

**THE WAKE FOREST UNIVERSITY
MATERNAL SERUM SCREENING PROGRAM FOR
NEURAL TUBE DEFECTS, DOWN SYNDROME and TRISOMY 18:**

THE PROTOCOL

SECTION ON MEDICAL GENETICS
WAKE FOREST UNIVERSITY SCHOOL of MEDICINE
MEDICAL CENTER BLVD.
WINSTON-SALEM, N.C. 27157-1080

Phone: (336) 713-7530
Fax: (336) 713-7577
Web site: www.wfubmc.edu/medicalgenetics/

Table of Contents

Introduction.....	3
Informing the Patient.....	6
Sample Preparation.....	8
Required Paperwork.....	10
Billing.....	12
Results.....	13
Diagnostic Testing for Abnormal Results.....	16
Family History.....	19
Follow-up.....	20

INTRODUCTION

The Section on Medical Genetics of the Department of Pediatrics at the Wake Forest University School of Medicine established a MSAFP Program in 1979. It is the first MSAFP program in the state of North Carolina and one of the first MSAFP Programs in the United States to provide maternal serum alpha fetoprotein screening for pregnant women. We provide a comprehensive service for approximately 12,000 pregnant women per year in the Piedmont and Western North Carolina. This screening service is coordinated by genetic counselors and directed by Dr. Mark Pettenati. Participants have access to genetic counselors, as well as Ph.D. and M.D. geneticists. Currently, our Program utilizes a "quad screen" assay for maternal serum levels of alpha fetoprotein, human chorionic gonadotropin, unconjugated estriol and inhibin-A. By comparing the patients' levels to data previously collected, it is possible to estimate a woman's chance to have a child with an open neural tube defect, Down syndrome or Trisomy 18.

Background on the "Quad Screen"

Maternal serum screening began as a "single screen" by looking only at maternal serum alpha fetoprotein. Alpha fetoprotein is produced by the fetal liver where it is excreted into the amniotic fluid. Next, the serum diffuses into the mother's bloodstream where it is detected as maternal serum alpha fetoprotein (MSAFP). The presence of an open fetal defect typically leads to increased leakage of AFP into the amniotic cavity. As a result, a greater amount of AFP may diffuse into the mother's bloodstream resulting in an increased level of MSAFP. Measurement of MSAFP detects approximately **85% of open neural tube defects** (anencephaly or spina bifida). Defects that are "closed" or skin-covered, such as an encephalocele, do not always produce an elevated MSAFP level and therefore, may not be detected by this blood test. Other birth defects involving abnormalities of the abdominal wall, urinary system and some chromosome abnormalities may also be associated with an elevated MSAFP level. From 1985 to 1991, for women under 35 years of age, a combination of **low** levels of **MSAFP** and maternal age was used to screen for **Down syndrome**. Using MSAFP alone in combination with maternal age, our Program was able to detect **20-25%** of fetuses with Down syndrome.

In January of 1991, we added the quantitative measurement of **human chorionic gonadotropin (hCG)** to our screening program because it was shown to be a better prenatal predictor of **Down syndrome**. Human chorionic gonadotropin is produced by the placenta. An elevated hCG level alone is able to detect **30%** of pregnancies affected with Down syndrome. Using a combination of maternal age, hCG and MSAFP we were able to detect approximately **60%** of fetuses with **Down syndrome**. In addition to using these chemicals to detect Down syndrome, we began screening for another chromosome abnormality, **Trisomy 18**, using cutoff values for AFP and hCG in April of 1992.

In November of 1993, the measurement of **unconjugated estriol (uE₃)** was added to our screening program. Unconjugated estriol is found in the placenta, fetal adrenal glands and fetal liver. The

combination of MSAFP/hCG/uE₃ for maternal serum screening is also known as the "**Triple Screen.**" Several studies found that the addition of uE₃ increased the detection rate for Down syndrome to 65% and decreased the false-positive rate. Furthermore, some literature suggested that adding uE₃ to MSAFP/hCG increased the detection rate for **Trisomy 18**. In addition to Trisomy 18, literature suggests an association between low/undetectable maternal serum unconjugated estriol (MSuE₃) and **steroid sulfatase deficiency (X-linked Icthyosis)**. In April of 1997 after careful review of the literature, our Program decided to include a comment stating that the possible explanation for low/undetectable MSuE₃ could be steroid sulfatase deficiency in the fetus.

In February of 2007, the measurement of **inhibin-A (DIA)** was added to our Screening Program. Inhibin-A is primarily produced by the placenta. The addition of **DIA** to MSAFP/hCG/uE₃ for maternal serum screening is known as the "**Quad Screen.**" Recent literature has shown that the addition of **DIA** increases the detection rate for Down syndrome to 75-80% and decreases the false positive rate. The addition of DIA does not influence the detection rates of open neural tube defects or Trisomy 18.

Current Detection of Maternal Serum Screening

Maternal Serum AFP is the only chemical within the "Quad screen" that can detect **open neural tube defects**. The detection rate for **open neural tube defects** using maternal serum screening is approximately 85%. In addition, with our Program the detection rate for ventral wall defects is greater than 90%. Using a cut-off laboratory value of 2.5 Multiples of the Median (MoM), approximately 2-3% of patients screened will have an elevated MSAFP level and will be offered genetic counseling and further testing, such as a targeted ultrasound and amniocentesis. Of note, approximately one quarter of our samples with an elevated MSAFP are based on incorrect gestational dating.

A patient-specific risk for **Down syndrome** is calculated using the levels of MSAFP/hCG/uE₃/DIA as well as the maternal age at delivery. Approximately 4-5% of patients screened will have an increased risk for **Down syndrome** and will be offered further diagnostic testing such as ultrasound and amniocentesis. Currently, we recommend genetic counseling and the consideration of amniocentesis if the patient's **Down syndrome** risk is equal to or greater than 1 in 270. This cut-off risk is specific to our Screening Program and was chosen to create the lowest false positive rate while optimizing our detection rate. We continue to monitor our Program's screening sensitivity and specificity, and for this reason, the cut-off risk may change over time. The current detection rate for **Down syndrome** using the WFUSM Maternal Serum Screening Program is 75-80% with a false-positive rate of 5%. Of note, approximately one half of our samples with an increased Down syndrome risk are based on incorrect gestational dating.

Literature reports suggest that low levels of maternal serum AFP, hCG and uE₃ are associated with an increased risk for **Trisomy 18**, and that a patient-specific risk for **Trisomy 18** can be calculated using maternal age in conjunction with AFP, hCG and uE₃ values. The current detection rate for Trisomy 18 using the WFUSM Maternal Serum Screening Program is approximately 90%, with a

false-positive rate of 0.2%. Patients with a risk for **Trisomy 18** equal to or greater than 1 in 100 will be offered the option of amniocentesis.

Any low/undetectable maternal serum unconjugated estriol (MSuE₃) value less than or equal to 0.10 MoM (multiple of the median) will have an additional comment that includes possible explanations for a low/undetectable MSuE₃ value such as **Steroid Sulfatase Deficiency**. Because our Program has not had many cases of steroid sulfatase deficiency, a reasonable detection and false-positive rate cannot be determined with any significance. Thus, a patient-specific risk for steroid sulfatase deficiency is not calculated.

*Detection and false-positive rates determined by data collected from 2000-2004.

INFORMING THE PATIENT

Maternal serum screening should be offered to all patients at their first prenatal visit.

The current recommendation of the American College of Obstetricians and Gynecologists (ACOG) is that all screening and diagnostic options should be made available to women of any age. The rationale is that women of any age can have a baby with Down syndrome or another extra chromosome condition, although the risk increases with a woman's age.

With the Quad Screen, the detection rate of Down syndrome for women who are 35 years of age or older is increased; however, the false positive rate is approximately 25%.

The Quad Screen will not identify all pregnancies affected with Down syndrome and is not able to detect most other extra chromosome conditions, while amniocentesis or CVS will detect nearly 100%.

Pamphlets containing information about our Screening Program can be supplied to your office and this information should be discussed with the patient. This pamphlet is available in Spanish and will be provided upon request.

Although maternal serum screening is considered "standard of care", the test is voluntary, and the following issues should be considered by the patient:

1. A normal result does not guarantee a "perfect" baby. Again, maternal serum screening detects approximately 85% of open neural tube defects, 75-80% of fetuses with Down syndrome, and 60% of fetuses with Trisomy 18 for women who are less than 35 years of age. These statistics show that a baby may be born with these birth defects even after a normal screening result. Furthermore, this test is not designed to detect all birth defects. All women face a 3-5% baseline risk for having a baby with a birth defect and/or mental retardation, even when the family history is not significant for any physical and/or mental differences.

2. An abnormal result does not necessarily mean that the baby has a birth defect or chromosome condition. Most patients with an abnormal screening result will have a healthy baby, but these patients do have the option of pursuing diagnostic testing. For those patients with an increased risk for Down syndrome, Trisomy 18 or a neural tube defect, a patient specific risk is calculated.

3. Some patients would want to know in advance if the baby has a neural tube defect or a chromosome abnormality. The identification of a neural tube defect or chromosome abnormality through diagnostic prenatal testing may present a patient with options regarding the pregnancy. If the pregnancy is continued, it allows the parents to prepare for medical, financial and emotional issues. In other cases, a patient may choose to terminate the pregnancy.

4. Some patients would not want to know in advance if the baby has a neural tube defect or a chromosome abnormality. In this case, diagnostic prenatal testing options may not be desired by the patient.

5. Some women will decline maternal serum screening and that is an acceptable decision.

SAMPLE PREPARATION

The screening sensitivity for open neural tube defects and Down syndrome is greatest at 16-18 weeks of gestation. For this reason, we recommend that the sample be drawn within this time period. However, interpretation of a sample is possible between 15-20.9 weeks gestation. For patients whose samples are drawn before 15 weeks or after 20.9 weeks, the levels of MSAFP/hCG/uE₃/DIA will be quantified, but an interpretation of the results is not possible. We recommend that samples drawn before 15 weeks gestation be re-drawn between 15.0-20.9 weeks.

An adequate blood sample is necessary to perform the screen. A **full sample** of peripheral blood drawn in a gold, red “tiger top” with SST gel and clot activator (serum separator), or a plain red top tube is required from all patients requesting screening. A gold, red “tiger top” tube is preferred. Our Laboratory prefers that you spin the sample before shipping. A **full 3.5 ml tube** of serum is necessary for all samples. **If the sample is placed in a tube other than a red top tube, or if the sample is drawn in a different tube and transferred into a red top tube, the results of the maternal serum screen may be significantly altered.**

As of March 2004, we began offering courier service to most practices who participate in our Screening Program. For courier pick-up, please place sample tubes inside a biohazard plastic bag along with the required paperwork (paperwork goes in outside pouch of bag). Our Program can provide your Practice with the biohazard bags. The bags should then be placed at a pick-up point that has been mutually designated by your staff and the courier.

Several practices are now sending samples through Federal Express. These practices should follow the same instructions for samples that are picked up by the courier in conjunction with the required Federal Express packaging.

Some practices submit their samples by USPS Two Day Priority Mail. **Because of OSHA regulations, our mailroom cannot accept samples unless they are packaged to the specifications outlined below.** The OSHA regulations contain strict penalties for those sending and receiving improperly packaged biohazardous materials. We provide the following items for mailing of samples:

- large cardboard cylinders
- small metal cylinders
- Two Day Priority envelopes with appropriate label
- biohazard labels
- Two Day Priority Postage

Steps required to properly assemble these mailers according to OSHA guidelines:

1. Label the patient's blood sample with her name and the date the sample was drawn. If a patient has a known infectious disease, this information must be clearly noted on the sample.

2. The samples must be wrapped with an appropriate amount of absorbent material (paper towels or tissue paper, for example) to minimize breakage. Glass blood tubes are especially prone to breakage so extra padding should be placed in the bottom and top of the small metal cylinders.
3. The wrapped samples should be placed in the small metal cylinder. Each metal cylinder can hold up to 5 tubes. The cylinder should be closed securely.
4. The metal cylinder is then inserted into the larger cardboard cylinder, which should also be tightly secured.
5. The larger cardboard cylinder should then be labeled with a biohazard sticker.
6. The patient's paperwork and the larger cardboard cylinder should then be placed in the green and white mailing envelope. The envelope should also be labeled with a biohazard sticker. Once these instructions are completed, the envelope is ready for mailing.

Please use the supply requisition form to notify us when your supplies are getting low.

Screening is most accurate on freshly drawn samples. For this reason, it is important to send samples immediately after they are drawn. Thus, the small metal cylinder does not need to be completely full prior to mailing.

If the sample cannot be shipped immediately, we recommend that blood samples be kept in a refrigerator (approximately 32°C). If the samples need to be kept over the weekend before shipping, we recommend that the samples are spun down in a centrifuge in order to isolate the serum. The serum should be poured into a cyro tube and frozen in a freezer (approximately – 10°C).

If in doubt, redtops should be refrigerated and separated tubes should be spun and then refrigerated.

Samples received more than two weeks after they were drawn may yield inaccurate results and are thus not acceptable for screening. In the event that a sample is received two weeks or more after the date drawn, our Laboratory will call your Office and request a new sample to insure the most accurate interpretation.

REQUIRED PAPERWORK

Laboratory requisition form: A completed laboratory requisition form must accompany all samples, and the first three of the four copies of the form should be sent with the sample to our laboratory. Your Practice should keep the "D" (last) copy of the requisition form. Each question is designed to give us the information we need to provide an accurate result and interpretation for your patient. We **cannot** provide an interpretation if the laboratory requisition form is incomplete.

In order to calculate and appropriately interpret the patient's risk we need the following information for each patient:

1. **Patient's full name (first, given middle, given maiden, and last names)**
2. **Social security numbers are requested but are not required**
3. **Most accurate gestational dating and the method by which the dating was obtained**
4. **Date of birth**
5. **Patient's weight on the date the sample was drawn**
6. **Date the sample was drawn**
7. **Whether there is a family history of a neural tube defect and if so, the relationship of this relative to the patient and the type of defect**
8. **Whether the patient is an insulin dependent diabetic**
9. **Whether this is a known multiple gestation**
10. **Patient's race**
11. **Family history of chromosome abnormality (e.g. Down syndrome or Trisomy 18)**

When questions are left blank, the answer is considered to be "no". When the race is left blank, we default to Caucasian.

Please pay special attention to the gestational dating. It is imperative that the BEST estimate of a patient's dating be indicated on the laboratory slip. If available, ultrasound dating is preferred. We will use the **LMP** unless other dating is provided, either by ultrasound or physical examination of the patient. If the LMP is unknown, we recommend an ultrasound be performed prior to screening. Please **do not** send any samples without a gestational dating listed on the laboratory requisition form. Results cannot be calculated and an interpretation cannot be provided if gestational dating is not available.

Patients with insulin-dependent diabetes mellitus (IDDM) are known to be at increased risk for birth defects, particularly neural tube defects. Studies have documented a reduced MSAFP level in diabetic women. A correction factor is programmed into our computer to compensate for this phenomenon. Therefore, women with IDDM have a chance equal to the general population of having an elevated result and being offered further testing.

Studies have shown that individuals of African-American ancestry have higher levels of

MSAFP than Caucasian individuals. For this reason, a correction factor is present in our computer program to counterbalance this phenomenon allowing women of African-American ancestry to have a chance equal to the general population of an elevated result, which may lead to further testing.

As with all patients screened, insulin-dependent diabetic women and women of African-American ancestry will be offered further testing when their MSAFP result is greater than or equal to 2.5 MoM.

If any of this pertinent information is absent from the lab slip, the processing of the patient's sample will be slowed down considerably. We must then place a call to your office to obtain the information. Sometimes, the person we need to speak to is not available to give us this information, and we have to wait for a return call. This means that the results and interpretation for your patient could be delayed by 2 or 3 days while we collect the information.

In addition to insurance information, the laboratory requisition form contains a consent statement for patients to sign. The purpose of this consent statement is to inform patients of the limitations of the screening test. Furthermore, this consent statement will allow our staff to obtain pregnancy outcome information. This information is essential because it provides your patients with the most accurate screening sensitivity and low false positive/low false negative rates specific for our patient population.

BILLING

Medicaid

1. If your patient has Medicaid, please enter their recipient identification number at the appropriate location on the laboratory requisition form and attach a current copy of their card that is legible.
2. If your patient has Medicaid “pending”, the insurance information should not be completed. If the patient becomes eligible, Medicaid will be billed for the test.
3. Indigent patients who are referred by a health department and who are not eligible for Medicaid will not be charged. Please indicate on the requisition form that the patient is indigent.

Private Insurance

1. If we are to bill your patient's insurance company, have them to fill in ALL applicable sections of the insurance information section.
2. Please make sure to have your patient sign and date the authorization statement and send a current copy of their insurance card(s) to be billed.

Doctor Billing

1. If your practice prefers to handle billing of your patients, the insurance information section should not be completed. A statement will be mailed to you at the end of each month with the total cost.

PLEASE NOTE:

If we receive the requisition form without pertinent insurance information (Subscriber ID#, Group or Plan NO., Effective Date, and Claims Address) the patient will be billed.

As of March 5, 2007 we do charge for second samples. To the best of our knowledge, insurance companies and Medicaid are providing coverage for second sample analysis. As always, your indigent patients will not be billed for first or second sample analysis. Please notify us if you have questions regarding this policy.

RESULTS

All samples are assayed within 24 hours of receipt in our Laboratory (Monday-Friday). Results are initially expressed as raw values in nanograms per milliliter (ng/ml) for AFP and uE₃, in picograms per milliliter (pg/ml) for DIA, and in international units per milliliter (IU/ml) for hCG. The raw value of each marker is then converted to a Multiple of the Median (MoM). MoM values allow comparison of results for the patient's population based on race, weight, diabetic status and gestational age.

The recommendations we provide for patients are based on information obtained from the literature, our experience, and the patient population screened through our Program.

If an ultrasound evaluation alters your patient's gestational dating by two or more weeks following maternal serum screening, please contact our office so the patient's results can be reinterpreted. We feel that the gestational age is best determined by the BPD (biparietal diameter) rather than the composite mean gestational age. This allows us to increase the sensitivity of our screening because: a) BPDs are smaller in fetuses with spina bifida and thus, reinterpretation will have the effect of keeping these women in the "at risk" pool and b) presuming femurs are shorter in babies with Down syndrome, use of femur lengths would lead to a younger mean gestational age. Reinterpretation would falsely indicate that these women should not be offered an amniocentesis. When the BPD and LMP are less than 2.0 weeks different, we consider them to be in agreement and feel that the patient's result is correctly interpreted.

Normal Results

A normal result means that the pregnancy is not considered to be at increased risk for an open neural tube defect, Down syndrome or Trisomy 18. However, this screening is not 100% sensitive, and it is still possible for the baby to have one of these conditions. Normal results are sent to your office by mail. The report can be faxed to your office upon request. **If gestational dating is subsequently changed by 2 or more weeks, then the results and interpretation may be incorrect.** Please notify us if a change in gestational dating occurs before a second sample is drawn. We will reinterpret the results and send you an amended copy of the report.

Because any baby can have a chromosome abnormality, it is possible to calculate a Down syndrome risk for your patient, even when her result is considered to be "normal". Normal means that the risk for Down syndrome is less than 1 in 270 (1:270). For Trisomy 18 syndrome it is also possible to calculate a risk for your patient even when her result is considered to be "normal." Normal means that the risk for Trisomy 18 is less than 1 in 100 (1:100).

Abnormal Results

Neural tube screening:

1. Elevated MSAFP MSAFP values greater than or equal to 2.5 Multiples of the Median (≥ 2.5 MoM) will be interpreted as elevated. The results and our recommendations will be called to your

office within 48 hours after completion of the assay, and the final report will be mailed. Please do not wait for the written report to schedule diagnostic testing. The report can be faxed to your office upon request.

A second sample will be requested on patients having MSAFP results between 2.50-3.49 Multiples of the Median (MoM) who are less than 17.5 weeks gestation. These patients have approximately a 50% chance that a second sample will be normal. Proceeding directly to diagnostic testing will be recommended for patients who have a MSAFP value greater than 3.50 MoM or are equal to or greater than 17.5 weeks gestation.

When requested, second samples should be drawn no sooner than 6 days after the first sample was drawn. This is based on the five-day half-life of AFP. If a fetal-maternal bleed is the cause of the elevated MSAFP, it is important to leave sufficient time for its clearance. Results of the second sample will be called to your office, and the final report will be mailed. The report can be faxed to your office upon request.

For a twin gestation, MSAFP values between 0-4.99 MoM are considered to be within normal limits. Because screening sensitivity is reduced in a twin gestation, we recommend a detailed ultrasound when the MSAFP levels are elevated between 2.5-4.99 MoM. MSAFP values greater than or equal to 5.0 MoM are considered elevated even for a twin gestation. For this reason, we recommend that the option of diagnostic testing be offered to patients with results in this range.

2. Borderline elevated MSAFP MSAFP values between 2.0-2.49 MoM are considered to be borderline elevated. For patients with a borderline value of MSAFP, a diagnostic ultrasound is recommended because the majority of undetected open neural tube defects have MSAFP levels between 2.0-2.49 MoM. A diagnostic ultrasound performed for patients with a borderline MSAFP value will improve the screening sensitivity for open neural tube defects. Unless a change in dating has occurred, a second sample is not recommended for patients with a borderline elevated MSAFP level.

3. Down syndrome screening: The likelihood of Down syndrome in the fetus is calculated using a combination of maternal age and the levels of MSAFP/hCG/uE₃/DIA. If the result suggests a risk for Down syndrome greater than or equal to 1 in 270 ($\geq 1:270$), diagnostic testing will be recommended. This result will be called to your office within 48 hours after completion of the assay, and the final report will be mailed. Please do not wait for the written report to schedule diagnostic testing. The report can be faxed to your office upon request.

4. Trisomy 18 screening: Patient specific risks for Trisomy 18 are calculated using maternal age in conjunction with MSAFP/hCG/uE₃ values. If the result suggests a risk for Trisomy 18 greater than or equal to 1 in 100 ($\geq 1:100$), diagnostic testing will be recommended. The results will be called to your office within 48 hours after completion of the assay and the final report will be mailed. The report can be faxed to your office upon request. It is not necessary to wait for the

written report to schedule diagnostic testing. In some cases, atypically low values of all three markers can be consistent with those of a non-pregnant individual. In this case, it is important to confirm the presence of a viable intrauterine pregnancy. If the pregnancy is viable, a sample mix up should be ruled out.

DIAGNOSTIC TESTING FOR ABNORMAL RESULTS

Diagnostic Testing for Elevated Maternal Serum Screening

If a patient is scheduled through one of the Medical Centers for genetic counseling and diagnostic testing, a genetic counselor will meet with the patient to discuss:

- possible causes of an elevated MSAFP, from a normal variation to the possibility of a birth defect
- the patient's specific risk for an open neural tube defect
- diagnostic testing options including the benefits, risks and limitations of ultrasound and amniocentesis
- information regarding ultrasound findings and/or the reinterpretation of results in the event of a significant change in gestational dating
- information regarding prognosis and support to patients as needed in the event of an abnormal finding
- the option of consultations with other specialists
- review of family and pregnancy history

In addition, the genetic counselor will communicate the test results and recommendations to the health care provider.

Targeted Ultrasound is available following genetic counseling in order to try to determine an explanation for the elevated MSAFP. Explanations for an elevated MSAFP level include: multiple gestation, more advanced gestational age, open neural tube defects, fetal demise, ventral wall defects, urinary tract abnormalities, other rare conditions, and normal variation for the pregnancy. If dating is confirmed and the patient is found to have a singleton pregnancy, amniocentesis is offered to the patient even if an abnormality is not identified by ultrasound. In about 1% of patients with an unexplained MSAFP elevation, a chromosome abnormality is identified in the fetus. Approximately 1-2% of patients screened are candidates for amniocentesis following ultrasound. *Even with using the most state-of-the-art equipment and experienced medical professionals, spinal defects can be missed. However, ultrasound can detect up to 85% of neural tube defects.*

Amniocentesis involves removing a sample of amniotic fluid (AF) for the measurement of AFP and acetylcholinesterase (AChE). Together, these tests are greater than 99% sensitive for the detection of open neural tube defects. Because elevated MSAFP results can be associated with an increased risk for a chromosome abnormality, chromosome analysis will be performed on the amniotic fluid. The results will be available 10 calendar days after the amniocentesis procedure and called to the patient and the health care provider. A copy of the results will be mailed to your office.

If the AF-AFP is elevated (greater than or equal to 2.5 MoM) or borderline elevated (between 2.0 and 2.49 MoM), another targeted ultrasound is recommended and genetic counseling should be made available.

Patients with an elevated MSAFP level and a normal AF-AFP level should be followed more

closely because they are at increased risk for adverse pregnancy outcomes such as preterm labor, low birth weight, intrauterine growth retardation, stillbirth and other birth defects.

Diagnostic Testing for Borderline Elevated MSAFP

MSAFP values between 2.0-2.49 MoM are considered to be borderline elevated. For those patients with a borderline elevated result, genetic counseling is not necessary. Because the majority of undetected open neural tube defects have MSAFP levels between 2.0-2.49 MoM, a diagnostic ultrasound to improve screening sensitivity is recommended. The health care provider can schedule a diagnostic ultrasound.

Diagnostic Testing for Down Syndrome and Trisomy 18

For those patients with an increased risk for Down syndrome or Trisomy 18, we request that patients have a local ultrasound to confirm dating and fetal viability prior to their appointment at one of the Medical Centers.

For patients with an increased risk for Down syndrome, a recalculation can be performed prior to drawing a second sample if the gestational dating changes by at least 14 days. In this case, please call our office so that the results can be recalculated, and a revised report will be sent to you. For increased Trisomy 18 risks, however, medical literature suggests that reinterpretation is not advised. However, if you feel the most accurate dating differs by 2 or more weeks from the sample dating, please contact our office. Again, a second sample is not necessary if dating is not altered as repeating the sample can lead to a false negative result.

If a patient is scheduled for genetic counseling and diagnostic testing at one of the Medical Centers, a genetic counselor will meet with the patient to discuss:

- the multiple possible causes of the increased risk
- for patients with an increased risk for Down syndrome, a patient specific risk for Down syndrome will be provided
- for patients with an increased risk for Trisomy 18, a patient specific risk will be provided
- diagnostic testing options including the benefits, risks and limitations of ultrasound and amniocentesis
- information regarding ultrasound findings and/or the reinterpretation of results in the event of a significant change in gestational dating
- information regarding prognosis and support to patients in the event of an abnormal result
- the option of consultations with other specialists
- review of pregnancy and family history

In addition, the genetic counselor will communicate the test results and recommendations to the health care provider.

Targeted Ultrasound is available following genetic counseling to rule out incorrect gestational

dating, a fetal demise, and other birth defects as possible explanations for the increased risk for Down syndrome or Trisomy 18. If gestational dating is confirmed, amniocentesis should be offered. *Although ultrasound technology is an excellent method to get a general view of the fetal anatomy, literature suggests that it is not an accurate method to screen for features of Down syndrome. In addition, ultrasound cannot detect fetal differences in all cases of Trisomy 18.*

Amniocentesis can rule out a chromosome abnormality with greater than 99% sensitivity. Final results are available in approximately 10 days. Results will be called to the health care provider, and the final report will be mailed. For increased sensitivity in detecting open neural tube defects, amniotic fluid AFP will be assayed for all samples, and the results will be mailed to your office.

Diagnostic Testing for Elevated hCG Results

MShCG values greater than or equal to 5.0 MoM are considered to be elevated. However, numerous literature reports suggest that hCG values above 2.0 MoM are associated with an increased risk of pregnancy complications such as preeclampsia, preterm delivery, placental abnormalities, intrauterine growth retardation (or small size for gestational age), and stillbirth. It is recommended that patients with elevated MShCG values have an ultrasound to assess fetal well-being.

Diagnostic Testing for low/undetectable uE₃ Results

Atypically low or undetectable MSuE₃ values can be consistent with inaccurate gestational dating, poor pregnancy outcome, or a fetal demise. Therefore, an ultrasound evaluation is recommended for gestational dating and to assess fetal well being. If dates and a viable pregnancy are confirmed, please contact our office and submit another blood sample.

Low/undetectable MSuE₃ values can also be consistent with steroid sulfatase deficiency for which prenatal testing is available. Options for testing include the measurement of placental sulfatase activity and dehydroepiandrosterone sulfate (DHEAS) in amniotic fluid cells, and fluorescence in situ hybridization (FISH) analysis to detect a deletion of the steroid sulfatase gene found on the X chromosome. This common deletion occurs in approximately 90% of individuals who have steroid sulfatase deficiency. We are happy to discuss these options with any of your patients who may be at an increased risk for having a baby with steroid sulfatase deficiency based on low/undetectable MSuE₃ value or positive family history.

FAMILY HISTORY

Neural Tube Defects: Patients with a significant family history of a neural tube defect are offered the option of an amniocentesis rather than participation in MSAFP screening, because they are at a higher risk for having a baby with a neural tube defect based on their family history alone. Amniocentesis can be performed earlier in gestation (14+ weeks) than MSAFP screening (15-20.9 weeks). In addition, the amniotic fluid AFP and acetylcholinesterase assays are more sensitive in detecting open neural tube defects than MSAFP screening. Ultrasonography is also needed to evaluate the fetus for skin-covered or closed defects that will not be identified by amniocentesis or MSAFP screening.

If a patient declines amniocentesis, then MSAFP screening is a prenatal testing option. This is also an appropriate time to offer patients genetic counseling, if they have not been offered counseling previously.

Different cut-offs are used for patients with a significant family history of a neural tube defect. If the MSAFP value is less than 2.0 MoM, a risk reduction will be calculated. For MSAFP values equal to or above 2.0 MoM, the risk may be increased. Therefore, a patient with a significant family history of a neural tube defect and a MSAFP value equal to or above 2.0 MoM should be offered the option of ultrasound and amniocentesis. Amniotic fluid AFP analysis in conjunction with an assay for acetylcholinesterase is greater than 99% accurate in the detection of open neural tube defects. Completion of these tests will take 10-14 days. Results will be called and mailed to your office when completed.

Chromosome Abnormalities: A normal maternal serum screening result does not guarantee a chromosomally normal fetus. In contrast to neural tube defect screening, we are unable to reduce a prior risk when there is a family history of Down syndrome after a normal MSAFP/hCG/uE₃/DIA result. For this reason, karyotyping via amniocentesis or chorionic villus sampling (CVS) remains the best option for women who desire reassurance that their baby's chromosomes are normal.

Chromosome abnormalities can be detected prenatally through CVS between 10 and 12 weeks gestation or through amniocentesis at 14 + weeks gestation. CVS does not allow for amniotic fluid AFP quantitation; therefore, patients opting for CVS may also want to consider MSAFP screening at 16-18 weeks gestation for open neural tube defect screening. Women who have an elevated MSAFP result will be offered the options of a targeted ultrasound and an amniocentesis for quantitation of amniotic fluid AFP. Genetic counselors at each Medical Center are available to answer questions about the benefits, risks and limitations of these procedures.

FOLLOW-UP

You will receive an outcome form (example attached) after delivery, which will request specific information about the pregnancy and its outcome on all patients with an abnormal screening result or a reported significant family history of a neural tube defect. This information allows us to provide you with the most accurate information regarding our Program's sensitivity, specificity, false positive and false negative rate for our patient population. If difficulties arise in completing this form or if you have any suggestions as to how we might expedite this process, please let us know.

For those patients with an abnormal screening result who decline diagnostic testing or **when a baby is born with any birth defect after a normal screening result**, particularly a neural tube defect or a chromosome abnormality, we ask that you **contact us directly**. This information is **essential** for optimal program performance.

For those patients who elect to terminate the pregnancy based on a prenatally detected abnormality, **we would appreciate a copy of the prenatal ultrasound report documenting the complication and a copy of the complete autopsy report**. Not only does this information provide us with outcome data, it is also useful for providing your patient with an accurate recurrence risk. Genetic counseling is available for all patients who have a prenatally detected abnormality. Again, by having the patient sign the original requisition form for MSAFP/hCG/uE₃/DIA screening, they are giving your office permission to release this information to us.

We are proud of our strong record of quality screening and patient care. We recognize that our success is dependent upon your continued interest and involvement in our Screening Program. If you have further questions about the Wake Forest University Maternal Serum Screening Program, or about a specific patient, please call one of the genetic counselors at (336) 713-7530.