



SALIVARY ESTRIOL/HS ESTRIOL ENZYME IMMUNOASSAY KIT

Catalog No. 1-2812, (Single) 96-Well Kit;
1-2812-5, (5-Pack) 480 Wells

Intended Use

The Salimetrics™ estriol/high sensitivity (HS) estriol kit is a competitive immunoassay specifically designed and validated for the quantitative *in vitro* diagnostic measurement of salivary estriol. Salivary estriol accurately reflects the amount of serum estriol in the circulation. Salimetrics has not validated this kit for use with serum/plasma samples. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics.

This instruction sheet contains two assay protocols. Use "Method A: Salivary High Sensitivity Estriol Procedure" for expected values below 20 pg/mL, such as in normal adults. Use "Method B: Salivary Estriol Procedure" for expected values in the range of 20 pg/mL or higher, such as in pregnancy.

Introduction

Estriol (1,3,5(10)-estratriene-3,16 α ,17 β -triol; E₃) is a female sex steroid hormone produced almost exclusively by the placenta during pregnancy, and is the major estrogen produced in the normal human fetus. During pregnancy the production of estriol depends on an intact maternal-placental-fetal unit. Steroid precursors from the maternal circulation are first converted to progesterone in the placenta. Progesterone is subsequently converted to dehydroepiandrosterone sulfate (DHEA-S) in fetal adrenal tissue and then 16 α -hydroxylated in the fetal liver. Subsequently, 16 α -hydroxy-DHEA-S is converted to estriol in the placenta. In non-pregnant circumstances the majority of estriol is derived from 17 β -estradiol. Estriol exists in biological matrix in unconjugated (~9%) and conjugated forms (~91%). In the maternal circulation, estriol is conjugated with glucuronic or sulfuric acid in the liver followed by urinary excretion with a half-life of ~20 minutes.

Fetal placenta production leads to a progressive rise in maternal circulating estriol levels reaching a peak in the 3rd trimester. 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. Estriol concentrations are subject to diurnal and episodic variation. With respect to estrogenic activity, estriol is less potent than estradiol, and the physiologic role of estriol is not known.

Research has established links between individual differences in estriol during pregnancy and birth characteristics such as birth weight and length, placenta weight, gestation duration, and the onset of labor. Many immunoassay and bioassay methods have been used to quantify estrogens. Recent studies suggest that estriol can be accurately measured in saliva of healthy pregnant women, and that there is a nonlinear rise in salivary estriol concentrations beginning from 30 weeks gestation until term (an increase of ~200%). At 35 weeks gestation the salivary estriol concentration median value increases sharply (positive inflection point, ~50-90% increase over baseline) at a demarcation between a slower increase during early pregnancy and a more rapid increase during late pregnancy. Other studies show that betamethasone suppresses salivary estriol consistent with the effect of glucocorticoids on fetal adrenal estriol precursors.

In the blood, only 1 to 15% of estriol is in its unbound or biologically active form. The remaining estriol is bound to serum proteins. Unbound serum estriol enters the saliva via intracellular mechanisms, and in saliva the majority of estriol remains unbound to protein. Salivary estriol levels are unaffected by salivary flow rate or salivary enzymes (1).

This kit is designed to measure estriol levels in saliva. The standard is in a saliva-like matrix. In addition, a built-in pH indicator warns the user of acidic or basic samples.

Test Principle

A microtitre plate is coated with rabbit antibodies to estriol. Estriol in standards and unknowns competes with estriol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound estriol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 2 molar-sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of estriol peroxidase detected is inversely proportional to the amount of estriol present (2).

Precautions

1. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.
2. Saliva samples are to be run at a 2x dilution for the **HS estriol procedure only**. Do not dilute the samples on the plate. Prepare the sample dilution outside of the sample well prior to testing.
3. Liquid stop solution is a 2-molar solution of sulfuric acid. This solution is caustic; use with care.

4. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
5. Do not mix components from different lots of kits.
6. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
7. See "Material Safety Data" at the end of procedure.
8. We recommend that samples be screened for possible blood contamination (3,4) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Cat No: 1-1302/1-1312). Do not use dipsticks, which result in false positive values due to salivary enzymes.
9. Routine calibration of pipettes is critical for the best possible assay performance.
10. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
11. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
12. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
13. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
14. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Anti-Estriol Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with rabbit anti-estriol antibodies in a resealable foil pouch.
2. **Estriol Standard:** 0.5 mL of estriol in a saliva-like matrix with a non-mercury preservative, at a concentration of 4860 pg/mL. Further dilution of the standard is necessary only for **Method A: Salivary HS Estriol Procedure** (see procedure for details).
3. **Estriol Controls:** Two vials, 0.5 mL each, containing high (3000 pg/mL) and low (50 pg/mL) concentrations of estriol in a saliva-like matrix with a non-mercury preservative. Further dilution of the controls is necessary to obtain the high and low controls only for **Method A: Salivary HS Estriol Procedure** (see procedure for details).
4. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H₂O). (*Note: If precipitate has formed in the concentrated wash buffer, heat to 60°C for 15 minutes to dissolve crystals. Cool to room temperature before use in assay.*)
5. **Estriol Assay Diluent:** 60 mL of a phosphate buffered solution containing a non-mercury preservative.
6. **Enzyme Conjugate:** 50 μ L of a solution of estriol labeled with horseradish peroxidase. Dilute prior to use with assay diluent.
7. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 12.5 mL of a 2-molar solution of sulfuric acid.
9. **Non-specific Binding Wells (NSB):** One strip of wells that do not contain anti-estriol antibody. They are located in the foil pouch. Wells may be broken off and inserted as blanks (optional) where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 7 μ L to 500 μ L
- Precision multichannel pipette to deliver 50 μ L, 100 μ L, and 200 μ L
- Vortex
- Plate rotator with 0.08-0.17 inch orbit
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One 15 mL disposable tube
- Small disposable tubes for dilution of standard, controls, and samples
- Pipette tips
- Serological pipette to deliver 12 mL or 14 mL
- Refrigerator

Specimen Collection

Due to the episodic secretion pattern of steroid hormones, we can expect reproducible and reliable results only in cases of multiple sampling. Therefore, we recommend taking a minimum of 3 samples within at least a 2-hour period and pooling the samples before testing (5,6).

The preferred method for collecting whole saliva is by unstimulated passive drool. Collection protocols are available on request. **Do not use Salivettes, the Salimetrics Oral Swab (SOS), Sorbettes, cotton, or polyester materials to collect samples.** False readings will result (7). Do **not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected. Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH

and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Record the time and date of specimen collection. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20°C within 4 hours of collection. (Samples may be stored at -20°C or lower for long term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. It is important to avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Limitations

- Samples with estriol values less than 20 pg/mL should be run using the HS Estriol assay. If values obtained are greater than 1215 pg/mL, re-run sample using the regular assay protocol. Any values obtained greater than 4860 pg/mL should be further diluted with estriol assay diluent and rerun for accurate results. To obtain the final estriol concentration, multiply the concentration of the diluted sample by the dilution factor.
- See "Specimen Collection" recommendations to insure proper collection of saliva specimens and to avoid interfering substances.
- Samples collected with sodium azide are unsuitable for this assay.
- Diagnostic ranges have been determined only for female salivary samples.
- Salivary estriol concentrations in pregnant women greater than 2100 pg/mL indicate the possibility of pre-term labor. Any quantitative results above this level should be followed by additional testing and evaluation.

Quality Control

The Salimetrics' high and low salivary estriol controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Method A: Salivary HS Estriol Procedure (expected values < 20 pg/mL):

(Proceed to Method B if expected values are \geq 20 pg/mL.)

Bring all reagents to room temperature. **Note:** It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.

Refer to "Reagents and Reagent Preparation" for directions on preparation of the 1215 pg/mL standard, the 500 pg/mL high control, and the 50 pg/mL low control.

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1215 Std	1215 Std	C-H	C-H								
B	405 Std	405 Std	C-L	C-L								
C	135 Std	135 Std	Unk-1	Unk-1								
D	45 Std	45 Std	Unk-2	Unk-2								
E	15 Std	15 Std	Unk-3	Unk-3								
F	5 Std	5 Std	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB	NSB	Unk-6	Unk-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder. Break off the bottom wells in each strip. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.
2. Do not insert wells from one plate into a different plate.

Step 3:

- Dilute the 4860 pg/mL standard 1:8 by adding 100 μ L of the standard to 700 μ L of estriol assay diluent. Label this tube 1215 pg/mL (tube 1). **Note:** The actual concentration of estriol in the standard is 607.5 pg/mL. Given that samples are run at a 2x dilution, the concentration of the standard curve has been adjusted for your convenience in order to eliminate the need to multiply all sample results by 2.
- Label five microcentrifuge tubes or other small tubes 2 through 6.
- Pipette 300 μ L of estriol assay diluent into tubes 2 through 6. Serially dilute the standard 3X by adding 150 μ L of the 1215 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 150 μ L from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6, are respectively, 1215 pg/mL, 405 pg/mL, 135 pg/mL, 45 pg/mL, 15 pg/mL, and 5 pg/mL. Standard concentrations in pmol/L are 4213.34, 1404.45, 468.15, 156.05, 52.02, and 17.34, respectively.
- Pipette 14 mL of estriol assay diluent into a disposable tube. (Scale down proportionally if not using the entire plate.) Set aside for Step 5.

Step 4:

- Predilute the 3000 pg/mL high control 1:6, by adding 100 μ L of the high to 500 μ L of estriol assay diluent. Label this tube 500 pg/mL. Predilute the 50 pg/mL low control 1:3.333 by adding 150 μ L of the low to 350 μ L of estriol assay diluent. Label this tube 15 pg/mL.
- Using small disposable tubes, such as microcentrifuge tubes, further dilute the 500 and 15 pg/mL controls and dilute the unknown samples 2x using 150 μ L of estriol assay diluent and 150 μ L of control or unknown sample. Mix well.

Step 5:

- Pipette 100 μ L of standards, diluted controls, and diluted unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 100 μ L of estriol assay diluent into 2 wells to serve as the zero.
- Pipette 100 μ L of estriol assay diluent into each NSB well.

Step 6:

Dilute the enzyme conjugate 1:2000 by adding 7 μ L of the conjugate to the 14 mL of estriol assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and add 100 μ L to each well using a multichannel pipette.

Step 7:

Cover plate with adhesive cover provided. Incubate overnight (20 - 24 hours), at 4°C with constant mixing at 500 rpm.

Step 8:

Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 μ L of wash buffer into each well and then decanting the liquid into a sink. After each wash, blot plate on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 9:

Add 200 μ L of TMB solution to each well with a multichannel pipette.

Step 10:

Incubate in the dark at room temperature for 45 minutes with constant mixing at 500 rpm.

Step 11:

Add 50 μ L of stop solution with a multichannel pipette.

Step 12:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow. **Caution:** Do not mix at speeds over 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

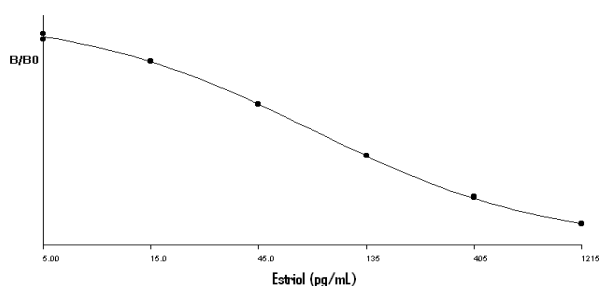
1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD from the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

Typical Results

The following results are shown for illustration only and *should not* be used to calculate results from another assay.

Well	Standard	Average OD	B	B/Bo	Estriol (pg/mL)
A1,A2	S1	0.119	0.096	0.061	1215
B1,B2	S2	0.318	0.295	0.188	405
C1,C2	S3	0.624	0.601	0.383	135
D1,D2	S4	1.004	0.981	0.626	45
E1,E2	S5	1.321	1.298	0.828	15
F1,F2	S6	1.503	1.480	0.944	5
G1,G2	Bo	1.591	1.568	NA	NA
H1,H2	NSB	0.023	NA	NA	NA

Example: HS Estriol 4-Parameter Sigmoid Minus Curve Fit



Salivary High Sensitivity Estriol Performance Characteristics

A. Recovery:

Three saliva samples were spiked with 3 different levels of estriol and assayed.

Sample	Endogenous (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
I	361.7	13.5	375.2	369.53	98.5
II	6.42	972	978.42	880.78	90.0
III	0	121.5	121.5	125.33	103.2

B. Linearity of Dilution

Two saliva samples were diluted with estriol assay diluent and assayed.

Sample	Dilution Factor	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
I			156.71	
	1:2	78.36	68.60	87.5
	1:4	39.18	34.76	88.7
	1:8	19.59	18.44	94.1
	1:16	9.79	9.69	99.0
II			789.84	
	1:2	394.92	409.60	103.7
	1:4	197.46	214.78	108.8
	1:8	98.73	113.3	114.8
	1:16	49.37	53.53	108.4

C. Sensitivity:

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 10 sets of duplicates at the 0 pg/mL level. The minimal concentration of estriol that can be distinguished from 0 is 1 pg/mL.

D. Precision:

The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
High	12	534.5	10.84	2.0
Low	12	15.04	1.25	8.3

The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
Low	12	18.19	2.63	14.5
High	12	488.30	20.32	4.2

E. Correlation with Serum

The correlation between saliva and serum estriol in pregnant and non-pregnant females was determined by assaying 35 matched samples. The saliva-serum correlation was highly significant, $r(33) = 0.87$, $p < 0.001$.

F. *Salivary Estriol Expected Ranges

Group	Time	N	Range +/- 2SD's (pg/mL)	Absolute Range (pg/mL)
Premenopausal Adult Females	AM	17	0 - 16.4	0 - 28.12
Premenopausal Adult Females	PM	16	0 - 4.8	0 - 6.88

*To be used as a guide only. Each laboratory should establish its own range.

Method B: Salivary Estriol Procedure (expected values \geq 20 pg/mL):

Bring all reagents to room temperature. **Note:** It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	4860 Std	4860 Std	C-H	C-H								
B	1620 Std	1620 Std	C-L	C-L								
C	540 Std	540 Std	Unk-1	Unk-1								
D	180 Std	180 Std	Unk-2	Unk-2								
E	60 Std	60 Std	Unk-3	Unk-3								
F	20 Std	20 Std	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB	NSB	Unk-6	Unk-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder. Break off the bottom wells in each strip. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.
2. Do not insert wells from one plate into a different plate.

Step 3:

- Label five microcentrifuge tubes or other small tubes 2 through 6.
- Pipette 100 μ L of estriol assay diluent into tubes 2 through 6. Serially dilute the standard 3X by adding 50 μ L of the 4860 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 50 μ L from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6, are respectively, 4860 pg/mL, 1620 pg/mL, 540 pg/mL, 180 pg/mL, 60 pg/mL, and 20 pg/mL. Standard concentrations in nmol/L are 16.85, 5.62, 1.87, 0.62, 0.21, and 0.07, respectively.
- Pipette 12 mL of estriol assay diluent into a disposable tube. (Scale down proportionally if not using the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 25 μ L of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 25 μ L of estriol assay diluent into 2 wells to serve as the zero.
- Pipette 25 μ L of estriol assay diluent into each NSB well.

Step 5: Dilute the enzyme conjugate 1:800 by adding 15 μ L of the conjugate to the 12 mL of estriol assay diluent prepared in Step 3. (Scale down proportionally if not using entire plate.) Immediately mix the diluted conjugate solution and add 100 μ L to each well using a multichannel pipette.

Step 6: Cover plate with adhesive cover provided. Incubate 2 hours at room temperature with constant mixing at 500 rpm.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 μ L of wash buffer into each well and then decanting the liquid into a sink. After each wash, blot plate on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 100 μ L of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix). Incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 100 μ L of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow. **Caution:** Do not mix at speeds over 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

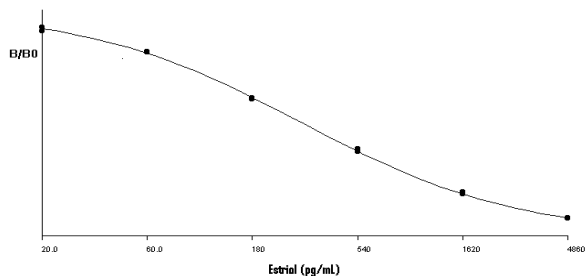
- Compute the average optical density (OD) for all duplicate wells.
- Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
- Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
- Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

Typical Results

The following results are shown for illustration only and *should not* be used to calculate results from another assay.

Well	Standard	Average OD	B	B/Bo	Estriol (pg/mL)
A1,A2	S1	0.113	0.097	0.076	4860
B1,B2	S2	0.257	0.241	0.188	1620
C1,C2	S3	0.502	0.486	0.379	540
D1,D2	S4	0.798	0.782	0.610	180
E1,E2	S5	1.063	1.047	0.817	60
F1,F2	S6	1.192	1.176	0.917	20
G1,G2	Bo	1.298	1.282	NA	NA
H1,H2	NSB	0.016	NA	NA	NA

Example: Estriol 4-Parameter Sigmoid Minus Curve Fit



Salivary Estriol Performance Characteristics

A. Sensitivity:

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 10 sets of duplicates at the 0 pg/mL level. The minimal concentration of estriol that can be distinguished from 0 is 16 pg/mL.

B. Linearity of Dilution:

Two saliva samples were diluted with estriol assay diluent and assayed.

Sample	Dilution Factor	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
I			539.52	
	1:2	269.76	244.36	90.6
	1:4	134.88	112.89	83.7
	1:8	67.44	56.47	83.7
II	1:16	33.72	31.09	92.2
			1912.24	
	1:2	956.12	941.92	98.5
	1:4	478.06	472.93	98.9
	1:8	239.03	248.18	103.8
	1:16	119.52	125.71	105.2

C. Recovery:

Three saliva samples were spiked with 3 different levels of estriol and assayed.

Sample	Endogenous (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
I	0	2000.00	2000.00	2099.10	105.0
	0	486.00	486.00	510.80	105.3
II	381.65	2000	2381.65	2678.39	112.5
	381.65	486.00	867.65	967.46	111.5
III	381.65	18.00	399.65	400.45	100.2
	921.00	2000	2921.00	3225.35	110.4
	921.00	486.00	1407.00	1532.18	108.9
	921.00	18.00	939.00	989.53	105.4

D. Precision:

The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
High	12	3049.47	94.53	3.1
Low	12	67.05	6.02	9.0

The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
Low	12	60.47	5.06	8.4
High	12	3173.1	182.79	5.8

E. Correlation with Serum

The correlation between saliva and serum estriol in pregnant and non-pregnant females was determined by assaying 35 matched samples. The saliva-serum correlation was highly significant, $r(33) = 0.87, p < 0.001$.

F. Salivary Estriol Expected Values

Salivary estriol concentrations in pregnant women (24 - 34 weeks' gestation) greater than 2100 pg/mL are associated with the occurrence of preterm labor. A marked increase in salivary estriol levels occurs three to four weeks before labor in women delivering preterm or at term (8,9).

Specificity of Antiserum

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Estriol EIA
Estradiol	50	1.4
Estrone	10	ND
Progesterone	100	ND
17 α -Hydroxyprogesterone	1000	ND
Testosterone	100	0.0145
Cortisol	1000	ND
DHEA	1000	ND
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Dexamethasone	1000	ND
Triamcinolone	1000	ND
Corticosterone	1000	ND
Prednisolone	1000	ND
Prednisone	1000	ND
Transferrin	1000	ND

ND = None detected (<0.004)

Material Safety Data*

Hazardous Ingredients

Liquid stop solution is caustic; use with care. We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics will not be liable for accidents or damage resulting from contact with reagents.

References

- Vining, R.F., & McGinley, R.A. (1987). The measurement of hormones in saliva: Possibilities and pitfalls. *Journal of Steroid Biochemistry*, 27, 81-94.
- Chard, T. (1990). *An introduction to radioimmunoassay and related techniques* (4th ed.). Amsterdam: Elsevier.
- Kivlighan, K.T., Granger, D.A., Schwartz, E.B., Nelson, V., & Curran, M. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior*, 46, 39-46.
- Schwartz, E., & Granger, D.A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clinical Chemistry*, 50, 654-656.
- West, C.D., Mahajan, D.K., Chavre, V.J., Nabors, C.J. (1973). Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion. *Journal of Clinical Endocrinology & Metabolism*, 36(6), 1230-1236.
- Brambilla, D.J., O'Donnell, A.B., Matsumoto, A.M., & McKinlay, J.B. (2007). Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology*, 67, 853-862.
- Shirtcliff, E.A., Granger, D.A., Schwartz, E., & Curran, M.J., (2001). Use of salivary biomarkers in biobehavioral research: Cotton based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology*, 26, 165-173.
- McGregor, J.A., Jackson, G.M., Lachelin, G.C., Goodwin, T.M., Artal, R., Hasings, C., & Dullien, V. (1999, Oct). Salivary estriol as risk assessment for preterm labor: A prospective trial. *Am J Obstet Gynecol*, 173(4), 1337-42.
- Reis, F.M., D'Antona, D., & Petraglia, F. (2002). Predictive value of hormone measurements in maternal and fetal complications of pregnancy. *Endocrine Reviews*, 23(2), 230-257.

Additional Reading Materials

Follingstad, A. H. (1978). Estriol, the forgotten estrogen? *Journal of the American Medical Association*, 239, 29-30.

Leff, R. P., & Goldkrand, J. W. (2002). The effect of betamethasone on salivary estriol. *Journal of Maternal and Fetal Neonatal Medicine*, 11, 192-195.

Wuu, J., Hellerstein, Lipworth L., Wide, L., Xu, B., Yu, G.P., Kuper, H., Lagiou, P., Hankinson, S.W., Ekblom, A., Carlström, K., Trichopoulos, D., Adami, H.O., & Hsieh, C.C. (2002). Correlates of pregnancy oestrogen, progesterone and sex hormone-binding globulin in the USA and China. *European Journal of Cancer Prevention*, 11, 283-293.

Hedriana, H.L., Munro, C.J., Eby-Wilkens, E.M., & Lasley, B.L. (2001). Changes in the rates of salivary estriol increases before parturition at term. *American Journal of Obstetrics and Gynecology*, 184, 123-130.

Mucci, L.A., Lagiou, P., Tamimi, R.M., Hsieh, C.C., Adami, H.O., & Trichopoulos, D. (2003). Pregnancy estriol, estradiol, progesterone and prolactin in relation to birth weight and other birth size variables (United States). *Cancer Causes Control*, 14, 311-318.

Peck, J.D., Hulka, B.S., Savitz, D. A., Baird, D., Poole, C., & Richardson, B.E. (2003). Accuracy of fetal growth indicators as surrogate measures of steroid hormone levels during pregnancy. *American Journal of Epidemiology*, 157, 258-266.

Tijssen, P. (1985). *Practice and theory of enzyme immunoassays*. Amsterdam: Elsevier.

Buster, J.E. (1983). Gestational changes in steroid hormone biosynthesis, secretion, metabolism, and action. *Clinical Perinatology*, 10, 527-552.

Canez, M.S., Lee, K.J., & Olive, D.L. (1992). Progestogens and estrogens. *Infertility and Reproductive Medical Clinics of North America*, 3, 59-78.

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“Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties.”

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