MAYO CLINIC LABORATORIES

Test Definition: ESTF

Estrogens, Estrone (E1) and Estradiol (E2), Fractionated, Serum

Overview

Useful For

Simultaneous high-sensitivity determination of serum estrone and estradiol levels

Situations requiring either higher sensitivity estradiol measurement, estrone measurement, or both, including
-As part of the diagnosis and workup of precocious and delayed puberty in females and, to a lesser degree, males
-As part of the diagnosis and workup of suspected disorders of sex steroid metabolism, (eg, aromatase deficiency and 17 alpha-hydroxylase deficiency)
-As an adjunct to clinical assessment, imaging studies, and bone mineral density measurement in the fracture risk assessment of postmenopausal women and, to a lesser degree, older men
-Monitoring low-dose female hormone replacement therapy in postmenopausal women
-Monitoring antiestrogen therapy (eg, aromatase inhibitor therapy)

Applications that require moderately sensitive measurement of estradiol including:
-Evaluation of hypogonadism and oligo-amenorrhea in females
-Assessing ovarian status, including follicle development, for assisted reproduction protocols (eg, in vitro fertilization)

In conjunction with luteinizing hormone measurements, monitoring of estrogen replacement therapy in hypogonadal premenopausal women

Evaluation of feminization, including gynecomastia, in males

Diagnosis of estrogen-producing neoplasms in males, and, to a lesser degree, females

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
E1	Estrone, S	Yes	Yes
EEST	Estradiol, Mass Spectrometry, S	Yes	Yes

Special Instructions

- Steroid Pathways

Method Name

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Test Definition: ESTF

Estrogens, Estrone (E1) and Estradiol (E2), Fractionated, Serum

Specimen

Specimen Type
Serum Red

Specimen Required
Supplies: Sarstedt Aliquot Tube 5 mL (T914)
Collection Container/Tube: Red top (Serum gel/SST is **not** acceptable)
Submission Container/Tube: Plastic vial
Specimen Volume: 1.2 mL
Collection Instructions: Within 2 hours of collection, centrifuge and aliquot serum in plastic vial.

Forms
If not ordering electronically, complete, print, and send General Test Request (T239) with the specimen.

Specimen Minimum Volume
0.7 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum Red	Refrigerated (preferred)	28 days	
	Ambient	28 days	
	Frozen	28 days	

Clinical & Interpretive

Clinical Information
Estrogens are involved in development and maintenance of the female phenotype, germ cell maturation, and pregnancy. They also are important for many other, non-gender-specific processes, including growth, nervous system maturation, bone metabolism/remodeling, and endothelial responsiveness. The 2 major biologically active estrogens in nonpregnant humans are estrone (E1) and estradiol (E2). A third bioactive estrogen, estriol (E3), is the main pregnancy estrogen, but plays no significant role in nonpregnant women or men.

Estradiol is produced primarily in ovaries and testes by aromatization of testosterone. Small amounts are produced in the adrenal glands and some peripheral tissues, most notably fat. By contrast, most of the circulating E1 is derived from peripheral aromatization of androstenedione (mainly adrenal). E2 and E1 can be converted into each other, and both can be inactivated via hydroxylation and conjugation. E2 demonstrates 1.25 to 5 times the biological potency of E1. E2 circulates at 1.5 to 4 times the concentration of E1 in premenopausal, nonpregnant women. E2 levels in men and postmenopausal women are much lower than in nonpregnant women, while E1 levels differ less, resulting in a reversal of the premenopausal E2:E1 ratio. E2 levels in premenopausal women fluctuate during the menstrual cycle. They are lowest during the early follicular phase. E2 levels then rise gradually until 2 to 3 days before ovulation, at which stage they start to increase much more rapidly and peak just before the ovulation-inducing luteinizing hormone/follicle stimulating hormone surge at 5 to 10 times the early follicular levels. This is followed by a modest decline during the ovulatory phase. E2 levels then gradually increase again until the midpoint of the luteal phase and thereafter decline to trough, early follicular levels.

Measurement of serum E2 forms an integral part of the assessment of reproductive function in females, including assessment of infertility, oligo-amenorrhea, and menopausal status. In addition, it is widely used for monitoring ovulation induction, as well as during preparation for in vitro fertilization. For these applications E2 measurements with modestly sensitive assays suffice. However, extra sensitive E2 assays, simultaneous measurement of E1, or both are needed in a number of other clinical situations. These include inborn errors of sex steroid metabolism, disorders of puberty, estrogen deficiency in men, fracture risk assessment in menopausal women, and increasingly, therapeutic drug monitoring, either in the context of low-dose female hormone replacement therapy or antiestrogen treatment.

For more information see Steroid Pathways.

Reference Values

ESTRONE (E1)

CHILDREN*

1 to14 days: Estrone levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days.

Males

Tanner stages#	Mean age	Reference range
Stage I (>14 days and prepubertal)	7.1 years	Undetectable-16 pg/mL
Stage II	11.5 years	Undetectable-22 pg/mL
Stage III	13.6 years	10-25 pg/mL
Stage IV	15.1 years	10-46 pg/mL
Stage V	18 years	10-60 pg/mL

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (+/- 2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females

Tanner stages#	Mean age	Reference range
Stage I (>14 days and prepubertal)	7.1 years	Undetectable-29 pg/mL
Stage II	10.5 years	10-33 pg/mL

Stage III	11.6 years	15-43 pg/mL
Stage IV	12.3 years	16-77 pg/mL
Stage V	14.5 years	17-200 pg/mL

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (+/- 2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.
*The reference ranges for children are based on the published literature,(1,2) cross-correlation of our assay with assays used to generate the literature data and on our data for young adults.

ADULTS
Males: 10-60 pg/mL
Females
Premenopausal: 17-200 pg/mL
Postmenopausal: 7-40 pg/mL
Conversion factor
E1: pg/mL x 3.704=pmol/L (molecular weight=270)

ESTRADIOL (E2)
CHILDREN*
1 to14 days: Estradiol levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days.
Males

Tanner stages#	**Mean age**	**Reference range**
Stage I (>14 days and prepubertal)	7.1 years	Undetectable-13 pg/mL
Stage II	12.1 years	Undetectable-16 pg/mL
Stage III	13.6 years	Undetectable-26 pg/mL
Stage IV	15.1 years	Undetectable-38 pg/mL
Stage V	18 years	10-40 pg/mL

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (+/- 2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females

Tanner stages#	**Mean age**	**Reference range**
Stage I (>14 days and prepubertal)	7.1 years	Undetectable-20 pg/mL
Stage II	10.5 years	Undetectable-24 pg/mL
Stage III	11.6 years	Undetectable-60 pg/mL
Stage IV	12.3 years	15-85 pg/mL

Stage V	14.5 years	15-350 pg/mL**

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (+/- 2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.
*The reference ranges for children are based on the published literature,(1,2) cross-correlation of our assay with assays used to generate the literature data and on our data for young adults.

ADULTS
Males: 10-40 pg/mL
Females
Premenopausal: 15-350 pg/mL**
Postmenopausal: <10 pg/mL
**E2 levels vary widely through the menstrual cycle.
Conversion factor
E2: pg/mL x 3.676=pmol/L (molecular weight=272)

For International System of Units (SI) conversion for Reference Values, see
https://www.mayocliniclabs.com/order-tests/si-unit-conversion.html

Interpretation

Estradiol (E2) levels below the premenopausal reference range in young females indicate hypogonadism. If luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels are elevated, primary gonadal failure is diagnosed. The main causes are genetic (eg, Turner syndrome, familial premature ovarian failure), autoimmune (eg, autoimmune ovarian failure, possibly as part of autoimmune polyglandular endocrine failure syndrome type II), and toxic (eg, related to chemotherapy or radiation therapy for malignant disease). If LH/FSH levels are low or inappropriately "normal," a diagnosis of hypogonadotrophic hypogonadism is made. This can have functional causes, such as starvation, overexercise, severe physical or emotional stress, and heavy drug and/or alcohol use. It also can be caused by organic disease of the hypothalamus or pituitary. Further work-up is usually necessary, typically including measurement of pituitary hormones (particularly prolactin), and possibly imaging.

Irregular or absent menstrual periods with normal or high E2 levels (and often high estrone: E1 levels) are indicative of possible polycystic ovarian syndrome, androgen producing tumors, or estrogen producing tumors. Further work-up is required and usually includes measurement of total and bioavailable testosterone, androstenedione, dehydroepiandrosterone (sulfate), sex hormone-binding globulin, and possibly imaging.

Estradiol analysis may be helpful in establishing time of ovulation and optimal time for conception. Optimal time for conception is within 48 to 72 hours following the midcycle E2 peak. Serial specimens must be collected over several days to evaluate baseline and peak total estrogen (E1 + E2) levels. Low baseline levels and a lack of rise, as well as persistent high levels without midcycle rise are indicative of anovulatory cycles.

For determining the timing of initiation of ovarian stimulation in in vitro fertilization studies, low levels (around 30 pg/mL) before stimulation are critical, as higher values often are associated with poor stimulation cycles.

Estrogen replacement in reproductive age women should aim to mimic natural estrogen levels as closely as possible. E2

levels should be within the reference range for premenopausal women, LH/FSH should be within the normal range, and E2 levels should ideally be higher than E1 levels.

The current recommendations for postmenopausal female hormone replacement are to administer therapy in the smallest beneficial doses for as briefly as possible. Ideally, E2 and E1 levels should be held below, or near, the lower limit of the premenopausal female reference range.

Postmenopausal women and older men in the lowest quartile of E2 levels are at increased risk of osteoporotic fractures. E2 levels are typically less than 5 pg/mL.

Antiestrogen therapy with central or peripheral acting agents that are not pure receptor antagonists usually aims for complete suppression of E2 production, and in the case of aromatase inhibitors, complete E1 and E2 suppression.

Gynecomastia or other signs of feminization in males may be due to an absolute or relative (in relation to androgens) surplus of estrogens. Gynecomastia is common during puberty in boys. Unless E1, E2, or testosterone levels exceed the adult male reference range, the condition is usually not due to hormonal disease (though it sometimes may still result in persistent breast tissue, which later needs to be surgically removed). For adults with gynecomastia, the workup should include testosterone and adrenal androgen measurements, in addition to E2 and E1 measurements. Causes for increased E1 or E2 levels include:
-High androgen levels caused by tumors or androgen therapy (medical or sport performance enhancing), with secondary elevations in E1 and E2 due to aromatization
-Obesity with increased tissue production of E1
-Decreased E1 and E2 clearance in liver disease
-Estrogen producing tumors
-Estrogen ingestion

Normal E1 and E2 levels in males may also be associated with feminization or gynecomastia, if bioavailable testosterone levels are low due to primary/secondary testicular failure. This may occur, for example, when patients are receiving antiandrogen therapy or other drugs with antiandrogenic effects (eg, spironolactone, digitalis preparations).

The gonadotrophin-releasing hormone (GnRH) stimulation test remains the central part of the workup for precocious puberty. However, baseline sex steroid and gonadotrophin measurements also are important. Prepubertal girls have E2 levels less than 10 pg/mL (most <5 pg/mL). Levels in prepubertal boys are less than half the levels seen in girls. LH/FSH are very low or undetectable. E1 levels also are low, but may rise slightly, in obese children after onset of adrenarche. E2, which is produced in the gonads, should remain low in these children. In true precocious puberty, both E2 and LH/FSH levels are elevated above the prepubertal range. Elevation of E2 or E1 alone suggests pseudo precocious puberty, possibly due to a sex steroid-producing tumor.

In delayed puberty, estrogens and gonadotrophins are in the prepubertal range. A rise over time predicts the spontaneous onset of puberty. Persistently low estrogens and elevated gonadotrophins suggest primary ovarian failure, while low gonadotrophins suggest hypogonadotrophic hypogonadism. In this latter case, Kallman syndrome (or related disorders) or hypothalamic/pituitary tumors should be excluded in well-nourished children.

Inherited disorders of sex steroid metabolism are usually associated with production abnormalities of other steroids,

most notably a lack of cortisol. Aromatase deficiency is not associated with cortisol abnormalities and usually results in some degree of masculinization in affected females, as well as primary failure of puberty. Males may show delayed puberty and delayed epiphyseal closure, as well as low bone-density. E2 and E1 levels are very low or undetectable. Various forms of testicular feminization are due to problems in androgen signaling pathways and are associated with female (or feminized) phenotypes in genetic males. E2 and E1 levels are above the male reference range, usually within the female reference range, and testosterone levels are very high.

For more information see Steroid Pathways.

Cautions

Fulvestrant is a member of a new class of drugs called "selective estrogen receptor degraders" (SERDS). Fulvestrant has modest cross-reactivity (1%-5%) in estradiol immunoassays, but because the peak dose levels of this drug are between 10-fold (reproductive age women) and more than 200-fold (postmenopausal women) higher than the naturally circulating estradiol concentrations in the treated women, this causes dramatically false-high results in estradiol immunoassays, when blood sampling occurs in close temporal proximity to dosing. By contrast, estradiol measurements by mass spectrometry display more than 1000-fold lower cross-reactivity (<0.001%), meaning that the impact of Fulvestrant on serum estradiol measurements by mass spectrometry is negligible, even if blood sampling occurs at peak dose.

Clinical Reference

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12. Richardson H, Ho V, Pasquet R, et al. Baseline estrogen levels in postmenopausal women participating in the MAP.3

breast cancer chemoprevention trial. Menopause. 2020;27(6):693-700. doi:10.1097/GME.0000000000001568

Performance

Method Description

Estrogens are fractionated into estradiol and estrone by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The LC-MS/MS method employs an organic extraction to remove water-soluble conjugates and to allow for concentration of the specimen. The method is free from interference and represents a reference methodology.

17beta-estradiol and estrone are extracted from 0.5 mL of serum with the organic solvent ethyl acetate. Deuterated 17beta-estradiol-d5 and estrone-d4 are added to each sample before the liquid extraction and serve as internal standards. After derivatization with dansyl chloride, high-pressure liquid chromatography is used prior to introduction of the derivatized sample extract into the LC-MS/MS.(1, 2) The mass spectrometer has an APCI (Atmospheric Pressure Chemical Ionization) interface and is operated in the multiple- reaction monitoring positive mode. The calibration utilizes an eight-point standard curve over a concentration range of 0-600 pg/mL.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

2 to 6 days

Specimen Retention Time

2 weeks

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

82671-Estrogens, fractionated
82670-Estradiol (If appropriate)
82679-Estrone (If appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ESTF	Estrogens, E1+E2, fractionated, S	53766-2

Result ID	Test Result Name	Result LOINC® Value
81418	Estrone, S	2258-2
81816	Estradiol, Mass Spectrometry, S	2243-4