



FREE ESTRIOL Elisa

Enzymeimmunoassay for the quantitative measurement of Free Estriol in human serum



BL-44-E

IN VITRO DIAGNOSTIC USE

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1 INTENDED USE

The Estriol (unconjugated) enzyme immunoassay (EIA) test kit is to be used for the in-vitro determination of unconjugated estriol in human serum during the second half of pregnancy.

Although Free Estriol measurement may also be of clinical interest in monitoring of pregnancy when applied to serum or amniotic fluid, this assay is not intended to be used as a screening parameter in Trisomie 21.

2 EXPLANATION OF THE TEST

Estriol (E3) is the major estrogen formed by the fetoplacental unit during pregnancy. ^{1/2} Unconjugated E3 passes through the placenta into the maternal circulation, where it is rapidly converted into glucuronide and sulfate derivatives to facilitate its excretion. ^{4/5} The half-life of estriol in the maternal bloodstream is only 20-30 minutes. Its measurement, therefore offers a convenient and quick evaluation of current fetal status. ^{5/7} Plasma estriol levels increase steadily throughout pregnancy and most rapidly during the third trimester (28-40 weeks)⁵⁻⁸. A sudden decrease in fetoplacental E3 production will result in a rapid fall in unconjugated E3 in the maternal serum. ^{9/10} There are several potential advantages to measuring unconjugated E3 rather than total serum or urinary E3. Unconjugated estriol levels are free from effects related to maternal renal or hepatic disease, ¹¹⁻¹² and are not altered by the administration of certain antibiotics. Unconjugated E3 more accurately reflects fetal outcome in diabetic pregnancies - and since no hydrolysis of unconjugated E3 is required, a more rapid turnaround for the test result is possible.

The E3 EIA provides a direct non-isotopic method for measuring unconjugated estriol in unextracted human serum. This method uses a highly specific estriol antibody and an enzyme-labeled analyte. The colored end-product may be measured on a spectrophotometer. The long shelf life of this product, together with the elimination of radioisotopes, radiation counter and necessary licensing requirements make this method applicable to both large and small laboratories.

3 PRINCIPLE OF THE METHOD

The Estriol (unconjugated) procedure is an enzyme immunoassay, which is based on the principle of competitive binding. In the procedure, estriol is conjugated to the enzyme Horse Radish Peroxidase. Serum containing estriol (i.e. calibrator or patient) is mixed with estriol HRP conjugate and added to the wells on a microtiter strip. Estriol antibody is already precoated in the microwells. After one hour, the Free estriol Horse Radish Peroxidase and unbound estriol are washed from the wells with buffer. Substrate is added to the antibody bound estriol Horse Radish Peroxidase which is immobilized in the wells. After a brief incubation period a stopping reagent is added, which gives rise to a colored product which is measured spectrophotometrically utilizing either a microplate or microstrip reader.

4 REAGENTS

4.1 Contents of the kit

Note: Store reagents at 2-8 °C.


- | | |
|-----|---|
| CAL | 0 |
|-----|---|

 0 ng/ml
 1 vial, 1 ml
 Contains human serum, buffered saline and preservatives.
- | | |
|-----|---|
| CAL | N |
|-----|---|

 N=1 to 5, 1 ml each
 See exact value on the vial label
 Each vial contains Estriol in human serum, buffered saline and preservatives.

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING HEPATITIS AND HUMAN IMMUNO-DEFICIENCY VIRUS. Source materials from which the calibrators were derived were found to be non-reactive for HBsAg and HIV when tested with licensed reagents. However, no known test method can assure that a product derived from human sources does not contain these viruses.

- | | |
|----|-----|
| Ag | HRP |
|----|-----|

 14 ml
 Each vial contains estriol-labeled Horse Radish Peroxidase.
- | |
|---|
|  |
|---|

 Rabbit anti estriol antibody coated microwells
 (8 x 12 well strips per plate)
 Contains estriol antibody bound to wells.
- | | |
|-------|-----|
| CHROM | TMB |
|-------|-----|

 TMB
 14 ml
- | | |
|------|------|
| STOP | SOLN |
|------|------|

 0.5 M H₂SO₄
 14 ml
- | | | |
|------|------|------|
| WASH | SOLN | CONC |
|------|------|------|

 (40X concentrated)
 30 ml.

NOTE: DISPOSAL This kit contains materials such as human serum which may necessitate special disposal procedures. Before disposing of leftover reagents, check local regulations regarding disposal of medical waste to ensure compliance with local laws.

4.2 Materials needed but not provided

1. Calibrated micropipettes (10 & 100 microliters)
2. Microwell strip or plate reading spectrophotometer capable of reading at a wavelength of 450 nm.
3. Absorbent Paper.

5 SPECIMEN COLLECTION AND HANDLING

1. Handle all blood and serum as if capable of transmitting Hepatitis and HIV.
2. Serum is required for use in E3 assay procedure.
3. Separate the serum fraction immediately and store tightly capped.
4. Specimens may be stored refrigerated (2-8 C) for 4 days. If storage time exceeds 4 days then frozen storage is recommended.
5. AVOID MULTIPLE FREEZE-THAW CYCLES FOR ANY SPECIMEN.
6. Prior to assay, frozen sera should be completely thawed and mixed well.
7. Do not use grossly lipemic specimens. (An attempt should be made to clarify grossly lipemic sera prior to assay).
8. Moderately lipemic, hemolyzed and icteric specimens will not interfere with the assay.

6 PREPARATION OF THE WASH SOLUTION

Dilute the 40X-concentrated Wash Buffer by addition of 1170 ml Aqua dest. to a final volume of 1200 ml
Store at 2-8°C. Stable until expiration date on the label.

7 ASSAY PROCEDURE

1. Pipette 10 µl of estriol calibrators into the appropriate well.
2. Pipette 10 µl of each control and patient serum into appropriate well.
3. Pipette 100 µl of estriol enzyme conjugate into all wells.
4. Incubate at room temperature for 1 hour.
5. Decant and wash 4 times with wash buffer (300 µl wash buffer per well) and blot on absorbent paper to remove excess liquid from the wells.
6. Add 100 µl of substrate reagent.
7. Incubate 30 minutes at room temperature.
8. Add 100 µl of stopping reagent.
9. Read absorbance at 450 ± 10 nm.

8 PROCEDURAL NOTES

1. When pipetting reagents, maintain a consistent order of addition from well to well. This will ensure equal incubation times for all wells. Carry out each addition step without pausing.
2. Pipet all reagents directly to the bottom of the well to prevent liquid from remaining on the side wall.
3. For optimal results, duplicate determinations of calibrators and unknown samples are recommended.
4. All pipets used including multi-channel or automated pipetting stations should have a precision coefficient of variation (CV) of less than 1 %.

9 RESULTS

Manual Calculation

1. To construct the calibrator curve, plot the absorbance for the estriol calibrators (vertical axis) versus the estriol calibrator concentration (horizontal Axis) on the semi-logarithmic paper supplied.
2. Draw the best curve through the points taken as a set.
3. Using the calibrator curve, interpolate the control and unknown serum value for each absorbance measured. Record the value for each control or unknown sample.

10 LIMITATIONS OF THE PROCEDURE

The user of this kit should have read and understood the package insert prior to running the test. Strict adherence to the protocol is necessary to obtain reliable results with this test kit. In particular, careful reagent handling, storage, pipetting and decanting are essential for achieving accurate and precise determinations.

Improper handling of patient samples may cause spurious results. Always mix the thawed samples thoroughly prior to assay. Avoid using old or mistreated serum specimens. Sample degradation as well as multiple freeze-thaw cycles may cause inaccurate determinations. Patient specimens should be assayed as soon as possible. Severely hemolytic, lipemic or icteric samples may result in poor precision or inaccurate values. Results obtained from these types of samples should be viewed with caution. Confirmation of values should be determined from a better quality specimen.

11 RECOMMENDATIONS FOR LABORATORY QUALITY CONTROL

1. Do not mix or interchange reagent lots with any other kit or different kit lots.
2. Do not use reagents beyond the expiration date printed on each vial or bottle.
3. Each laboratory should establish its own criteria for precision and accuracy by running controls in the normal range, as well as in the low and elevated ranges.
4. Trend charts and statistical methods should be updated to assure that performance is reliable and consistent from lot to lot.

12 CLINICAL SIGNIFICANCE

The measurement of Estriol (E3) in body fluids has routinely been used for the monitoring and management of fetal well-being, particularly in the high-risk pregnant patient.^{9,16,17} The concentration of E3 in plasma increases gradually during the first 20 weeks gestation and more rapidly during the third trimester.^{3,19} Since the ranges for normal and abnormal serum conjugated E3 are wide and overlap considerably, a single E3 determination is of little value. The patient should be monitored frequently to establish any individual trend.

Consistently low levels or a sudden and continual decrease of serum E3 during the third trimester is highly indicative of fetal distress and possibly intrauterine death.^{6,8,9} When such observations are made, the status of the fetus should be assessed by alternative methods.

Interpretation of serum unconjugated E3 levels should be made in conjunction with other clinical tests or diagnostic procedures such as amniocentesis and ultrasound. Subnormal E3 levels may also be observed in patients being administered certain antibiotics or corticosteroids or in patients with impaired maternal hepatic function.¹¹⁻¹⁴

13 EXPECTED VALUES

| Week of gestation p.m. | Expected range (ng/ml) | Week of gestation p.m. | Expected range (ng/ml) | Twins pregnancy (ng/ml) |
|------------------------|------------------------|------------------------|------------------------|-------------------------|
| 12 | 0.3 - 1.0 | 22 - 23 | 2.7 - 16 | 3 - 18 |
| 13 | 0.3 - 1.1 | 24 - 25 | 2.9 - 17 | 3 - 20 |
| 14 | 0.4 - 1.6 | 26 - 27 | 3.0 - 18 | 4 - 21 |
| 15 | 1.0 - 4.4 | 28 - 29 | 3.2 - 20 | 4 - 22 |
| 16 | 1.4 - 6.5 | 30 - 31 | 3.6 - 22 | 5 - 25 |
| 17 | 1.5 - 6.6 | 32 - 33 | 4.6 - 23 | 6 - 39 |
| 18 | 1.6 - 8.5 | 34 - 35 | 5.1 - 25 | 7 - 39 |
| 19 | 1.9 - 11 | 36 - 37 | 7.2 - 29 | 9 - 38 |
| 20 | 2.1 - 13 | 38 - 39 | 7.8 - 37 | 13 - 40 |
| 21 | 2.6 - 14 | 40 - 42 | 8.0 - 39 | --- --- |

14 EXAMPLE OF TYPICAL CALIBRATOR CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

| Calibrator | Optical Units |
|----------------------------|---------------|
| Calibrator 0 (0 ng/ml) | 1.91 |
| Calibrator 1 (0.3 ng/ml) | 1.58 |
| Calibrator 2 (1.2 ng/ml) | 1.25 |
| Calibrator 3 (4.0 ng/ml) | 0.90 |
| Calibrator 4 (15.00 ng/ml) | 0.58 |
| Calibrator 5 (40.00 ng/ml) | 0.36 |

15 PERFORMANCE CHARACTERISTICS

15.1 Sensitivity

The lowest detectable level of Free Estriol was assessed to be ≤ 0.02 ng/ml

15.2 Specificity

The Specificity of the Free Estriol ELISA has been tested according to the method described by Abraham.

| Steroid | Crossreactivity (%) |
|------------------------|---------------------|
| Estriol | 100 |
| 16-Epiestriol | 2.70 |
| 16,17-Epiestriol | 0.080 |
| Estradiol-17 β | 0.038 |
| Estradiol-17a | 0.037 |
| 17-Epiestriol | 0.0128 |
| Corticosterone | 0.0003 |
| 5a-Dihydrotestosterone | 0.00004 |
| Testosterone | n.d. |
| 11-Desoxycortisol | n.d. |
| Estrone | n.d. |

n.d. = non detectable

15.3 Precision

| Serum | Intraassay | | | Interassay | | |
|-------|------------|-------------------|---------|------------|-------------------|---------|
| | n | <X> ± SD ng/ml | CV % | n | <X> ± SD ng/ml | CV % |
| 1 | 12 | 3.19 ± 0.14 | 4.27 | 18 | 3.19 ± 0.19 | 5.96 |
| 2 | 12 | 12.36 ± 0.54 | 4.36 | 18 | 12.68 ± 0.62 | 4.88 |
| 3 | 12 | 26.14 ± 1.19 | 4.54 | 18 | 26.83 ± 1.48 | 5.53 |

15.4 Accuracy

The accuracy of the assay was evaluated by recovery and dilution tests.

15.5 Recovery test

| Serum | Endogenous Free Estriol ng/ml | Added Free Estriol ng/ml | Recovery % |
|-------|-------------------------------------|--------------------------------|------------|
| 1 | 1.47 | 2.00 | 100.1 |
| | | 7.50 | 108.1 |
| | | 20.00 | 99.2 |
| 2 | 6.44 | 2.00 | 95.0 |
| | | 7.50 | 103.1 |
| | | 20.00 | 99.0 |
| 3 | 13.66 | 2.00 | 95.8 |
| | | 7.50 | 107.3 |
| | | 20.00 | 101.9 |

15.6 Dilution test

| Serum | Dilution factor | Measured conc. ng/ml Free Estriol | Recovery % |
|-------|-----------------|--------------------------------------|------------|
| 1 | Undiluted | 22.20 | |
| | 1:2 | 11.11 | 100.1 |
| | 1:4 | 5.29 | 95.3 |
| | 1:8 | 2.85 | 102.7 |
| | 1:16 | 1.41 | 101.6 |
| 2 | Undiluted | 26.80 | |
| | 1:2 | 14.20 | 106.0 |
| | 1:4 | 6.54 | 97.6 |
| | 1:8 | 3.34 | 99.7 |
| | 1:16 | 1.77 | 105.7 |
| 3 | Undiluted | 34.35 | |
| | 1:2 | 17.81 | 103.7 |
| | 1:4 | 8.56 | 99.7 |
| | 1:8 | 4.30 | 100.1 |
| | 1:16 | 2.08 | 96.9 |

15.7 Quality Control

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

We recommend to use BIO RAD Lyphochek Immunoassay Control Sera .

PROCEDURE FLOW SHEET free Estriol Elisa BL-44-E

| Description | Calibrator Sample µl | Enzyme Conjugate µl | | TMB Substrate Solution µl | | Stop Solution µl | | Results ng/ml |
|--------------|----------------------------|---------------------------|------------------|---------------------------------|-------------|------------------------|-------------|------------------|
| Calibrator 0 | 10 | 100 | Incubate | 100 | Incubate | 100 | OD at | 0 |
| Calibrator 1 | 10 | 100 | for 60 min | 100 | for | 100 | 450 ± 10 nm | 0,3 |
| Calibrator 2 | 10 | 100 | at room | 100 | 30 min | 100 | with a | 1,2 |
| Calibrator 3 | 10 | 100 | temperature | 100 | at room | 100 | Microtiter- | 4 |
| Calibrator 4 | 10 | 100 | | 100 | temperature | 100 | plate | 15 |
| Calibrator 5 | 10 | 100 | Rinse wells | 100 | | 100 | reader | 40 |
| Sample 1 | 10 | 100 | 3 times with | 100 | | 100 | | - |
| Sample 2 | 10 | 100 | Wash Solution | 100 | | 100 | | - |
| Sample 3 | 10 | 100 | | 100 | | 100 | | - |