BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Unconjugated Estriol

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REF 33570

FOR PROFESSIONAL USE ONLY

Rx Only

PRINCIPLE

INTENDED USE

The Access Unconjugated Estriol assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of unconjugated estriol levels in human serum using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

The presence of unconjugated estriol (1,3,5(10)-Estratrien-3,16α,17β-triol) in maternal serum is due primarily to the secretion of estriol by the fetal liver and placenta. Estriol precursors, cholesterol and pregnenolone are derived from the mother and the placenta. The fetal adrenal glands convert pregnenolone to dehydroepiandrosterone (DHEA), which is then converted to 16-OH-DHEA-sulfate by the fetal liver. The sulfated derivative then passes to the placenta where it is converted to estriol and enters the maternal plasma.[1] Once in the maternal circulation its half-life is approximately 20 minutes before conjugation in the liver.

Estriol comprises 90% of the circulating estrogens in normal pregnancies. Determining the serum levels of the unconjugated form provides a sensitive indicator of fetal well-being and placental function.[2]

METHODOLOGY

The Access Unconjugated Estriol assay is a competitive binding immunoenzymatic assay. Sample is added to a reaction vessel with estriol-alkaline phosphatase conjugate, paramagnetic particles coated with goat anti-rabbit IgG, and polyclonal rabbit anti-estriol. Estriol in the sample competes with the estriol-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-estriol antibody. The resulting antigen: antibody complexes are bound to the capture antibody on the solid phase.

After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of estriol in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum is the recommended sample.
2. Observe the following recommendations for handling, processing, and storing blood samples:[3]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.

- Keep tubes stoppered at all times.
- Physically separate serum from contact with cells as soon as possible.
- Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than 8 hours.
- If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
- If the assay will not be completed within 14 days, or for shipment of samples, freeze at -20°C or colder.

3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Thaw samples no more than two times.

REAGENTS

PRODUCT INFORMATION

Access Unconjugated Estriol Reagent Pack

Cat. No. 33570: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of 2 hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-rabbit IgG suspended in TRIS buffered saline with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.0125% Cosmocil* CQ.
R1b:	Rabbit anti-estriol in TRIS buffered saline with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide and 0.0125% Cosmocil CQ.
R1c:	Estriol-alkaline phosphatase (bovine) conjugate in HEPES buffered saline with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide and 0.0125% Cosmocil CQ.

*Cosmocil is a trademark of Arch Chemicals, Inc.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

Avoid skin contact with reagent. Use water to wash reagent from skin.

> ⚠ **CAUTION**
>
> **Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
> **To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Unconjugated Estriol Calibrators
   Provided at zero and approximately 0.07, 0.17, 0.34, 0.86, 3.4, and 6.9 ng/mL (0.24, 0.60, 1.2, 3.0, 12, and 24 nmol/L).
   Cat. No. 33575
2. Quality Control (QC) materials: commercial control material
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1	Access Unconjugated Estriol Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access Unconjugated Estriol assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[4] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use twenty-five (25) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 3.467.

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

EXPECTED RESULTS

Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.017-6.9 ng/mL [0.059-24.0 nmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.017 ng/mL [< 0.059 nmol/L]).
   - If a sample contains more than the stated value of the highest Access Unconjugated Estriol Calibrator (S6), report the result as greater than that value (i.e., > 6.9 ng/mL [> 24.0 nmol/L]). Alternatively, dilute one volume of sample with one or two volumes of Access Unconjugated Estriol Calibrator S0 (zero). Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[5,6]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[7,8] Carefully evaluate the results of patients suspected of having these types of interferences.
4. The Access Unconjugated Estriol results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

A comparison of serum unconjugated estriol values using the Access Unconjugated Estriol assay on the Access Immunoassay system and a commercially available radioimmunoassay kit gave the following statistical data using Deming calculations:[9]

n	Range of Observations (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
80	0.040 - 6.423	0.212	1.062	0.949

DILUTION RECOVERY (LINEARITY)

Serial dilution of four patient samples with Access Unconjugated Estriol Calibrator S0 (zero) resulted in the following data:

Sample 1	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	1.742	N/A
1/2	0.871	0.807	92.7
1/4	0.436	0.391	89.7
		Mean % Recovery	**91.2**

Sample 2	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	3.191	N/A
1/2	1.596	1.514	94.9
1/4	0.798	0.676	84.7
		Mean % Recovery	**89.8**

Sample 3	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	3.976	N/A
1/2	1.988	1.759	88.5
1/4	0.994	0.870	87.5
		Mean % Recovery	**88.0**

Sample 4	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	6.006	N/A
1/2	3.003	2.840	94.6
1/4	1.502	1.306	87.0
		Mean % Recovery	**90.8**

IMPRECISION

One study, using commercially available human serum based control material generating a total of 20 assays, 3 replicates per assay, over 10 days provided the following data, analyzed via analysis of variance (ANOVA).[10,11]

Sample	Grand Mean (n=60) (ng/mL)	Within Run SD (ng/mL)	Within Run (%CV)	Total SD (ng/mL)	Total Imprecision (% CV)
Low	0.267	0.016	6.15	0.029	10.75
Medium	2.594	0.065	2.51	0.123	4.73
High	4.832	0.085	1.76	0.165	3.42

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 20 mg/dL (342 µmol/L) bilirubin, 500 mg/dL (5 g/L) hemoglobin, the equivalent of 1,800 mg/dL (20.32 mmol/L) triglycerides (Triolein), 10 g/dL (100 g/L) total protein do not significantly affect the measurement of unconjugated estriol (± 10%).

The percentage of cross-reactivity to estriol conjugated compounds and other steroids were determined by spiking 50 ng/mL of the potential cross-reactant into a calibrator matrix containing approximately 0.4 ng/mL of unconjugated estriol.

Substance	Analyte Added (ng/mL)	Cross-reactivity (%)
Estriol-3 sulfate	50	1.14
Estriol-3β glucuronide	50	1.47
Estriol-16β glucuronide	50	0.44
Estriol-17β glucuronide	50	ND†
16-Epiestriol	50	0.70
17-Epiestriol	50	0.41
β-Estradiol	50	0.33
Estrone	50	0.03
Estrone sulfate	50	ND
Estrone glucuronide	50	ND
Cortisol	50	ND
11-deoxycortisol	50	0.06
Progesterone	50	ND
17α-hydroxyprogesterone	50	ND
Testosterone	50	ND
5α-dihydrotestosterone	50	ND
DHEA-S	50	ND
Dexamethasone	50	ND

† ND = None detected

ANALYTICAL SENSITIVITY

The lowest detectable level of unconjugated estriol distinguishable from zero (Access Unconjugated Estriol Calibrator S0) with 95% confidence is 0.017 ng/mL (0.059 nmol/L). This value is determined by processing a complete seven point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

ADDITIONAL INFORMATION

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REVISION HISTORY

Revision M

IFU updated to add Dutch, Macedonian, and Traditional Chinese

Revision N

Remove CE Mark and EC REP Address, Update Website Address

Revision P

Added Translations.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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