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Performance of an Improved Automated Chemiluminescent Assay for Unconjugated Estriol on the IMMULITE 2000 Analyzer

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Abstract

Background: Unconjugated estriol is present in maternal serum as a result of the secretion of estriol by the fetus and placenta. On traversing the placenta, estriol is rapidly metabolized to conjugated forms. Unconjugated estriol accounts for about 9% of the total estriol in circulation. Normally, as the fetus develops, estriol production increases. Serum levels of estriol provide a sensitive indicator of fetal maturity and well-being, and reduced levels of estriol have been found to indicate fetal distress.

Method: An improved unconjugated estriol assay on the IMMULITE® 2000 system has been developed for the quantitative measurement of unconjugated estriol in serum. The assay is designed as a one-step, solid-phase competitive chemiluminescent enzyme immunoassay with a 30-minute incubation time. Estriol in the sample competes with an estriol–alkaline phosphatase conjugate for limited binding sites on an antiestriol polyclonal antibody adsorbed to a polystyrene bead. Unbound material is removed during a centrifugal wash procedure. The test requires a sample volume of 40 µL. The assay calibration was based on GC-MS-measured unconjugated estriol in pregnancy samples.

Results: Method comparison to GC-MS with 58 samples yielded the following weighted Deming regression characteristics: slope = 0.98, intercept = 0.02 ng/mL, and r = 0.97. The reportable range was 0.07–12 ng/mL. The LoB of the assay was 0.01 ng/mL, the LoD was 0.05 ng/mL, and the functional sensitivity at the 20% CV was 0.1 ng/mL. Precision was assessed according to the CLSI protocol EP5-A (n = 80) on unconjugated estriol–spiked normal female serum pools using two IMMULITE 2000 kits comprising two different reagent and bead lots. The mean within-run imprecision at 0.27, 1.0, and 9.6 ng/mL was 11.1%, 6.5%, and 5.6%, respectively. The mean total imprecision at the same concentrations was 12.4%, 7.4%, and 6.1%, respectively. Dilutional linearity of six pregnancy serum samples with the zero calibrator matrix provided mean recoveries of 94%–111%, whereas those for unconjugated estriol spiked into six pregnancy serum pools were 96%–104%.

Conclusions: The data demonstrate that the improved IMMULITE 2000 unconjugated estriol assay offers a faster assay time, improved precision at the low end, and a clinically reliable automated method for the routine measurement of unconjugated estriol in pregnancy serum samples.

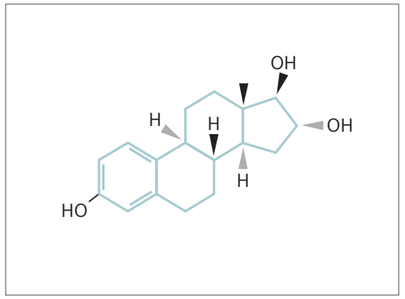
Background

- Estriol [1,3,5(10)-estriene-3,16-α, 17-β-triol] (MW 288 Da) is an estrogen with three hydroxyl groups at positions 3, 16 and 17 (Figure 1).

- Most of the estriol circulating or excreted during the third trimester of pregnancy is the joint product of fetus and placenta, originating from a precursor synthesized in the fetus by the adrenal glands and transformed by the fetal liver and the placenta into estriol. Therefore, estriol production is a function of the fetoplacental unit. On traversing the placenta, estriol is rapidly metabolized, primarily in the maternal liver, to conjugated forms: the estriol sulfates and glucuronides. As a result, “free” estriol, the unconjugated form, accounts for barely nine percent of the total estriol in circulation.

- Normally, as the fetus develops, estriol production increases, resulting in a nearly threefold rise in circulating estriol levels during the final trimester. Serum levels of estriol provide a sensitive indicator of fetal maturity and well-being, and persistently low or rapidly falling estriol levels suggest fetal distress.

Figure 1. Unconjugated estriol chemical structure



- In combination with other techniques for fetal surveillance, serial determinations have been used in the management of pregnancies complicated by diabetes,[1,2] hypertension, prolonged gestation and uncertain dates. These clinical applications have been reviewed.[1–4]

- The IMMULITE 2000 unconjugated estriol assay is intended as an aid in monitoring fetal maturity and well-being in the context of high-risk and poorly dated pregnancies.

- The purpose of this study was to evaluate the analytical performance of an improved unconjugated estriol assay on the IMMULITE 2000 analyzer.

Assay Principle

- An improved unconjugated estriol assay on the IMMULITE 2000 system has been developed for the quantitative measurement of unconjugated estriol in serum.

- The assay is designed as a one-step, solid-phase competitive chemiluminescent enzyme immunoassay with a 30-minute incubation time (Figure 2).

- Unconjugated estriol in the sample competes with an estriol-alkaline phosphatase conjugate for limited binding sites of an antiestriol polyclonal antibody adsorbed to a polystyrene bead.

- After incubation, unbound material is removed during a centrifugal wash procedure.

- The substrate is added and the chemiluminescent signal is measured.

- The test requires a sample volume of 40 µL.

- This format gives the first result in 35 minutes. The IMMULITE 2000 system has a throughput of up to 200 tests per hour.

Results

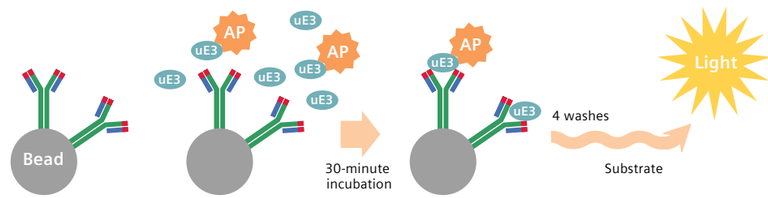
Reportable range

Assay Reportable range	0.07 to 12 ng/mL

Standardization to Gas Chromatography–Mass Spectroscopy (GC-MS)

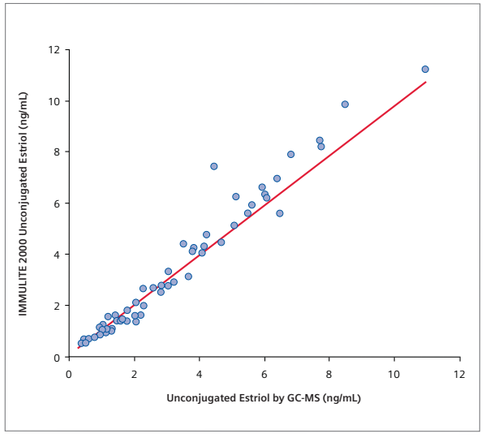
- The assay calibration was determined on the basis of unconjugated estriol measurement in pregnancy samples by GC-MS. The assay's traceability to GC-MS is shown in Figure 3.

Figure 2. IMMULITE 2000 Unconjugated Estriol Assay Principle



- Comparison of IMMULITE 2000 to GC-MS results with 58 serum samples yielded the following weighted Deming regression equation: IMMULITE 2000 = 0.98(GC-MS) + 0.02 ng/mL, R = 0.98.

Figure 3. Method comparison between the IMMULITE 2000 unconjugated estriol assay and GC-MS



Analytical Sensitivity and Functional Sensitivity

- The limit of blank (LoB), determined in accordance with CLSI EP17-A, was estimated as the highest value expected for a sample with no analyte.

- The limit of detection (LoD), determined in accordance with CLSI EP17-A, was estimated as the lowest consistently detectable concentration.

- The functional sensitivity was estimated as the lowest concentration at or above the LoD that can be expected to yield a total coefficient of variation (CV) deemed optimal for the assay's intended applications. For this assay, that CV was deemed to be 20%.

- Estimates of the LoB, LoD, and functional sensitivity, determined from the pooled results of two IMMULITE 2000 kits comprising two different reagent and bead lots, are shown in Table 1.

Table 1. Analytical sensitivity and functional sensitivity of the unconjugated estriol assay on the IMMULITE 2000 system.

LoB (ng uE3/mL)	LoB (ng uE3/mL)	Functional Sensitivity (ng uE3/mL)
0.01	0.04	0.09

Assay Imprecision

- Precision was assessed according to the CLSI protocol EP5-A from the results of 80 replicates on unconjugated estriol–spiked normal female serum pools.

- The precision data, representing the pooled results of two IMMULITE 2000 kits comprising two different reagent and bead lots, is shown in Table 2.

Table 2. Within-run and total imprecision for the IMMULITE 2000 unconjugated estriol assay

Sample	Mean ng uE3/mL	Within-run SD	Within-run CV	Total SD	Total CV
1	0.12	0.02	14.6%	0.02	16.7%
2	0.27	0.03	11.1%	0.03	12.4%
3	0.59	0.05	8.2%	0.05	9.2%
4	1.03	0.07	6.5%	0.08	7.4%
5	2.21	0.14	6.3%	0.14	6.4%
6	6.72	0.31	4.6%	0.37	5.6%
7	9.59	0.53	5.6%	0.58	6.1%

Linearity

- Dilutional linearity of six pregnancy serum samples tested undiluted and diluted at several levels with the zero calibrator matrix is shown in Table 3.
- The mean recoveries ranged from 94% to 111%.

Table 3. Dilutional linearity for the IMMULITE 2000 unconjugated estriol assay

Sample	Dilution	Observed	Expected	% O/E	Mean
1	8 in 8	1.56	-	-	
	4 in 8	0.83	0.78	106%	
	2 in 8	0.44	0.39	113%	111%
	1 in 8	0.22	0.20	113%	
2	8 in 8	2.60	-	-	
	4 in 8	1.23	1.30	95%	
	2 in 8	0.62	0.65	95%	94%
	1 in 8	0.30	0.33	92%	
3	8 in 8	3.85	-	-	
	4 in 8	1.98	1.93	103%	
	2 in 8	0.90	0.96	94%	97%
	1 in 8	0.46	0.48	96%	
4	8 in 8	5.85	-	-	
	4 in 8	2.74	2.93	94%	
	2 in 8	1.39	1.46	95%	94%
	1 in 8	0.68	0.73	93%	
5	8 in 8	6.23	-	-	
	4 in 8	3.14	3.12	101%	
	2 in 8	1.55	1.56	100%	102%
	1 in 8	0.82	0.78	105%	
6	8 in 8	7.53	-	-	
	4 in 8	3.72	3.77	99%	
	2 in 8	2.02	1.88	107%	101%
	1 in 8	0.90	0.94	96%	

Analyte Recovery

- Six pregnancy serum pools spiked 1 to 19 with three unconjugated estriol solutions (A, 12.0; B, 24.8; and C, 44.2 ng/mL) were assayed for analyte recovery.
- The average recoveries ranged from 96% to 104% and are shown in Table 4.

Table 4. Analyte recovery for the IMMULITE 2000 unconjugated estriol assay

Sample	Dilution	Observed	Expected	% O/E	Mean
1	-	1.86	-	-	
	A	2.21	2.37	93%	
	B	2.78	3.01	92%	96%
	C	4.08	3.98	103%	
2	-	2.32	-	-	
	A	2.92	2.80	104%	
	B	3.75	3.44	109%	104%
	C	4.40	4.41	100%	
3	-	3.99	-	-	
	A	4.17	4.39	95%	
	B	5.28	5.03	105%	101%
	C	6.09	6.00	102%	
4	-	5.10	-	-	
	A	5.33	5.45	98%	
	B	5.72	6.09	94%	99%
	C	7.46	7.06	106%	
5	-	6.70	-	-	
	A	6.91	6.97	99%	
	B	7.41	7.61	97%	101%
	C	9.11	8.58	106%	
6	-	7.80	-	-	
	A	8.05	8.01	100%	
	B	8.55	8.65	99%	100%
	C	9.78	9.62	102%	

Conclusions

The improved IMMULITE 2000 unconjugated estriol assay offers:

- Standardization to GC-MS
- Improved precision at the low end
- A faster time-to-first-result
- A clinically reliable automated method for the routine measurement of unconjugated estriol in pregnancy serum samples.

IMMULITE 2000

Acknowledgements

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