Patient Brochure for Alpha-Fetoprotein (AFP) Testing

What is AFP testing?

There is a blood test called alpha-Fetoprotein (AFP) that is available to help identify a small number of women whose unborn babies may have certain defects of the brain and spinal cord. These are known as neural tube defects. The test also offers the remaining majority of women tested the added assurance that their babies are not likely to have this type of birth defect.

The test is being offered for those who want it. You are free to choose to take an AFP test or you may say that you do not want the test. If you are thinking of having the AFP blood test, you should be aware that the test itself is not a guarantee. It possible that a neural tube defect may be missed, and there is a very slight chance, even with extensive follow-up testing, that a healthy unborn baby may be incorrectly identified as having a neural tube defect. After reading this brochure, discuss the possibility of incorrect results with your doctor before you ask for an AFP test.

What are neural tube defects?

Neural tube defects affect the central nervous system. This system includes the spinal cord and the brain and is derived from a structure in the developing embryo called the neural tube. As a normal embryo develops in its mother, the neural tube closes completely.

If, however, all or part of the neural tube fails to close, leaving an opening, the baby has a neural tube defect. In some cases, the opening in the neural tube may be left exposed (open) or in others it may be covered with bone and skin (closed). Most closed neural tube defects are not detected by the AFP test.

What are some types of neural tube defects?

Two common and serious types of neural tube defects are anencephaly and spina bifida.

Anencephaly. This is a serious condition where the brain, head and possibly spinal cord do not develop normally. Newborns affected with this severe disorder usually die shortly after birth.

Spina bifida. This disorder is a defect of the spinal column, which is also called "open spine". Normal development of a child with spina bifida is possible. Often, however, disabilities are severe. Paralysis of the lower limbs, repeated urinary tract infections, hydrocephalus ("water on the brain"), mental retardation and incontinence (inability to control bladder or bowel movements) occur.

Additionally, the spinal malformation and nerve damage caused by this disorder hamper movement in varying degrees. Some individuals with spina bifida can walk by themselves; others use braces, crutches or wheelchairs. Modern surgical and corrective techniques can help many children born with spina bifida lead healthy and productive lives.

What causes neural tube defects?

The cause of neural tube defects is not known. The defects may be inherited; they may also be caused by environmental factors. More research must be conducted to find out the cause of these disorders.

What are the chances of a baby being born with a neural tube defect?

In the United States, about one to two live births per thousand involve a neural tube defect. Therefore, of the 3,000,000 babies born each year, about 3,000 to 6,000 will have a neural tube defect. About 90 percent of these will be open NTDs, evenly split between anencephaly and spina bifida.

Of the births that result in a neural tube defect, approximately 90 to 95 percent of babies with such a defect will be born to women with no "special risk" characteristic. The remaining 5 to 10 percent of babies with neural tube defects will be born to parents who have "special risk" characteristics such as:

- women who have already given birth to a child with a neural tube defect
- one or both parents have neural tube defects; and
- women whose mothers or fathers have a history of neural tube defects.

What is alpha-fetoprotein (AFP)?

Alpha-fetoprotein is a substance that is produced by the unborn baby as it grows.

When the neural tube is not properly formed, large amounts of alpha-fetoprotein pass into the amniotic fluid and reach the mother's blood. By measuring AFP in the mother's blood and amniotic fluid, it is possible to tell whether or not there is a chance that the unborn baby has an open neural tube defect.

What is the AFP blood test?

The AFP blood test is a simple laboratory procedure. A sample of blood is taken from your arm and sent to a laboratory. The laboratory tests the sample to see how much AFP is present and then sends the results to your doctor. If the result of the first blood test is elevated, then other tests are required before your doctor can determine whether your unborn baby has an open neural tube defect.

When should a pregnant woman have the AFP blood test?

The AFP blood test should be conducted between 15 to 22 weeks after the last menstrual period. The best time is 16 to 18 weeks. Unreliable results may be obtained if the blood sample is taken too early during pregnancy.

What do the test results mean?

If your blood has normal levels of AFP, the test is considered negative and there will be no need for further tests. Parents should understand that an AFP blood test result in the normal

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range can not guarantee a normal baby at birth. The first AFP blood test may miss as many as 20 percent of those babies affected with open neural tube defects. It can offer much greater assurance, however, that the baby is not likely to have an open neural tube defect.

An elevated result indicates that there are high levels of AFP in your blood. This does not always mean that a neural tube defect is present. It means that you should have additional tests to see whether or not your unborn baby has a neural tube defect.

After a first elevated blood sample, a second sample of blood may be taken from your arm and an ultrasound may be performed. If the result of the second AFP test is also elevated, there is only about a 4 to 10 percent chance that your unborn baby has an open neural tube defect. More likely, you may be carrying twins or your pregnancy may be farther advanced than you think. It is for these reasons that the doctor will want to conduct additional tests.

What are additional tests that may be needed if the AFP blood test is elevated?

Ultrasound. This is a procedure in which sound waves are used to obtain a television-like picture of the unborn baby. The picture will enable your doctor to tell whether the blood test was elevated because of twins or because the week of pregnancy was estimated incorrectly.

Ultrasound can also show if an unborn baby has died and often whether a neural tube defect is present. If the ultrasound does not give a reason for the elevated AFP values, amniocentesis may be recommended by your doctor.

Amniocentesis. In this procedure, the doctor will take a sample of amniotic fluid (the fluid filling the sac around the unborn baby) and send it to the laboratory to measure the AFP level in the fluid. If the AFP level is high and other possible causes (twins or an incorrect estimate of the week of pregnancy) have been ruled out, there is a high chance that the unborn baby has an open neural tube defect.

This test cannot tell how severe the defect will be or the possible degree of disability. Although relatively safe, amniocentesis presents a small risk. You may want to discuss the nature of this risk with your physician.

Other Tests. If the level of AFP in the amniotic fluid is elevated, the physician may wish to attempt to locate the possible defect by means of high resolution ultrasound (a more advanced and accurate type of ultrasound) or by amniography, in which the unborn baby is outlined by radiopaque dye injected into the uterus. To further reduce the chance of an incorrect diagnosis, a chemical test may be used in which amniotic fluid is analyzed for the enzyme acetylcholinesterase.

What should I consider when deciding whether or not to have an AFP test?

You should ask your doctor about these tests, the risks that they present, how much they cost, where these tests are given, genetic counseling that is available, and other questions that may concern you. Your doctor has received a more detailed brochure about AFP testing and should be able to provide you with additional information.

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2001-08-06 (ISO 8601)
August 6, 2001
ZS1106 – B

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Physician Brochure for Alpha-Fetoprotein (AFP) Testing

DPC IMMULITE® and IMMULITE® 2000 AFP

The concentration of AFP in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. Therefore, values obtained with different AFP assays cannot be used interchangeably. The results reported by the laboratory to the physician must include the identity of the AFP assay used. Prior to changing assays, the laboratory must establish a range of normal values for the new assay, based on normal sera and amniotic fluids from pregnant women with confirmed gestational age

Table of Contents

Purpose

This booklet has been written by Diagnostic Products Corporation (DPC) to familiarize you and your staff with pregnancy screening using alpha-fetoprotein (AFP) testing. Pregnancy screening using AFP testing, in conjunction with the appropriate and necessary follow-up testing, will provide you and your patients with valuable information concerning their pregnancies, including identification of most open neural tube defects (NTDs). It is recommended that each patient considering an AFP screening test be provided with a copy of the patient booklet "Patient Brochure for Alpha-Fetoprotein (AFP) Testing." Patient booklets and additional physician booklets are available from Diagnostic Products Corporation.

To obtain booklets, please call DPC's Customer Services at 1-800-372-1782.

Introduction

Neural tube defects (NTDs) are among the most common major fetal malformations found at birth. The incidence in the western United States is between 1 and 2 per 1,000 births. The incidence is higher in some other geographic areas. Anencephaly and spina bifida occur equally in approximately 94% of NTDs. Anencephaly is an open lesion that is incompatible with life. Open spina bifida occurs in approximately 80% of spina bifida cases and is associated with significant morbidity and mortality. Only approximately 30% of spina bifida patients survive beyond five years and approximately 94% of these patients have moderate or severe disabilities. Disabilities include difficulties with ambulation, bowel incontinence, bladder incontinence, urinary tract infections, hydrocephalus, and mental retardation.[1]

The causes of NTDs are not known. However, it is known that women who have delivered infants with NTDs are at a higher risk for recurrence (approximately 3%). Nevertheless, approximately 95% of the NTD births occur to women without such a history.

Alpha-fetoprotein (AFP) is the dominant serum protein early in fetal life but its functional role has not been clearly defined.[2] Its concentration decreases rapidly toward birth, and after birth to a baseline level that is maintained throughout adult life. In normal pregnancy, the fetal serum AFP concentration peaks at 10 to 13 weeks gestation. During pregnancy AFP is normally found in both the maternal serum and amniotic fluid. Maternal serum AFP (MSAFP) peaks at approximately 32 weeks gestation.[3]

Apparent abnormal elevations of MSAFP and amniotic fluid AFP (AFAFP) occur more often than true elevations, due primarily to multiple pregnancy and to inappropriate normal ranges based upon incorrect gestational age. Truly abnormal elevations of MSAFP and AFAFP occur in most women bearing a fetus with an open NTD. Excess AFP gains access to amniotic fluid, and to a lesser extent to the maternal serum, by transudation across the exposed surface of the fetus or across damaged glomeruli.[1,4] Most closed NTDs are not accompanied by abnormally high AFP concentrations and thus are not detectable by AFP testing.

A protocol for AFP prenatal testing, which is recommended, is found on page 8. Patient counseling precedes AFP testing and patient consent for testing is obtained. The optimal time for obtaining a maternal serum specimen is gestational weeks 16, 17, or 18.[1]

If the MSAFP is greater than the cutoff level established by the AFP laboratory, the MSAFP may be repeated on a second specimen. Based upon a single or repeated

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elevated MSAFP, ultrasound can be employed to verify the gestational age, the absence of multiple fetuses or other circumstances that may explain the abnormal MSAFP result. If no explanation for the abnormal MSAFP result is revealed by ultrasound, an AFAFP measurement is usually made following an amniocentesis procedure. If an elevated AFAFP concentration is found, it is very likely that the fetus has an open NTD. Acetylcholinesterase testing and high-resolution ultrasound or amniography can be used to confirm the presence of an NTD.

When to test

The timing of all AFP tests and related test procedures is crucial. The patient, physician, medical staff and laboratory personnel must collaborate to provide effective screening for open NTDs. Accurate gestational dating is essential for interpretation of MSAFP results. Gestational age is typically calculated by counting from the first day of the last menstrual period (LMP). The number of gestational weeks is considered to be the number of completed gestational weeks.

If an ultrasound has already been performed, this would be the preferred method for dating. Maternal serum AFP testing can be performed at any time between gestational weeks 15 and 20, weeks 16 and 18 being the optimal time for testing.[1]

Expected MSAFP values

The use of multiples of the median has been established as the preferred method to express AFP results. The median value is a number which is greater than half of the values of a sample population and less than the remaining half (50 percentile). Calculation of the multiple of the median for an individual patient requires knowing the gestational week. The patient's AFP concentration is divided by the population median for that same gestational week. Each AFP screening program or laboratory should establish its own cutoff. Cutoff values of 2.0 and 2.5 are examples of levels which have been employed in various populations.

Population AFP medians and patient results should be obtained with the same AFP assay. Population medians should be applied only to the same population from which it was derived. These should be obtained with a sufficient number of patients and should be updated on a regular basis to verify their continued applicability.

Maternal serum AFP concentrations from unaffected, singleton pregnancies expressed as the median and multiples of the median for gestational weeks 15 to 20 are displayed in the following table. These values were calculated by the weighted log-linear regression on maternal serum AFP measurements obtained from three clinical sites in the United States using DPC's IMMULITE and IMMULITE 2000 AFP assays.

Gestational Week	No. of Specimens	Regressed Medians IU/mL	Multiples of Regressed Medians (IU/mL) 2.0	2.5	3.0
15	370	24.9	49.8	62.3	74.7
16	605	28.5	57.0	71.3	85.5
17	569	32.6	65.2	81.5	97.8
18	431	37.2	74.4	93.0	111.6
19	221	42.5	85.0	106.3	127.5
20	91	48.6	97.2	121.5	145.8

Clinical sensitivity and specificity—MSAFP

The sensitivity and specificity of MSAFP testing for open NTDs vary as a function of the chosen cutoff. In general, the specificity of a test increases when higher cutoffs are selected, while the reverse is true for the test sensitivity.

The sensitivity of MSAFP testing is defined as the percentage of the population carrying fetuses with an open NTD that had MSAFP concentrations greater than the selected cutoff.[5] The following table displays the *clinical sensitivity* for IMMULITE and IMMULITE 2000 assays at different cutoffs for *maternal serum* samples:

Assay	*n* of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
IML AFP	13	92.3%	69.2%	69.2%
IML 2000 AFP	9	100.0%	77.8%	66.7%

Specificity of MSAFP testing is defined as the percentage of the pregnant population sampled carrying unaffected, singleton fetuses that had MSAFP concentrations less than or equal to the cutoff. Specificity of MSAFP testing is generally in the high 90th percentile. The following tables display the *clinical specificity* for IMMULITE and IMMULITE 2000 assay at different cutoffs for *maternal serum* samples.

IMMULITE AFP Clinical Specificity for Maternal Serum:

Gestation. Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	173	96.0%	98.8%	98.8%
16	411	98.1%	99.3%	99.5%
17	372	96.5%	99.7%	100%
18	204	95.1%	99.0%	100%
19	108	94.4%	99.1%	100%
20	50	100%	100%	100%
15 – 20	1318	96.7%	99.3%	99.7%
95% CI for All Samples		95.5% – 97.6%	98.7% – 99.7%	99.2% – 99.9%

IMMULITE 2000 AFP Clinical Specificity for Maternal Serum:

Gestational Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	276	94.2%	97.5%	98.6%
16	304	96.1%	99.0%	99.7%
17	272	97.1%	99.3%	99.6%
18	287	95.8%	98.6%	99.3%
19	152	93.4%	98.0%	99.3%
20	41	95.1%	100%	100%
15 – 20	1332	95.5%	98.6%	99.3%
95% CI for All Samples		94.2% – 96.5%	97.8% – 99.1%	98.7% – 99.7%

Interpretation of MSAFP results

Elevated MSAFP results are not diagnostic for NTDs and should not be considered a cause for termination of pregnancy. An overlap exists in the distributions of AFP concentrations from pregnancies with and without open NTDs. After ruling out multiple fetuses and incorrect gestational age, the following fetal conditions should be considered as suspect with elevated levels of MSAFP: fetal distress or demise, congenital nephrosis, ventral wall defects including omphalocele and gastroschisis, esophageal or duodenal atresia, polyhydramnios, annular pancreas, tetralogy of Fallot, Meckel's syndrome, pilonidal sinus, sacrococcygeal teratoma and Turner's syndrome.[1,6,7] Additionally, maternal conditions such as feto-maternal transfusion, liver disease, severe Rh isoimmunization and toxemia can result in elevated levels of MSAFP.

Other factors should be considered when assessing the risk of an NTD. The overall rate of NTDs decreases geographically from east to west within the United States.[8] There is an inverse correlation between maternal weight and MSAFP concentrations; higher maternal weights, reflective of blood volume, result in lower MSAFP concentrations.[9] Women with insulin-dependent diabetes have also been reported to have approximately 21 percent lower MSAFP than noninsulin-dependent diabetic women.[10] Racial differences also pose another consideration. MSAFP concentrations in pregnant African-American women has been reported to be approximately 10 percent higher than those in their Caucasian counterparts.[11] In addition, prevalence of NTD is approximately two to three times higher among births to white families than among those to black families.[12]

Ultrasound

Ultrasound is a useful method in determining the probable cause of abnormal MSAFP values. For example, the correct gestational age and the presence of multiple fetuses can be determined by ultrasound. In addition, ultrasound may detect some NTDs such as anencephaly. However, ultrasound cannot detect all NTDs.

When to perform AFAFP testing

Gestational weeks 15 through 20 are recommended for detecting an open NTD via AFAFP testing.

A pre-amniocentesis maternal serum or plasma specimen should be analyzed for AFP. The specimen must be drawn before amniocentesis to avoid fetal blood contaminating the maternal specimen and thereby producing a false MSAFP elevation. The pre-amniocentesis specimen can be of additional value if the amniotic fluid specimen is contaminated with fetal blood.

Expected AFAFP values

Amniotic fluid AFP values from singleton pregnancies unaffected by NTDs, expressed as medians and multiples of the median for gestational weeks 15 to 20, are displayed in the following table. These values were calculated by the weighted log-linear regression on amniotic fluid AFP measurements obtained from two clinical sites in the United States using DPC's IMMULITE and IMMULITE 2000 AFP assays.

Gestational Week	No. of Specimens	Regressed Medians kIU/mL	Multiples of Regressed Medians (kIU/mL) 2.0	2.5	3.0
15	76	13.0	26.0	32.5	39.0
16	89	10.7	21.4	26.8	32.1
17	53	8.73	17.5	21.8	26.2
18	54	7.14	14.3	17.9	21.4
19	46	5.84	11.7	14.6	17.5
20	23	4.78	9.56	12.0	14.3

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Clinical sensitivity and specificity—AFAFP

The United Kingdom Collaborative Study on Alpha-Fetoprotein in Relation To Neural Tube Defects evaluated the sensitivity of AFAFP at multiple cutoffs. With a cutoff of 3.0 MoM at weeks 16–18, more than 98 percent of the samples associated with open NTDs were correctly identified.[4]

The following table displays the *clinical sensitivity* for IMMULITE and IMMULITE 2000 assays for different cutoffs for *amniotic fluid* samples:

Assay	*n* of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
IML AFP	10	90.0%	90.0%	90.0%
IML 2000 AFP	8	87.5%	87.5%	87.5%

Specificity of AFAFP testing is defined as the percentage of the pregnant population sampled carrying unaffected, singleton fetuses that had AFAFP concentrations less than or equal to the cutoff. Specificity of AFAFP testing is usually very high. The following tables display the *clinical specificity* for IMMULITE and IMMULITE 2000 AFP assays at different cutoffs for *amniotic fluid* samples:

IMMULITE AFP Clinical Specificity for Amniotic Fluid:

Gestation. Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	23	100%	100%	100%
16	39	97.4%	100%	100%
17	25	100%	100%	100%
18	34	94.1%	97.1%	100%
19	33	100%	100%	100%
20	13	100%	100%	100%
15 – 20	167	98.2%	99.4%	100%
95% CI for All Samples		94.8% – 99.6%	96.7% – 100%	97.8% – 100%

IMMULITE 2000 AFP Clinical Specificity for Amniotic Fluid:

Gestation. Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	53	100%	100%	100%
16	50	98.0%	100%	100%
17	28	100%	100%	100%
18	20	100%	100%	100%
19	13	92.3%	100%	100%
20	10	100%	100%	100%
15 – 20	174	98.9%	100%	100%
95% CI for All Samples		95.9% – 99.9%	97.9% – 100%	97.9% – 100%

Interpretation of AFAFP results

An elevated amniotic fluid AFP concentration usually identifies the presence of an open NTD, if fetal blood contamination has been ruled out. (See the section below on specimen collection and handling.) The following are conditions other than NTDs that can be associated with elevated levels of AFP: ventral wall defects including omphalocele and gastrochisis, fetal distress or demise, fetal congenital nephrosis, esophageal or duodenal atresia, oligohydramnios, tetralogy of Fallot, Meckel's syndrome, sacrococcygeal teratoma and Turner's syndrome.[1,6,7]

In addition, rare idiopathic AFAFP elevations can occur; confirmatory testing should always be performed when amniotic fluid AFP levels are elevated.

Confirmatory tests

Acetylcholinesterase (AchE) has been found to be present in the amniotic fluid of fetuses with open NTDs and can serve as a confirmatory test for open NTDs.[13] High resolution ultrasound or amniography can also be helpful in visualizing the site and extent of an open NTD, if present.

Specimen collection and handling

Maternal serum and plasma:

- Fasting is not required.
- Collect blood by standard venipuncture techniques into plain, EDTA or heparin tubes.
- Separate serum or plasma from the cells immediately to avoid hemolysis. (Serum separator tubes may be employed.)
- Specimens must be obtained prior to amniocentesis since this procedure may lead to spuriously elevated

MSAFP levels persisting for 2 to 3 weeks. If a repeat analysis is required, the original type of specimen should be taken to maintain consistency of results.

- Serum or plasma samples may be stored at 2–8°C for up to 3 days. Samples not assayed within 3 days should be stored at –20°C.[14]

Amniotic fluid:

- Collect amniotic fluid by amniocentesis into plain tubes. Samples should be obtained by aseptic transabdominal amniocentesis performed by an experienced obstetrician during the second trimester of pregnancy in women with confirmed gestational age.
- Centrifuge the specimen, retaining a portion of the clear supernatant.
- Inspect supernatant and sediment for signs of blood or hemoglobin. Contamination by even trace amounts of fetal material will raise the apparent AFP concentration of the sample, rendering it unsuitable for analysis.
- The origin of the fetal material should be determined by a test for fetal hemoglobin. If fetal contamination has occurred and the AFAFP concentration is elevated, an additional specimen should be obtained after 7 to 10 days for evaluation. Amniotic fluid contamination by maternal serum may reflect accurate AFP levels provided the degree of contamination is not sufficient to dilute the sample.
- Amniotic fluid samples may be stored at 2–8°C for up to 3 days. Samples not assayed within 3 days should be stored at –20°C.[14]

Sample Physician Record for AFP Prenatal Testing

The following form is useful to record information related to AFP prenatal testing. Physicians may consider using a similar form to track their patients through AFP testing:

Patient Name		Patient ID	
Maternal Age			
First Day of Last Menstrual Period (LMP)			
Serum Specimen #1			
Draw Date		Gestational Age (GA)	
Assay Date		AFP IU/mL =	= MoM
		Corrected MoM	= MoM
Interpretation			
Serum Specimen #2			
Draw Date		Gestational Age	
Assay Date		AFP IU/mL =	= MoM
		Corrected MoM	= MoM
Interpretation			
Ultrasonography			
Date	Location	No. of fetuses	
GA (by LMP)		GA (by U/S)	
Comments / Interpretation			
Amniocentesis			
Draw Date	Location	Performed by:	
Sample Adequacy			
Assay Date		GA / method	
kIU/mL =	MoM		
Comments / Interpretation			
Other Tests			
Test	Date	Location	
Performed by:			
Comments / Interpretation			

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Patient Data Collection Form for AFP Prenatal Testing

The following information is to be completed by the patient:

Patient Name	Date of Birth
Address	
Race (please specify)	

To be completed by physician:

Visit Date			
Maternal Height			Maternal Weight
Gestational Age (last completed week of pregnancy)			
Gestational Age by:	LMP	PE	U/S (date)
Patient Diabetic	Yes	No	
Other History			
Reason for Referral			

To be completed by Laboratory:

Date Sample Collected			Institution where sample collected	
Sample Type:	Serum		Plasma (type)	
	Initial		Repeat	
Amniotic Fluid(*)	Is fluid blood stained:		Yes	No
If yes, are fetal red blood cells or fetal hemoglobin present?			Yes	No
Date sample received				
Date sample tested			Assay Method	
Result: MSAFP =		IU/mL =	MoM	
MSAFP at cutoff for gestational week		is	IU/mL	
Normal		Elevated (cutoff determination)		
If elevated, telephone physician immediately				
Amniotic Fluid Result:	AFAFP =		kIU/mL =	MoM
AFAFP at cutoff for gestational week		is	kIU/mL	
Normal		Elevated (cutoff determination)		
If elevated, telephone physician immediately				

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References

1. J.E. Haddow, et al, "Fetal Disorders Associated with elevated MSAFP Values" Prenatal Screening for Major Fetal Disorders, J.E. Haddow et al., ed. (Foundation For Blood Research, 1990) Volume 1.
2. B. F. Crandall, "Alpha-fetoprotein: A review" CRC Critical Reviews in Clinical Laboratory Sciences (September 1981) 127-185.
3. D. Gitlin, Normal biology of AFP, Ann NY Acad Sci 259:7 (1975).
4. N.J. Wald and H.S. Cuckle, "Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second Report of the U.K. Collaborative Study on Alpha-fetoprotein in Relation To Neural Tube Defects" Lancet ii:651-661 (1979).
5. B.K. Burton, S.G. Sowers and L.W. Nelson: Maternal serum alpha-fetoprotein screening in North Carolina. Experience with more than twelve thousand pregnancies. Am J Obstet Gynec 146:439 (1983).
6. D.J.H. Brock, "Prenatal diagnosis-chemical methods" British Medical Bulletin 32:16 (1976).
7. M. Seppälä, "Fetal pathophysiology of human alpha-fetoprotein" Ann NY Acad Sci 259:59-73 (1975).
8. F. Greenburg, L.M. James, G.P. Oakley. Estimates of birth prevalence rates of spina bifida in the United States from computer-generated map. Am J Ob Gynec 145 (1983) 570.
9. J.E. Haddow, E.M. Kloza, G.J. Knight, D.E. Smith, "Relation between maternal weight and serum alpha-fetoprotein concentration during the second trimester" Clin Chem 27:133 (1981).
10. M.F. Green, J.E. Haddow, G.E. Palomaki, G.J. Knight, "Maternal serum alpha-fetoprotein levels in diabetic pregnancies" Lancet 2 (8606):345-6 (Aug. 6, 1988).
11. B.F. Crandall, R.B. Lehbhez, P.C. Schroth, et al., "Alpha-fetoprotein concentration in maternal serum: Relation to race and body weight" Clin Chem 20:531 (1983).
12. J.D.Erikson, "Racial variations in the incidence of congenital malformations" Annals of Human Genetics 39:315 (1976).
13. A.D. Smith, N.J. Wald, H.S. Cuckle, G.M. Stirrat, M. Bobrow and H Lagercrantz, "Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural tube defects in early pregnancy" Lancet 1:685 (1979).
14. D.R. Pollard and G. Kamlesh, "Stability of Alpha-fetoprotein in Stored and Frozen-thawed Aliquots" Clin Biochem (5) 266-267 (1982).

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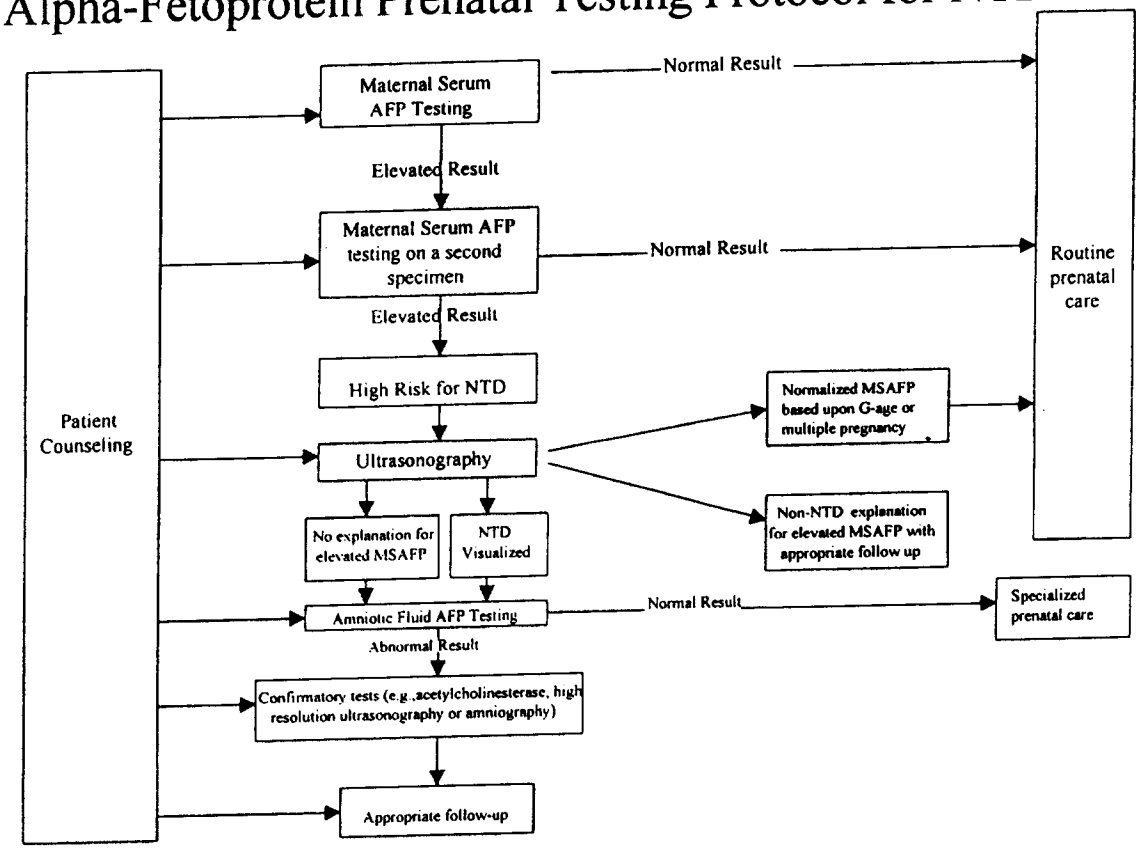
Alpha-Fetoprotein Prenatal Testing Protocol for NTD Detection



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2001-09-17 (ISO 8601)

September 17, 2001

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