Test Definition: QUAD1

Quad Screen (Second Trimester) Maternal, Serum

Overview

Useful For

Prenatal screening for open neural tube defect (alpha-fetoprotein only), trisomy 21 (alpha-fetoprotein, human chorionic gonadotropin, estriol, and inhibin A) and trisomy 18 (alpha-fetoprotein, human chorionic gonadotropin, and estriol)

Special Instructions

- Second Trimester Maternal Screening Alpha-Fetoprotein / Quad Screen Patient Information

Method Name

Immunoenzymatic Assay

NY State Available

Yes

Specimen

Specimen Type

Serum

Necessary Information

To provide the best result interpretation, either answer the order entry questions or provide the required information using the Second Trimester Maternal Screening Alpha-Fetoprotein / Quad Screen Patient Information (T595).

Specimen Required

Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Instructions:
1. Do not collect specimen after amniocentesis as this could affect results.
2. Within 2 hours of collection, centrifuge and aliquot serum into a plastic vial.
Additional Information:
1. For an assessment that includes neural tube defect results, gestational age must be between 15 weeks, 0 days and 22 weeks, 6 days.
2. Assessments for trisomy 21 (Down syndrome) and trisomy 18 (Edwards syndrome) only are available between 14 weeks, 0 days and 22 weeks, 6 days.
3. Initial or repeat testing is determined in the laboratory at the time of report and will be reported accordingly. To be considered a repeat test for the patient, the testing must be within the same pregnancy and trimester, with

interpretable results for the same tests, and both tests are performed at Mayo Clinic.
4. Maternal Serum Screening patient education brochure (T522) is available upon request.

Forms

If not ordering electronically, **Second Trimester Maternal Screening Alpha-Fetoprotein / Quad Screen Patient Information (T595) is required.**

Specimen Minimum Volume

0.75 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	7 days	
	Ambient	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, including neural tube defects (NTD), trisomy 21 (Down syndrome), and trisomy 18 (Edwards syndrome). The screen is performed by measuring analytes in maternal serum that are produced by the fetus and the placenta. The analyte values along with maternal demographic information such as age, weight, gestational age, diabetic status, and race are combined in a mathematical model to derive a risk estimate. A specific cutoff for each condition is used to classify the risk estimate as either screen-positive or screen-negative. A screen-positive result indicates that the value obtained exceeds the established cutoff. A positive screen does not provide a diagnosis but rather indicates that further evaluation should be considered.

Analytes:
Alpha-Fetoprotein:
Alpha-fetoprotein (AFP) is a fetal protein that is initially produced in the fetal yolk sac and liver. A small amount is produced by the gastrointestinal tract. By the end of the first trimester, nearly all 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 non-pregnancy level of 0.2 ng/mL 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 kidney disease, esophageal atresia, and other fetal distress situations (eg, threatened abortion and fetal demise) also may result in maternal serum AFP elevations. Increased maternal serum AFP concentrations also may be seen in multiple pregnancies and in unaffected singleton pregnancies in which the gestational age has been underestimated.

Lower maternal serum AFP concentrations have been associated with an increased risk for genetic conditions such as trisomy 21 and trisomy 18.

Estriol:
Estriol (E3), the principal circulatory estrogen hormone in the blood during pregnancy, is synthesized by the intact feto-placental unit. E3r exists in maternal blood as a mixture of the unconjugated form and a number of conjugates. The half-life of unconjugated estriol (uE3) in the maternal blood system is 20 to 30 minutes because the maternal liver quickly conjugates E3 to make it more water soluble for urinary excretion. E3 levels increase during the course of pregnancy. Decreased uE3 has been shown to be a marker for trisomy 21 and trisomy 18. Low levels of E3 also have been associated with pregnancy loss, Smith-Lemli-Opitz, and X-linked ichthyosis (placental sulfatase deficiency).

Decreased second trimester uE3 has been shown to be a marker for trisomy-21 and trisomy-18 syndromes. uE3 is a part of multiple marker prenatal biochemical screening, together with alpha-fetoprotein, human chorionic gonadotropin, and inhibin-A measurements. Low levels of uE3 also have been associated with pregnancy loss, Smith-Lemli-Opitz syndrome (defect in cholesterol biosynthesis), X-linked ichthyosis and contiguous gene syndrome (placental sulfatase deficiency disorders), aromatase deficiency, and primary or secondary fetal adrenal insufficiency.

Human Chorionic Gonadotropin:
Human chorionic gonadotropin (hCG) is a glycoprotein consisting of 2 noncovalently bound subunits. The alpha subunit is identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyrotropin (TSH, previously 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. hCG 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 hCG levels are associated with trisomy 21, while decreased levels may be seen in trisomy 18. Elevations of hCG also can be seen in multiple pregnancies, 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 feto-placental 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 trisomy 21 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 trisomy 21.

Reference Values

Neural Tube Defect Risk Estimate:
An alpha-fetoprotein (AFP) multiple of the median (MoM) <2.5 is reported as screen negative.
AFP MoM > or =2.5 (singleton and twin pregnancies) are reported as screen positive.

Down Syndrome Risk Estimate:
Calculated screen risks <1/270 are reported as screen negative, risks > or =1/270 are reported as screen positive.

Trisomy 18 Risk Estimate:
Calculated screen risks <1/100 are reported as screen negative, risks > or =1/100 are reported as screen positive.

An interpretive report will be provided.

Interpretation

Neural Tube Defects
A screen-negative result indicates that the calculated alpha-fetoprotein (AFP) multiple of the median (MoM) falls below the established cutoff of 2.50 MoM. A negative screen does not guarantee the absence of neural tube defects (NTD).

A screen-positive result indicates that the calculated AFP MoM is 2.50 or greater 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 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 NTD but indicates that further evaluation should be considered. Approximately 80% of pregnancies affected with NTD have elevated AFP, MoM values greater than 2.5.

Trisomy 21 (Down syndrome) and Trisomy 18 (Edwards syndrome):
A screen-negative result indicates that the calculated screen risk is below the established cutoff of 1/270 for trisomy 21 and 1/100 for trisomy 18. A negative screen does not guarantee the absence of trisomy 21 or trisomy 18.

When a trisomy 21 second-trimester risk cutoff of 1/270 is used for follow-up, the combination of maternal age, AFP, estriol, human chorionic gonadotropin, and inhibin A has an overall detection rate of approximately 77% to 81% with a false-positive rate of 6% to 7%. In practice, both the detection rate and false-positive rate increase with age. The detection rate ranges from 66% (early teens) to 99% (late 40s), with false-positive rates of between 3% and 62%,

respectively.

The detection rate for trisomy 18 is 60% to 80% using a second trimester 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.

Ultrasound is recommended to confirm dates for NTD or trisomy 21 screen-positive results. Many pregnancies affected with trisomy 18 are small for gestational age. Recalculations that lower the gestational age may decrease the detection rate for trisomy 18. If ultrasound yields new dates that differ by at least 7 days, a recalculation should be considered. If dates are confirmed, high-resolution ultrasound and amniocentesis (including amniotic fluid AFP and acetylcholinesterase measurements for NTD) are typically offered.

Cautions

Variables Affecting Marker Levels:
Race, weight, smoking, multiple fetus pregnancy, insulin-dependent diabetes (IDD), and in vitro fertilization (IVF) may affect marker concentrations. Black mothers tend to have higher alpha-fetoprotein (AFP) levels but lower risk of neural tube defects and are assigned to a separate AFP median set. All multiples of the median (MoM) are adjusted for maternal weight (to account for dilution effects in heavier mothers). The AFP, unconjugated estriol (uE3), and inhibin MoM are adjusted upward in IDD to account for lower values in diabetic pregnancies. Human chorionic gonadotropin (hCG) levels are higher and uE3 levels are lower in pregnancies conceived by IVF, MoM are adjusted accordingly to account for the alterations. Smoking results in higher second trimester maternal serum AFP and inhibin A levels and lower uE3 and hCG levels. MoM are adjusted accordingly to account for analyte differences in smokers.

The estimated risk calculations and screen results are dependent on accurate information for gestation, maternal age, race, IDD, and weight. Inaccurate information can lead to significant alterations in the estimated risk. In particular, erroneous assessment of gestational age can result in false-positive or false-negative screen results. Because of its increased accuracy, the determination of gestational age by ultrasound is recommended, when possible, rather than by last menstrual period.

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).

Valid measurements of AFP in maternal serum cannot be made after amniocentesis.

Triplet and higher multiple pregnancies cannot be interpreted.

Each center offering maternal serum screening to patients should establish a standard screening protocol that 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 uE3 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. Wald NJ, Cuckle HS, Densem JW, Stone RB. Maternal serum unconjugated oestriol and human chorionic gonadotrophin levels in pregnancies with insulin-dependent diabetes: implications for screening for Down's syndrome. Br J Obstet Gynaecol. 1992;99(1):51-53
2. American College of Obstetricians and Gynecologists. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. Obstet Gynecol. 2016;127(5):e123-137
3. 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;353(19):2001-2011
4. Wald NJ, Rodeck C, Hackshaw AK, et al. SURUSS in perspective. Semin Perinatol. 2005;29(4):225-235
5. Rudnicka AR, Wald NJ, Huttly W, Hackshaw AK. Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. Prenat Diagn. 2002;22(10):893-897
6. Zhang J, Lambert-Messerlian G, Palomaki GE, Canick JA. Impact of smoking on maternal serum markers and prenatal screening in the first and second trimesters. Prenat Diagn. 2011;31(6):583-588
7. 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 4-marker screen includes alpha-fetoprotein (AFP), estriol (uE3), human chorionic gonadotropin (total beta-hCG: ThCG), and inhibin A. Analyte values are compared to median values at a given gestational age and multiple of the median (MoM) results obtained. The MoM results are used in a multivariate algorithm that includes the mother's age to derive risk factors for trisomy 21 (Down syndrome) and trisomy 18 (Edwards syndrome). The screen for neural tube defects (NTD) uses the AFP MoM only. An interpretive report will be provided. The Beckman Access AFP, ThCG, uE3, and inhibin A assays are automated immunoenzymatic assays with paramagnetic separation and chemiluminescent detection.(Package inserts: Access AFP. Beckman Coulter; 2024; Access Total bhCG. Beckman Coulter; 2024; Access Unconjugated Estriol. Beckman Coulter; 2021; Access Inhibin A. Beckman Coulter; 2024)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available
4 to 6 days

Specimen Retention Time
3 months

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
81511

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
QUAD1	QUAD SCRN (2nd Tri) Maternal, S	48800-7

Result ID	Test Result Name	Result LOINC® Value
IDD	Insulin dependent diabetes	44877-9
IVFP	IVF pregnancy	47224-1
MULTF	Number of Fetuses	55281-0
10334	Down syndrome screen risk estimate	43995-0
10335	Down syndrome maternal age risk	49090-4
10337	Trisomy 18 screen risk estimate	43994-3
10356	INTERPRETATION	49092-0
10248	Additional comments	48767-8
10357	RECOMMENDED FOLLOW UP	80615-8
10358	GENERAL TEST INFORMATION	62364-5
7058	Recalculated Maternal Serum Screen	32399-8
3009	Specimen collection date	33882-2

7823	Maternal date of birth	21112-8
7834	Calculated age at EDD	43993-5
26717	Maternal Weight	29463-7
26718	Maternal Weight	29463-7
10353	hCG, TOTAL	83086-9
10054	EDD by U/S scan	11781-2
7753	EDD by LMP	11779-6
7203	GA on collection by U/S scan	11888-5
7204	GA on collection by dates	11885-1
7830	GA used in risk estimate	21299-3
10351	AFP	83073-7
10352	uE3	2250-9
10354	INHIBIN	2478-6
113146	Results Summary	32399-8
113147	Neural tube defect risk estimate	48803-1
113148	AFP MoM	23811-3
113149	uE3 MoM	21264-7
113150	hCG, TOTAL MoM	23841-0
113151	INHIBIN MoM	36904-1
RACE1	Patient race	21484-1
SMKNG	Current cigarette smoking status	64234-8
CHOR_	Number of Chorions	92568-5
PRHIS	Prev Down (T21) / Trisomy Pregnancy	53826-4
PRNTD	Prev Pregnancy w/ Neural Tube Defect	53827-2
PTNTD	Patient or father of baby has a NTD	53827-2
INTL	Initial or repeat testing	77202-0
DRPHN	Physician Phone Number	68340-9
601921	AFP MoM (14,0-14,6)	23811-3