Unconjugated Estriol (uE3) Test System
Product Code: 5075-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free (unconjugated) Estriol in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

The last few years have seen the development of screening for fetal Down syndrome by measurement of multiple markers in maternal serum (1). Although amniocentesis has been widely available for more than 40 years it can only be selectively used to diagnose the disorder because of the hazard to fetus. Of most importance in the diagnosis of Down syndrome is the determination of maternal serum levels of unconjugated estriol (uE3). Although amniocentesis has been widely used for the detection of Down syndrome, of most interest is the determination of maternal serum levels of unconjugated estriol (uE3). Although amniocentesis has been widely used for the detection of Down syndrome, of most interest is the determination of maternal serum levels of unconjugated estriol (uE3).

2.1 INTRODUCTION

The specimens shall be blood serum or heparanised plasma in a 24 hr urine sample. The specimens should be stored at 2-8°C. The specimens may be frozen and stored at -20°C for up to 60 days. The specimens shall be blood serum or heparanised plasma in a 24 hr urine sample. The specimens should be stored at 2-8°C. The specimens may be frozen and stored at -20°C for up to 60 days.

2.2 MATERIALS AND REAGENTS

A. uE2 Tracer Reagent – 6.0 ml/vial – Icon

B. uE3 Biotin Reagent – 6.0 ml – Icon

C. Wash Solution – 20 ml/vial – Icon

D. Wash Buffer

E. Wash Solution – 20ml/vial – Icon

F. Signal Reagent A – 7.0ml/vial - Icon A

G. Signal Reagent B – 7.0ml/vial - Icon B

H. Bovine Serum Albumin (BSA) – 1mg/ml – Icon

I. Buffer A – 10X

J. Buffer B

K. Buffer C

L. Buffer D

M. Buffer E

N. Buffer F

O. Buffer G

P. Buffer H

Q. Buffer I

R. Buffer J

S. Buffer K

T. Buffer L

U. Buffer M

V. Buffer N

W. Buffer O

X. Buffer P

Y. Buffer Q

Z. Buffer R

2.3 METHOD

(a) Wash the microplates.

(b) Add the sample, suspected of concentrations higher than 30 ng/ml, to the microplate wells. Avoid extended exposure to heat and light. The results should be read within 30 minutes after adding the specimen to the microplate wells.

(c) Wash the microplates.

(d) Read the relative light units in each well with a microplate reader as outlined in Example 1.

2.4 QUALITY CONTROL

Laboratory or hospital personnel must be capable of conducting test procedures. The results should be read within 30 minutes after adding the specimen to the microplate wells. The results should be read within 30 minutes after adding the specimen to the microplate wells.

2.5 CALCULATION OF RESULTS

A. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in triplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

B. Pipette 0.055 ml (50µl) of the appropriate calibrator, control or specimen into the assigned well.

C. Add 0.055 ml (50µl) of uE3 Biotin Reagent to all wells.

D. Swirl the microplate gently for 20-30 seconds to mix.

E. Add 0.055 ml (50µl) of u-E3 Tracer Reagent to all wells.

F. Add 0.055 ml (50µl) of u-E3 Biotin Reagent to all wells.

G. Add 0.055 ml (50µl) of u-E3 Tracer Reagent to all wells.

H. Add 0.055 ml (50µl) of u-E3 Biotin Reagent to all wells.

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Y. Add 0.055 ml (50µl) of u-E3 Tracer Reagent to all wells.

Z. Add 0.055 ml (50µl) of u-E3 Biotin Reagent to all wells.

2.6 RESULTS

(a) Read the relative light units in each well with a microplate reader as outlined in Example 1.

(b) The results should be read within 30 minutes after adding the specimen to the microplate wells. The results should be read within 30 minutes after adding the specimen to the microplate wells.

(c) The results should be read within 30 minutes after adding the specimen to the microplate wells.

(d) The results should be read within 30 minutes after adding the specimen to the microplate wells.

2.7 PRECAUTIONS

(a) Do not use reagents that are contaminated or have bacteria growth.

(b) Do not use reagents that are contaminated or have bacteria growth.

(c) Do not use reagents that are contaminated or have bacteria growth.

(d) Do not use reagents that are contaminated or have bacteria growth.

2.8 TEST PROCEDURE

(a) Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

(b) Two procedures may be performed by a skilled individual or trained professional.*

**TABLE 4**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>S</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>20</td>
<td>1.68</td>
<td>0.15</td>
<td>9.1%</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>2.04</td>
<td>0.42</td>
<td>21.2%</td>
</tr>
<tr>
<td>High</td>
<td>20</td>
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</tbody>
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**TABLE 2**

<table>
<thead>
<tr>
<th>Week</th>
<th>Expected Range (ng/ml)</th>
<th>Gestation 12</th>
<th>0.3-1.0</th>
<th>27.7-16.0</th>
<th>3.0-18.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.3-1.0</td>
<td>27.7-16.0</td>
<td>3.0-18.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4-1.6</td>
<td>26</td>
<td>3.0-18.0</td>
<td>4.0-21.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6-2.6</td>
<td>35</td>
<td>4.0-21.0</td>
<td>5.0-25.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.6-3.6</td>
<td>50</td>
<td>5.0-25.0</td>
<td>6.0-30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6-4.6</td>
<td>70</td>
<td>6.0-30.0</td>
<td>7.0-35.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values could be expected to be found by a given method for a population of "normal" persons dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be established using the method with a population indigenous to the area in which the laboratory is located.

**14.0 PERFORMANCE CHARACTERISTICS**

**14.1 Precision**

The within and between assay precision of the Unconjugated Estradiol AccuLite® CLIA Test System was evaluated by adding interfering substances to a serum matrix at three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these controls are presented in Table 2 and Table 3.

**TABLE 3**

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**TABLE 5**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind</td>
<td>4.30</td>
<td>Y = 0.97X + 0.52X</td>
<td>0.991</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

**14.4 Specificity**

The % cross reactivity of the Estradiol antibody to selected substances, for determination of Unconjugated Estradiol was evaluated by adding interfering substances to a serum matrix at three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these controls are presented in Table 2 and Table 3.

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According to ref. 1, 2.1 (20ml) 2 plates

Prednisone <0.001
Progesterone <0.001
Testosterone <0.001
