Estriol Saliva ELISA

Enzyme immunoassay for the quantitative determination of Estriol in human saliva.

REF
RE52291

Σ
96

2-8 °C

EU: IVD

For research use only.
Not for use in diagnostic procedures.

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INTENDED USE
Competitive immunoenzymatic colorimetric method for quantitative determination of Estriol concentration in saliva.

1 CLINICAL SIGNIFICANCE
Estriol (also Oestradiol) is one of the three main steroids produced by the human body. It is only produced in significant amounts during pregnancy as it is made by the fetus. During pregnancy the production of estriol depends on an intact maternal-placental-fetal unit. Fetal-placental production of estriol leads to a progressive rise in maternal circulating levels reaching a lategestational peak several orders of magnitude greater than non-pregnant levels. In the maternal circulation, estriol undergoes a rapid conjugation in the liver followed by urinary excretion with a half-life of about 20 minutes. Since normal estriol production depends on an intact maternal-placental-fetal circulation and functional fetal metabolism, maternal estriol levels have been used to monitor fetal status during pregnancy, particularly during the third trimester. DHEA-S is produced by the adrenal cortex of the fetus, this is converted to estriol by the placenta. If levels are abnormally low in a pregnant woman, this may indicate a problem with the development in the child.

2 PRINCIPLE
Estriol (antigen) in the sample competes with horseradish peroxidase estriol (enzyme-labelled antigen) for binding onto the limited number of antiestriol (antibody) sites on the microplate (solid phase). After incubation, the bound/free separation is performed by a simple solid-phase washing. The enzyme substrate ($H_2O_2$) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined. Estriol concentration in the sample is calculated based on a series of standards. The colour intensity is inversely proportional to the Estriol concentration in the sample.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 REAGENTS AND MATERIALS SUPPLIED IN THE KIT
1. Estriol Standards 6x (1 vial = 1 mL)
   STD0
   STD1
   STD2
   STD3
   STD4
   STD5
2. Controls (2 vials = 1mL each)
   L Control
   M Control
3. Incubation Buffer (1 bottle) 30 mL
   Phosphate buffer pH 7.4 BSA 1g/L; Stabiliser
4. Conjugate (1 vial) 1 mL
   Estriol-HRP conjugate
5. Coated Microplate 1x (breakable)
   Anti-Estriol IgG adsorbed on microplate
6. Wash solution Conc. 50X (1 bottle) 20 mL
   NaCl 45 g/L; Tween-20 55 g/L
7. TMB-substrate (1 vial) 15 mL
   H$_2$O$_2$-TMB 0.26 g/L (avoid any skin contact!)
8. Stop solution (1 vial) 15 mL
   0.15 mol/L H$_2$SO$_4$ (avoid any skin contact!)

3.2 REAGENTS NECESSARY NOT SUPPLIED
Distilled water

3.3 AUXILIARY MATERIALS AND INSTRUMENTATION
Automatic dispenser.
Microplates reader
Saliva Collection Device REF RE69991
Microplates reader

Note
Store all reagents at 2-8 °C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close immediately after use.

4 WARNINGS
• This kit is intended for in vitro use by professional persons only.
• Use appropriate personal protective equipment while working with the reagents provided.
• Some reagents contain small amounts of Proclin 300 as preservative. Avoid the contact with skin or mucosa.
• The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
• The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
• Avoid the exposure of reagent TMB/H$_2$O$_2$ to directed sunlight, metals or oxidants.
• This method allows the determination of Estriol from 2.5 pg/mL to 4000 pg/mL.
• The clinical significance of Estriol determination can be invalidated if the patient was treated with natural or synthetic steroids.
5 PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- All reagents should be stored refrigerated at 2-8 °C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated should not be used in the assay. Highly lipemic or haemolysed specimens should similarly not be used
- Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 Preparation of the Standard (S0...S5)

Before use, mix for 5 min. with rotating mixer

The standard has the following concentration of Estriol:

<table>
<thead>
<tr>
<th>S</th>
<th>pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>S1</td>
<td>15</td>
</tr>
<tr>
<td>S2</td>
<td>100</td>
</tr>
<tr>
<td>S3</td>
<td>600</td>
</tr>
<tr>
<td>S4</td>
<td>4000</td>
</tr>
</tbody>
</table>

Once open is stable at +4 °C until the kit expiration date.
For SI UNITS: pg/mL x 3.5 = pmol/mL

6.2 PREPARATION OF THE WASH SOLUTION

Dilate the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8 °C.

6.3 PREPARATION OF DILUTED CONJUGATE

Prepare immediately before use.
Add 10 µL Conjugate (reagent 4) to 1.0 mL of Incubation Buffer (reagent 3). Mix gently. Stable 3 hours at 22-28 °C.

6.4 PREPARATION OF THE SAMPLE

The determination of Estriol can be performed in saliva.
It is recommended to collect saliva samples with IBL International Saliva Collection Device Salicaps (REF RE69991 oder RE69995)

6.4.1 METHOD AND LIMITATIONS

Collect saliva samples at the times indicated. In order to have high reproducibility and accuracy, it is advisable to collect at least 3 samples in a period of not less than 2 hours and pooling the samples before testing.
If no specific instructions have been given oral fluid (saliva) samples may be collected at any time; for saliva collection, the following should be noted:

a) If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
b) During the day allow 1 hour after any food or drink before collecting saliva samples
c) It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

6.4.2 SALIVA PROCESSING INSTRUCTIONS

Let the saliva flow down through the straw into the centrifuge glass tube
1. Centrifuge the sample for 15 minutes at 3000 rpm
2. Store at −20 °C for at least 1 hour
3. Defrost samples
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2-8 °C for one week or at −20 °C for longer time.
6.5 PROCEDURE
As it is necessary to perform the determination in duplicate, prepare two wells for each point of the standard curve (S0-S5), two wells for each control and for each sample, one for Blank.
Pipette:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard Controls</th>
<th>Sample</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 µL</td>
<td></td>
</tr>
</tbody>
</table>

Incubate 60 minutes. at 22-28 °C.
Remove the contents from each well. Wash the wells with 300 µL of diluted wash solution. Repeat two additional times for a total of three washes, drain the solution completely.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Incubate at room temperature 22-28 °C for 15 minutes in the dark.

<table>
<thead>
<tr>
<th>Stop solution</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Read the absorbance (E) at 450 nm against Blank.

7 QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of Estriol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8 LIMITATION OF PROCEDURE
8.1 INTERPRETATION
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

9 RESULTS
9.1 MEAN ABSORBANCE
Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

9.2 STANDARD CURVE
Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

9.3 CALCULATION OF RESULTS
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

10 REFERENCE VALUE
As the Estriol Saliva values follow a cicaadian pattern we suggest to collect the samples at the same time (8 A.M.):
The following values should be used as preliminary guide until each laboratory has your own reference range.

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>Range ± 2SD [pg/mL]</th>
<th>Absolute Range [pg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>21</td>
<td>0 – 21.0</td>
<td>0 – 32.0</td>
</tr>
<tr>
<td>17:00</td>
<td>21</td>
<td>0 – 6.8</td>
<td>0 – 8.9</td>
</tr>
</tbody>
</table>

Pregnancy weeks

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>Estriol in Saliva (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>10</td>
<td>(700 ± 500)</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>(900 ± 600)</td>
</tr>
<tr>
<td>26</td>
<td>10</td>
<td>(1200 ± 700)</td>
</tr>
<tr>
<td>28</td>
<td>10</td>
<td>(1500 ± 800)</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>(1800 ± 800)</td>
</tr>
<tr>
<td>32</td>
<td>10</td>
<td>(2200 ± 1100)</td>
</tr>
<tr>
<td>34</td>
<td>10</td>
<td>(3200 ± 1300)</td>
</tr>
<tr>
<td>36</td>
<td>10</td>
<td>(4100 ± 1600)</td>
</tr>
<tr>
<td>37</td>
<td>10</td>
<td>(4500 ± 1700)</td>
</tr>
<tr>
<td>38</td>
<td>10</td>
<td>(5000 ± 2000)</td>
</tr>
<tr>
<td>39</td>
<td>10</td>
<td>(5300 ± 2000)</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>(5700 ± 2000)</td>
</tr>
</tbody>
</table>

11 PERFORMANCE AND CHARACTERISTICS
11.1 PRECISION

11.1.1 INTRA ASSAY VARIATION
Within run variation was determined by replicate measurements (16x) of two different saliva control in one assay. The within assay variability is 9.7%.

11.1.2 INTER ASSAY VARIATION
Between run variation was determined by replicate measurements (10x) of two different saliva control with different lots of kit. The between assay variability is ≤13.7%.

11.2 ACCURACY
The recovery of 50, 300, 2000 ng/mL of Estriol added to “saliva-free” sample gave an average value (±SD) of 100.6% ± 14.6% with reference to the original concentrations.

11.3 SENSITIVITY
The lowest detectable concentration of Estriol that can be distinguished from the zero standard is 1.0 pg/mL at the 95% confidence limit.

Version 10/2011
11.4 SPECIFICITY
The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Crossreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>100.0%</td>
</tr>
<tr>
<td>16-epi-estriol</td>
<td>10.5%</td>
</tr>
<tr>
<td>15α-OH-estriol</td>
<td>7.0%</td>
</tr>
<tr>
<td>Estriol-3-sulfat</td>
<td>2.0%</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.1%</td>
</tr>
<tr>
<td>17-epi-estriol</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estriol-3α-glucuronat</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estriol-16α-glucuronate</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estron</td>
<td>&lt;0.0001%</td>
</tr>
</tbody>
</table>

11.5 CORRELATION
The Estriol saliva ELISA kit was compared to another commercially available Estriol saliva assay. 30 saliva samples were analysed according in both test systems.

The linear regression curve was calculated:

\[ y = 1.03x + 0.68 \]
\[ r^2 = 0.988 \]

\[ y = \text{Estriol Saliva ELISA (IBL)} \]
\[ x = \text{Estriol Saliva ELISA (Salimetrics)} \]

12 WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

13 BIBLIOGRAPHY
LIABILITY: Complaints will be accepted in each mode – written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.