

Overview

Useful For

Prenatal screening for Down syndrome, neural tube defects, and trisomy 18

Identifying abnormal levels of alpha-fetoprotein in the second trimester

Method Name

Immunoenzymatic Assay

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

Do not order this test unless test SEQA / Sequential Maternal Screening, Part 1, Serum has already been ordered.

If a standalone second-trimester screen is desired, order QUAD1 / Quad Screen (Second Trimester) Maternal, Serum.

If a stand-alone neural tube defect risk assessment is desired, order MAFP1 / Alpha-Fetoprotein (AFP), Single Marker Screen, Maternal, Serum.

Additional Testing Requirements

Sequential maternal screening is a 2-step test that includes a first-trimester sample (SEQA / Sequential Maternal Screening, Part 1, Serum) and a second-trimester sample (SEQB / Sequential Maternal Screening, Part 2, Serum).

Necessary Information

Collection date is required.

Specimen Required

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 1 mL

Collection Instructions:

1. Collect blood between 15 weeks, 0 days and 22 weeks, 6 days. Do not collect blood after performing amniocentesis, as that may lead to an artificially increased serum alpha-fetoprotein level and unreliable results.

2. Centrifuge and aliquot serum into a plastic vial within 2 hours of collection.

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Ambient	7 days	
	Refrigerated (preferred)	7 days	
	Frozen	90 days	

Clinical & Interpretive
Clinical Information

Maternal serum screening is used to identify pregnancies that may have an increased risk for certain birth defects, such as trisomy 21 (Down syndrome), neural tube defect (NTD) and trisomy 18. Various options for maternal serum screening are available and include: first trimester, second trimester, and cross-trimester.

Sequential screening is a type of cross-trimester screening, which has an improved detection rate as compared to either first- or second-trimester screening. Sequential screening combines biochemical and ultrasound markers (nuchal translucency: NT) measured in both trimesters of the pregnancy.

SEQA / Sequential Maternal Screening, Part 1, Serum involves an ultrasound and a blood collection. The ultrasound measurement, referred to as the NT measurement, is difficult to perform accurately. Therefore, NT data is accepted only from NT-certified sonographers. Along with the NT measurement, a maternal serum specimen is collected to measure pregnancy-associated plasma protein A. The results of the ultrasound measurement and blood work along with the maternal age and demographic information are used to calculate Down syndrome and trisomy 18 risk estimates.

If the result from part 1 indicates a risk for Down syndrome that is higher than the screen cutoff, the screen is completed, and a report is issued. In that event, the patient is typically offered counseling and diagnostic testing. When the part 1 screen is completed, a NTD risk is not provided. For a stand-alone NTD-risk assessment, order MAFP1 / Alpha-Fetoprotein (AFP), Single Marker Screen, Maternal, Serum.

If the risk from the first trimester is below the established cutoff, an additional serum specimen is collected in the second trimester for this test. The blood sample is tested for AFP, unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and inhibin A. The information from both trimesters is combined and a report is issued. If results

are positive, the patient is typically offered counseling and diagnostic testing.

AFP:

AFP is a fetal protein that is initially produced in the fetal yolk sac and liver. A small amount also is produced by the gastrointestinal tract. By the end of the first trimester, nearly all of the AFP is produced by the fetal liver. The concentration of AFP peaks in fetal serum between 10 to 13 weeks. Fetal AFP diffuses across the placental barrier into the maternal circulation. A small amount also is transported from the amniotic cavity.

The AFP concentration in maternal serum rises throughout pregnancy, from a nonpregnancy level of 0.2 to about 250 ng/mL at 32 weeks gestation. If the fetus has an open NTD, AFP is thought to leak directly into the amniotic fluid causing unexpectedly high concentrations of AFP. Subsequently, the AFP reaches the maternal circulation, thus producing elevated serum levels. Other fetal abnormalities such as omphalocele, gastroschisis, congenital renal disease, esophageal atresia, and other fetal-distress situations such as threatened abortion and fetal demise, may also show AFP elevations. Additionally, increased maternal serum AFP values may be seen in pregnancies with multiple fetuses and in unaffected singleton pregnancies in which the gestational age has been underestimated. Lower maternal serum AFP values have been associated with an increased risk for genetic conditions such as Down syndrome and trisomy 18.

uE3:

Estriol, the principal circulatory estrogen hormone in the blood during pregnancy, is synthesized by the intact fetoplacental unit. Estriol exists in maternal blood as a mixture of the unconjugated form and a number of conjugates. The half-life of uE3 in the maternal blood system is 20 to 30 minutes because the maternal liver quickly conjugates estriol to make it more water soluble for urinary excretion. Estriol levels increase during the course of pregnancy. Decreased uE3 has been shown to be a marker for Down syndrome and trisomy 18. Low levels of estriol also have been associated with pregnancy loss, Smith-Lemli-Opitz, and X-linked ichthyosis (placental sulfatase deficiency).

hCG:

hCG is a glycoprotein consisting of 2 noncovalently-bound subunits. The alpha subunit is identical to the alpha subunits of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyrotropin (TSH, formerly thyroid-stimulating hormone), while the beta subunit has significant homology to the beta subunit of LH and limited similarity to the FSH and TSH beta subunits. The beta subunit determines the unique physiological, biochemical, and immunological properties of hCG.

The *CGA* gene (glycoprotein hormones, alpha polypeptide) is thought to have developed through gene duplication from the *LH* gene in a limited number of mammalian species. hCG only plays an important physiological role in primates (including humans), where it is synthesized by placental cells, starting very early in pregnancy, and serves to maintain the corpus luteum, and hence progesterone production, during the first trimester. Thereafter, the concentration of hCG begins to fall as the placenta begins to produce steroid hormones and the role of the corpus luteum in maintaining pregnancy diminishes.

Increased total beta hCG levels are associated with Down syndrome, while decreased levels may be seen in trisomy 18. Elevations of hCG also can be seen in pregnancies with multiple fetuses, unaffected singleton pregnancies in which the gestational age has been overestimated, triploidy, fetal loss, and hydrops fetalis.

Inhibin A:

Inhibins are a family of heterodimeric glycoproteins, primarily secreted by ovarian granulosa cells and testicular Sertoli

cells, which consist of disulfide-linked alpha and beta subunits. While the alpha subunits are identical in all inhibins, the beta subunits exist in 2 major forms, termed A and B, each of which can occur in different isoforms. Depending on whether an inhibin heterodimer contains a beta A or a beta B chain, they are designated as inhibin A or inhibin B, respectively. Together with the related activins, which are homodimers or heterodimers of beta A and B chains, the inhibins are involved in gonadal-pituitary feedback and in paracrine regulation of germ cell growth and maturation. During pregnancy, inhibins and activins are produced by the fetoplacental unit in increasing quantities, mirroring fetal growth. Their physiological role during pregnancy is uncertain. They are secreted into the coelomic and amniotic fluid, but only inhibin A is found in appreciable quantities in the maternal circulation during the first and second trimesters.

Maternal inhibin A levels are correlated with maternal hCG levels and are abnormal in the same conditions that are associated with abnormal hCG levels (eg, inhibin A levels are typically higher in Down syndrome pregnancies). However, despite their similar behavior, measuring maternal serum inhibin A concentrations in addition to maternal serum hCG concentrations further improves the sensitivity and specificity of maternal multiple marker screening for Down syndrome.

Reference Values

An interpretive report will be provided.

Interpretation

Maternal screens provide an estimation of risk, not a diagnosis. A negative result indicates that the estimated risk falls below the screen cutoff. A positive result indicates that the estimated risk exceeds the screen cutoff.

Neural Tube Defect:

Screen-negative results indicate that the alpha-fetoprotein (AFP) multiple of the median (MoM) falls below the screen cutoff of 2.50 MoM. A negative screen does not guarantee the absence of a neural tube defect (NTD).

A screen-positive result indicates that the calculated AFP MoM is greater or equal to 2.50 MoM and may indicate an increased risk for open NTD. The actual risk depends on the level of AFP and the individual's pretest risk of having a child with a NTD based on family history, geographical location, maternal conditions such as diabetes and epilepsy, and use of folate prior to conception. A screen-positive result does not infer a definitive diagnosis of a NTD but indicates that further evaluation should be considered. Approximately 80% of pregnancies affected with open NTDs have AFP MoM values of greater than 2.50.

Trisomy 21 (Down Syndrome):

Second-trimester results are negative when the calculated risk is below 1/270 (0.37%). Negative results mean that the risk is less than the established cutoff; they do not guarantee the absence of Down syndrome.

Results are positive when the risk is greater than the established cutoff ($>$ or $=$ 1/270). Positive results are not diagnostic.

When both sequential maternal screening parts 1 and 2 are performed with a screen cutoff of 1/270, the combination of maternal age, nuchal translucency, pregnancy-associated plasma protein A, AFP, unconjugated estriol, human chorionic gonadotropin, and inhibin A has an overall detection rate of approximately 90% with a false-positive rate of approximately 3% to 4%. In practice, both the detection rate and false-positive rate vary with maternal age.

Trisomy 18 (Edwards Syndrome):

The screen cutoff for trisomy 18 is 1/100 (1%) in the second trimester. Risks that are greater or equal to 1% are

screen-positive; positive results are not diagnostic. Risks less than 1% are screen-negative; negative results do not guarantee the absence of trisomy 18.

Use caution when revising trisomy 18 positive results with earlier dating. Babies with trisomy 18 tend to be small, which can lead to underestimation of gestational age and an increased risk of missing a true-positive.

When sequential maternal screening parts 1 and 2 are performed, the overall detection rate is approximately 90% with a false-positive rate of approximately 0.1% using a screen cutoff of 1/100.

Follow-up:

Upon receiving maternal serum screening results, all information used in the risk calculation should be reviewed for accuracy (maternal date of birth, gestational dating, etc). If any information is incorrect, the laboratory should be contacted for a recalculation of the estimated risks.

Screen-negative results typically do not warrant further evaluation.

If the results are positive, the patient is typically offered counseling, ultrasound, diagnostic testing, and, possibly, a referral to genetics counseling or a high-risk clinic.

Cautions

Incorrect or incomplete information may significantly alter results.

A screen-negative result does not guarantee the absence of fetal defects. A screen-positive result does not provide a diagnosis but indicates that further diagnostic testing should be considered (an unaffected fetus may have screen-positive result for unknown reasons). In fact, given the low prevalence of Down syndrome, the majority of women with a positive screen will not have a Down syndrome fetus.

Maternal serum alpha-fetoprotein (AFP) should not be measured after amniocentesis because maternal-fetal transfusion may occur, which would falsely increase the serum AFP.

In twin pregnancies, the risk for Down syndrome is approximated, using twin-adjusted medians. In cases where one twin has demised, results may be unreliable.

Results are not available for triplets or higher-multiple pregnancies.

Each center offering maternal serum screening to patients should establish a standard screening protocol, which provides pre- and post-screening education and appropriate follow-up for screen-positive results.

In a small percentage of samples, there is potential for alkaline phosphatase associated positive interference in the Beckman Access uE3 assay. This potential interference does not appear to be related to the amount of alkaline phosphatase in the patient sample. A falsely elevated unconjugated estriol test result can lead to inaccurately underestimating the relative risk of chromosomal abnormalities, such as trisomy 21 and 18.

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays.

Caution should be used in interpretation of results and the laboratory should be alerted if the result does not correlate with the clinical presentation.

Clinical Reference

1. Malone FD, Canick JA, Ball RH, et al: First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med*. 2005 Nov 10;353(19):2001-2011
2. Prenatal Diagnostic Testing for Genetic Disorders. ACOG Practice Bulletin No. 163. American College of Obstetricians and Gynecologists. *Obstet Gynecol*. 2016 May;127(5):979-981. doi: 10.1097/AOG.0000000000001439
3. Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A: SURUSS in perspective. *Semin Perinatol*. 2005 Aug;29(4):225-235
4. Palomaki GE, Steinort K, Knight GJ, Haddow JE: Comparing three screening strategies for combining first- and second-trimester Down syndrome markers. *Obstet Gynecol*. 2006 Feb;107(2 Pt 1):367-375
5. Palomaki GE, Neveux LM, Knight GJ, Haddow JE: Maternal serum-integrated screening for trisomy 18 using both first- and second-trimester markers. *Prenat Diagn*. 2003 Mar;23(3):243-247
6. Yarbrough ML, Stout M, Gronowski AM: Pregnancy and its disorders. In: Rifai N, Horvath AR, Wittwer CT, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th ed. Elsevier; 2018:1655-1696

Performance**Method Description**

This test includes measuring alpha-fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and inhibin A. These measurements are compared to median values for a given gestational age and a multiple of the median (MoM) is calculated for each. Then, results from both trimesters (part 1 and part 2) are entered into a multivariate algorithm that includes the mother's age to derive risk factors for Down syndrome, trisomy 18, and neural tube defects. An interpretive report will be provided.

AFP, beta hCG, uE3, and inhibin A assays are performed on the Beckman Access using an automated immunoenzymatic assays with paramagnetic separation and chemiluminescent detection.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

4 to 6 days

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

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

- 81511
- 82105 (if appropriate)
- 82677 (if appropriate)
- 84702 (if appropriate)
- 86336 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SEQB	Sequential Maternal Screen, Part 2	48800-7

Result ID	Test Result Name	Result LOINC® Value
29504	Additional Comments	48767-8
29476	Recalculated Maternal Serum Screen	43995-0
29477	Specimen Collection Date 1	33882-2
29478	Maternal Date of Birth	21112-8
29479	Maternal Weight	29463-7
29887	PAPP-A	48407-1
29494	AFP	20450-3
29495	uE3	20466-9
29496	hCG, Total	83086-9
29497	Inhibin	35738-4
29500	Down Syndrome Screen Risk Estimate	43995-0
29501	Down Syndrome Maternal Age Risk	49090-4
29502	Trisomy 18 Screen Risk Estimate	43994-3
29503	Interpretation	49092-0
29505	Recommended Follow Up	80615-8
29506	General Test Information	62364-5
29892	Calculated age at EDD	43993-5
29481	Insulin Dependent Diabetes	33248-6
29482	Patient Race	32624-9

29493	Specimen Collection Date 2	33882-2
29888	IVF	47224-1
29485	Scan Date	34970-4
29886	Number of Fetuses	11878-6
29488	CRL	11957-8
29489	CRL Twin	11957-8
29490	Chorions	92568-5
29893	GA on Collection by U/S Scan 1	11888-5
29894	GA on Collection by U/S Scan 2	11888-5
29491	NT	49035-9
29492	NT Twin	49035-9
601813	Results Summary	49092-0
602040	Neural Tube Defect Risk Estimate	49091-2
601814	NT MoM	49035-9
601815	NT Twin MoM	49035-9
601816	PAPP-A MoM	76348-2
601817	AFP MoM	23811-3
602805	AFP MoM (14,0-14,6)	23811-3
601818	uE3 MoM	21264-7
601819	hCG, Total MoM	23841-0
601820	Inhibin MoM	36904-1
601811	Current cigarette smoking status	72166-2
601808	Prev Down (T21) / Trisomy Pregnancy	53826-4
601809	Prev Pregnancy w/ Neural Tube Defects	53827-2
601807	Initial or repeat testing	89231-5
601810	Patient or father of baby has a NTD	53827-2
601803	Sonographer Name	49088-8
602041	Sonographer Code	No LOINC Needed
601812	Sonographer Reviewer ID	49089-6
601804	Physician Phone Number	68340-9