>25–150</td>
</tr>
<tr>
<td>Females</td>
<td>25–350</td>
</tr>
</tbody>
</table>

**INTER-ASSAY PRECISION**

Three samples were assayed ten times over a period of four weeks. The results (in pg/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77.6</td>
<td>9.08</td>
<td>11.7</td>
</tr>
<tr>
<td>2</td>
<td>272.4</td>
<td>24.2</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>823.6</td>
<td>89.77</td>
<td>10.9</td>
</tr>
</tbody>
</table>

**RECOVERY**

Spiked samples were prepared by adding defined amounts of estrone to three patient serum samples. The results (in pg/mL) are tabulated below:

**LINEARITY**

Three patient serum samples were diluted with calibrator A. The results (in pg/mL) are tabulated below:

**OTHER RELATED DBC KITS**

DBC Estrone ELISA Kit

**REFERENCE**


