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

2. Control materials should be included in every run at a high

PROCEDURAL CAUTIONS AND WARNINGS

1. All reagents within the kit are calibrated for the
determination of estriol in serum, plasma or other specimen of human.

LIMITATIONS

4. The results obtained with this kit should never be used as
the sole basis for a clinical diagnosis. For example, the
occurrence of heterophilic antibodies in patients regularly exposed
to livestock welfare, etc. These antibodies may produce
false positive results.

SAFETY CAUTIONS AND WARNINGS

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.

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with
plenty of water.

SPECIMEN COLLECTION AND STORAGE

1. Specimen tubes are to be placed into a freezer and allowed to

STABILITY

1. Calibrator A may be used to dilute any high saliva
specimen. The use of any other reagent may lead to false\nresults.

2. Preparation of conjugate working solution: Dilute HRP
conjugate concentrate 1:200 in assay buffer

S. Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate

5. All kit reagents and specimens should be brought to
room temperature and mixed thoroughly before use. Avoid
repeated freezing and thawing of reagents and specimens.

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

7. The kit control should be included in every run and fall
within established confidence limits.

8. Improper procedures or reagents, imprecise pipetting,
complete washing as well as improper reagent storage
may be indicated when assay values for the control do not
reflect established ranges.

9. The luminescence 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, standard and
control.

12. Do not mix various lots of reagent components within a
and do not use any component beyond the expiration
date printed on the label.

13. Kit reagents must be regarded as hazardous waste and
disposed of according to national regulations.
CALCULATIONS
1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibration curve on semi-log paper with the mean RLU on the Y-axis and the calibrator concentrations on the X-axis. If immunooassay 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.

If a sample reads more than 30 nmol, then dilute it with a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>RLUs/10⁶</th>
<th>SD%</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 pg/mL</td>
<td>4.38</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>1 mg/mL</td>
<td>3.44</td>
<td>9.9</td>
<td></td>
</tr>
</tbody>
</table>

RECOVERY
Samples were spiked by adding different estriol standards (1:1 volume/volume) to three patient saliva samples. The results in (ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.44</td>
<td>2.37</td>
</tr>
<tr>
<td>2</td>
<td>2.94</td>
<td>2.72</td>
</tr>
<tr>
<td>3</td>
<td>1.91</td>
<td>1.28</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.44</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>2.94</td>
<td>10.7</td>
</tr>
<tr>
<td>3</td>
<td>1.91</td>
<td>12.8</td>
</tr>
</tbody>
</table>

LINEARITY
Three saliva samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs.</th>
<th>Exp.</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.44</td>
<td>2.89</td>
<td>106.7</td>
</tr>
<tr>
<td>2</td>
<td>2.94</td>
<td>2.18</td>
<td>91.3</td>
</tr>
<tr>
<td>3</td>
<td>1.91</td>
<td>1.47</td>
<td>94.8</td>
</tr>
</tbody>
</table>

REFERENCE