Abbott AXSYM SYSTEM

en

TUMOR MARKERS AFP

REF 7K52

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B7K520

Read Highlighted Changes
Revised August, 2010

TUMOR MARKERS AFP

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	STANDARD CAL A	Standard Calibrator (A-F)
IVD	*In Vitro* Diagnostic Medical Device	CONTROL L	Control Low, Medium, High (L, M, H)
LOT	Lot Number	REAGENT PACK	Reagent Pack
	Expiration Date	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
15°C 30°C	Store at 15-30°C	MATRIX CELLS	Matrix Cells
	Caution	REACTION VESSELS	Reaction Vessels
	Manufacturer		Consult instructions for use

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

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WARNING: 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. The results reported by the laboratory to the physician must include the identity of the AFP assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining AFP levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST:

1. For Cancer Management - Confirm baseline values for patients being serially monitored.
2. For Prenatal Testing - Establish a range of normal values for the new assay based on normal serum, plasma and amniotic fluids from pregnant women with confirmed gestational age.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the AxSYM AFP assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this assay package insert.

NAME

AxSYM AFP - alpha-fetoprotein

INTENDED USE

AxSYM AFP is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of alpha-fetoprotein (AFP) in:

1. Human serum or plasma to aid in the management of patients with nonseminomatous testicular cancer.
2. Human serum, plasma and amniotic fluid at 15 to 21 weeks gestation to aid in the detection of fetal open neural tube defects (NTD). Test results when used in conjunction with ultrasonography or amniography are a safe and effective aid in the detection of fetal open NTD.

SUMMARY AND EXPLANATION OF THE TEST

The discovery of alpha-fetoprotein (AFP) in fetal serum was first recorded by Bergstrand and Czar in 1956.[1] Alpha-fetoprotein is a single polypeptide chain glycoprotein with a molecular weight of approximately 70,000 daltons. The physicochemical properties and amino acid composition are similar to those of albumin.[2,3] Synthesis of AFP occurs primarily in the liver and yolk sac of the fetus. It is secreted into fetal serum, reaching a peak at about 13 weeks gestation and gradually declining thereafter. Elevated serum AFP levels subsequently reappear during pregnancy and in conjunction with several malignant diseases.

Cancer Management

Alpha-fetoprotein (AFP) was first described as a human tumor-associated protein in 1964 by Tatarinov.[4] Since then it has been shown that elevation of serum AFP above values typically found in healthy individuals occurs in several malignant diseases,[5-8] most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease.[9,10] Elevated AFP levels also have been observed in patients diagnosed as having seminoma with nonseminomatous elements but have not been observed in patients with pure seminoma.[7,9,11,12] Human chorionic gonadotropin (hCG) and AFP are also important prognostic indicators of survival rate among patients with advanced nonseminomatous germ cell testicular tumors.[13]

The usefulness of AFP measurements in the management of patients with nonseminomatous testicular cancers has been well documented.[7,11,14] For patients in clinical remission following treatment, AFP levels generally decrease.[11] Post-operative AFP values which fail to return to normal strongly suggest the presence of residual tumor.[6,7,11] Tumor recurrence is often accompanied by a rise in AFP before progressive disease is clinically evident.[7,9]

Greater than 70% of patients with primary hepatocellular carcinoma have been reported to have elevated levels of serum AFP.[5,6,15]

Elevated AFP levels have occasionally been found in association with gastrointestinal tract cancers with and without liver metastases[16] and only rarely in other malignancies.[5,6] Serum AFP has been found to be elevated during pregnancy, in diseases such as ataxia telangiectasia, hereditary tyrosinemia, teratocarcinoma and in benign hepatic conditions, such as acute viral hepatitis, chronic active hepatitis and cirrhosis.[6,15,17] Elevation of serum AFP in benign hepatic diseases is usually transient.[5]

AFP testing is not recommended as a screening procedure to detect cancer in the general population.

Prenatal Testing

Many studies have confirmed the utility of AFP in the early detection of fetal open neural tube defects (NTD)[18-20]. Reports from the scientific literature suggest several factors to be considered when assessing the risk of NTD.[21-27] One factor is the effect of maternal weight. Maternal blood volume, as reflected by maternal weight, has been reported to affect maternal serum AFP (MSAFP) concentration in maternal circulation; the higher the maternal weight, the lower the MSAFP concentration.[25,28] Maternal serum AFP levels in the black population average about 10% higher than MSAFP values in the non black population. An adjustment factor or use of an appropriate normative database have been suggested in the literature.[24,25] Another factor to consider is maternal diabetes. Insulin dependent diabetic women reportedly have MSAFP levels significantly lower than non-diabetic women, and an increased incidence of NTD.[26,27,29]

Amniotic fluid AFP (AFAFP) levels peak at about 13 weeks gestation after which they rapidly decline until about 22 weeks of gestation and then gradually decline until term. Transfer of AFP into maternal circulation is accomplished primarily through diffusion across the placenta.[30] If the fetus has an open neural tube defect, AFP is thought to leak directly into the amniotic fluid causing unexpectedly high levels of AFAFP. Subsequently, the AFAFP reaches the maternal circulation, thus producing abnormally elevated levels of MSAFP. Other fetal abnormalities such as congenital renal disease and esophageal atresia also show AFAFP elevations.[31,32]

Abnormally high levels of MSAFP may also be exhibited by fetal distress situations such as omphalocele or gastroschisis, defective kidneys, threatened abortion, prematurity, and sometimes fetal demise[33-36]. Increased MSAFP values are also seen in multiple pregnancies[37] and normal singleton pregnancies in which the gestational age has been underestimated. Low MSAFP values have been associated with molar pregnancy, missed abortion, pseudocyesis, and overestimated gestational age.[38]

AFP testing during pregnancy is recommended as an effective way to identify those women potentially at risk of carrying a fetus affected with an open NTD. Used in conjunction with other confirmatory procedures such as ultrasonography or amniography, the measurement of AFP serves as an important tool in the care and management of these patients.

The reliability of MSAFP evaluation in prenatal testing is dependent upon the accurate determination of gestational age. An overestimated gestational age may result in the misinterpretation of MSAFP values, and thus, the underestimation of risk of NTD. On the other hand, underestimated gestational age may also result in the misinterpretation of MSAFP values, that is, overestimation of risk of NTD. Both situations may lead to unnecessary additional testing. When gestational age is uncertain, a reliable ultrasound examination is important.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM AFP is based on the Microparticle Enzyme Immunoassay (MEIA) technology.

The AxSYM AFP reagents and sample are pipetted in the following sequence:

Sample and all AxSYM AFP reagents required for one test are pipetted by the Sampling Probe into various wells of a reaction vessel (RV) in the Sampling Center. The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

The reactions occur in the following sequences:

- Sample, Specimen Diluent and Anti-AFP Coated Microparticles are delivered to one well of the reaction vessel. During the incubation of this reaction mixture the AFP in the specimen binds to the Anti-AFP Coated Microparticles forming an antibody-antigen complex.
- An aliquot of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- The matrix cell is washed to remove unbound materials.
- The Anti-AFP: Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds to the antibody-antigen complex.
- The matrix cell is washed to remove unbound materials.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.

For further information, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

Reagent Pack, 100 Tests

AxSYM AFP Reagent Pack (7K52-21)

- 1 Bottle (7.0 mL) Anti-AFP (Mouse, Monoclonal) Coated Microparticles in TRIS buffer with protein stabilizers. Preservative: sodium azide. (Reagent Bottle 1)
- 1 Bottle (13.2 mL) Anti-AFP (Mouse, Monoclonal): Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 µg/mL. Preservatives: sodium azide and antimicrobial agents. (Reagent Bottle 2)
- 1 Bottle (23.6 mL) Specimen Diluent, Phosphate buffered calf serum with protein stabilizers. Preservatives: sodium azide and antimicrobial agents. (Reagent Bottle 3)
- 1 Bottle (45.2 mL) Specimen Diluent (for Autodilutions), Phosphate buffered calf serum. Preservatives: sodium azide and antimicrobial agents. (Reagent Bottle 4)

Calibrators

AxSYM AFP Standard Calibrators (7K87-01)

6 Bottles (4 mL each) of AxSYM AFP Standard Calibrators: Calibrator A contains phosphate buffered calf serum. Calibrators B-F contain AFP (human) in pooled human serum prepared in phosphate buffered calf serum at the following concentrations:

Bottle	AFP Concentration (ng/mL)
STANDARD CAL A	0
STANDARD CAL B	15
STANDARD CAL C	50
STANDARD CAL D	100
STANDARD CAL E	200
STANDARD CAL F	350

Preservatives: sodium azide and antimicrobial agents.

The AFP calibrators are manufactured gravimetrically and are referenced to the World Health Organization (WHO) International Standard for alpha-fetoprotein at each concentration level. The conversion factor is 0.83 International Units per nanogram of AFP.

Controls

AxSYM AFP Controls (7K87-10)

3 Bottles (8 mL each) of AxSYM AFP Controls contain AFP in pooled human serum prepared in processed bovine serum to yield the following concentration ranges:

	AFP Concentration		Range	
Bottle	ng/mL	IU/mL	ng/mL	IU/mL
CONTROL L	20	16.60	15 - 25	12.45 - 20.75
CONTROL M	80	66.40	64 - 96	53.12 - 79.68
CONTROL H	175	145.25	140 - 210	116.20 - 174.30

Preservatives: sodium azide and antimicrobial agents.

AxSYM AFP Calibrators and Controls should be mixed by gentle inversion prior to use.

Specimen Diluent

AxSYM AFP Specimen Diluent (7K52-50)

SPECIMEN DILUENT 1 Bottle (100 mL) AxSYM AFP Specimen Diluent, phosphate buffered calf serum. Preservatives: sodium azide and antimicrobial agents.

Other Reagents

Solution 1 (MUP) (8A47-04)

SOLUTION 1 MUP 4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: sodium azide.

Solution 3 (Matrix Cell Wash) (8A81-04)

SOLUTION 3 MATRIX CELL WASH 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS Buffer. Preservatives: sodium azide and antimicrobial agents.

Solution 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate Buffer. Preservatives: sodium azide and antimicrobial agents.

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium hydroxide (TEAH).

WARNINGS AND PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[39] Biosafety Level 2[40] or other appropriate biosafety practices[41,42] should be used for materials that contain or are suspected of containing infectious agents.
- The pooled human serum used in Calibrators B-F and the Controls has been tested and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way. For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the AxSYM System Operations Manual, Section 7 and 8.

Handling Precautions

- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**
- Do not use kits beyond the expiration date or a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different reagent packs.
- AxSYM AFP Reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. Refer to the AxSYM System Operations Manual, Section 9.

Refer to the AxSYM System Operations Manual, Sections 7 and 8 for a more detailed discussion of the safety and handling precautions during system operation.

Storage Instructions

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The AxSYM AFP Reagent Pack, AxSYM AFP Calibrators, AxSYM AFP Controls and AxSYM AFP Specimen Diluent must be stored at 2 to 8˚C (do not freeze). The AxSYM AFP Reagent Pack, AxSYM AFP Calibrators, AxSYM AFP Controls and AxSYM AFP Specimen Diluent may be used immediately after removing them from the refrigerator. Calibrators, Controls and Specimen Diluent should be returned to 2 to 8˚C storage immediately after use. Reagents are stable until the expiration date when stored and handled as directed.
- The AxSYM AFP Reagent Pack may be on board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight hour shifts. After 112 hours the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2, 5 and Appendix C, for further information on tracking onboard time.
- **Solution 1 (MUP) must be stored at 2 to 8˚C. It may be on board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded. It may be used immediately after removing it from the refrigerator. Do not freeze MUP.**
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The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash) and Solution 4 (Line Diluent) must be stored at 15 to 30˚C.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM AFP assay file must be installed on the AxSYM System from the following software disk, prior to performing AFP assays:

- 3D50-06 and higher (112 hours onboard stability)

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM AFP Assay Parameters

The default values for the assay parameters used for the AxSYM AFP assay are listed in the following table. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen. Press PRINT to print the assay parameters.

	Assay Parameters
1	Long Assay Name (English): AFP
6	Abbrev Assay Name (English): AFP
11	Assay Number: 428
12	Assay Version: *
13	Calibration Version: *
14	Assay File Revision: *
15	Assay Enabled > ON
17	Assay Type: MEIA
18	Standard Cal Reps > 2
20	Cal Adjust Reps: 0
21	Cal A Concentration: 0.00
22	Cal B Concentration: 15.00
23	Cal C Concentration: 50.00
24	Cal D Concentration: 100.00
25	Cal E Concentration: 200.00
26	Cal F Concentration: 350.00
29	Cal Adjust Concentration: 0.00
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > ng/mL
46	Selected Result Decimal Places > 2
92	High Range Undiluted: *
97	High Range Dil1: *
102	High Range Dil2: *

NOTE: Parameter 45 can be edited to the alternate result unit IU/mL.

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Specimens with obvious microbial contamination should not be used.
- Do not test grossly hemolyzed specimens.
- Serum, plasma (Heparin, Citrate or EDTA), or amniotic fluid specimens may be used with the AxSYM AFP assay. When a patient is being monitored by assay of serial samples, the same type of specimen should be used throughout monitoring.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) is(are) used in the AxSYM AFP assay.
- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If a serum sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- Avoid repeated freezing and thawing. Mix thoroughly after thawing by low speed vortexing or inverting gently to ensure consistency in the results. Specimens showing particulate matter, erythrocytes, or turbidity must be clarified by centrifugation before testing.
- Samples may be stored for up to 24 hours at 2 to 8˚C prior to being tested. If testing will be delayed more than 24 hours, the specimen should be stored at -20˚C or colder. Samples that have been stored at -20˚C or colder for 12 months have shown no performance differences.
- Serum or plasma specimens should be collected aseptically in such a way as to avoid hemolysis. For maternal serum or plasma analysis, the blood specimen should be collected prior to the initiation of amniocentesis. It has been demonstrated that increased levels of AFP may occur in maternal serum or plasma following amniocentesis.[43]
- Amniotic fluid should be collected aseptically with appropriate precautions relative to both fetal and maternal safety by appropriately trained personnel. Visibly bloodstained specimens should be examined for the presence of fetal blood cells by using the Kleihauer-Betke technique[30] and/or for fetal hemoglobin by electrophoresis, immunoelectrophoresis or other available techniques. Amniotic fluid specimens contaminated with fetal blood may exhibit abnormally high AFP values which may lead to misinterpretation of test results.
- Performance has not been established using cadaver specimens or body fluids other than listed specimen types (i.e. human serum or plasma).
- All samples (patient samples, controls and calibrators) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.

Sample Volume

Sample volume required to perform a single AFP test on the AxSYM System varies according to the different sample containers. For sample cups, a ROUTINE test requires 150 µL and a STAT test requires 108 µL. For every additional AFP test performed (ROUTINE or STAT) from the same container, an additional 58 µL of sample is required.

The sample cup minimum volumes for both STAT and ROUTINE tests are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is(are) ordered. The STAT sample cup minimum volume is printed on the Orderlist Report. When using Host Order Query, the Order screen information and the Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5: Operating Instructions, Subsection: Ordering Patient Samples, for a description of the Host Order Query option.

If the assay is configured for auto retest/auto dilution, the additional sample volume needed for the retest will not be displayed on the order screen at the time the test(s) is(are) ordered. Therefore, the total sample volume should include the additional 58 µL of sample.

To obtain the recommended volume requirements for the AxSYM AFP Calibrators and Controls, invert, hold the bottles **vertically** and dispense 4 drops of each Calibrator or Control into each respective sample cup.

Refer to the AxSYM System Operations Manual, Section 5, for volume requirements in primary or aliquot tubes and calibrator/control requirements for multiple reagent lots.

AxSYM AFP PROCEDURE

Materials Provided

- 7K52-21 AxSYM AFP **REAGENT PACK**

Materials Required but Not Provided

- AxSYM System
- 7K87-10 AxSYM AFP Controls
- 7K87-01 AxSYM AFP Standard Calibrators
- 7K52-50 AxSYM AFP Specimen Diluent
- 8A47-04 **SOLUTION 1 MUP**
- 8A81-04 **SOLUTION 3 MATRIX CELL WASH**
- 8A46 **SOLUTION 4 LINE DILUENT**
- 9A35-05 AxSYM **PROBE CLEANING SOLUTION**
- 8A76-01 **SAMPLE CUPS**
- 8A75-02 **REACTION VESSELS**
- 8A73-02 **MATRIX CELLS**

CAUTION:

- When manually dispensing sample into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the system inventory of matrix cells, bulk solutions and waste levels are acceptable.

NOTE: The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum STAT sample cup volume requirements for all tests ordered. When using Host Order Query the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Operating Instructions, Subsection: Ordering Patient Samples, for a description of the Host Order Query option.

CAUTION: When operating the AxSYM System, always observe the following:

- The System status must be WARMING, PAUSED, READY or STOPPED before adding or removing sample segments, reagent packs or reaction vessels.
- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. Any tests will be terminated and must be repeated.
- The cap for reagent bottle 4 must be manually opened prior to running an AxSYM AFP assay. Upon completion of the run, close the reagent bottle 4 cap securely.
- When testing is completed, it is recommended that samples and the AxSYM AFP Reagent Pack be removed from the Sampling Center to maximize the onboard reagent pack use. Store at 2 to 8°C.

SAMPLE DILUTION PROCEDURES

Patient samples with an AFP value exceeding 350 ng/mL (High Range Undiluted, assay parameter 92) are flagged with the code " >350 ". To quantitate the concentration result, perform either an Automated Dilution Protocol or a Manual Dilution Protocol.

NOTE: AMNIOTIC FLUID samples must be diluted. Refer to the dilution protocols in this section for more information.

Automated Dilution Protocols

Automated Dilution Protocols are provided to assist in quantitating test results greater than 350 ng/mL up to 35350 ng/mL. A 1:3 dilution (Dilution Protocol 1) or 1:101 dilution (Dilution Protocol 2) can be selected. The AxSYM 1:3 Dilution Protocol allows quantification of samples up to 1050 ng/mL, while the 1:101 Dilution Protocol allows quantification of samples up to 35350 ng/mL. The 1:101 Auto Dilution Protocol is recommended for amniotic fluid samples. The AxSYM System performs a dilution of the unknown sample using one reaction vessel. The AxSYM System automatically calculates the concentration of the diluted sample and reports the results.

Refer to the AxSYM System Operations Manual, Section 5, for additional information on ordering sample dilutions.

Manual Dilution Protocols

A manual dilution can be performed by making a dilution of the specimen (serum, plasma or amniotic fluid) with the AxSYM AFP Specimen Diluent (7K52-50) before pipetting the sample into the sample cup. It is desirable to perform the dilution so that the diluted specimen reads above 15 ng/mL on the calibration curve.

Example 1: A twenty-fold dilution is prepared by adding 50 µL of the serum to 950 µL of AxSYM AFP Specimen Diluent. Mix thoroughly before assaying. To determine the concentration of AFP in the specimen, multiply the concentration of the diluted sample by the dilution factor.

Example 2: For an amniotic fluid sample, use a precision pipette to deliver 10 µL of clear amniotic fluid to 1.0 mL of the AxSYM AFP Specimen Diluent. Mix thoroughly before assaying. This is a 101-fold dilution. Multiply the concentration of the diluted sample by 101 to obtain the corrected AFP concentration (ng/mL). To report the values in µg/mL, divide the corrected AFP concentration (ng/mL) by 1000. An example of the dilution factor correction and conversion from ng/mL to µg/mL for AFP in amniotic fluid is listed in the following table.

AFP Concentration On Test Results Printout (ng/mL)	Correction for 101-fold Dilution (ng/mL)	AFP Concentration (µg/mL)
93.40	9433	9.43

QUALITY CONTROL PROCEDURES

Calibration

The AxSYM AFP assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

To perform an AFP Standard Calibration, test Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of the AFP Low, Medium and High Controls must be tested as a means of evaluating the assay calibration.

Once the AxSYM AFP calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used.
- Control values are out of their specified range.

Refer to the AxSYM System Operations Manual, Section 6, for:

- Setting up an assay calibration
- When recalibrations may be necessary
- Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for an error code. Refer to the AxSYM System Operations Manual, Appendices, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

Quality Control

The recommended control requirement for an AxSYM AFP Assay is a single sample of each of the Low, Medium or High AFP Controls tested once every 24 hours. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

The AFP Control values must be within the acceptable ranges specified in this package insert (see **REAGENTS, Controls** section).

Indications of Instability or Deterioration of Reagents

When an AFP control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require retesting. Assay recalibration may be indicated.

Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

The AxSYM System has a capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

Fluorescence Background Acceptance Criteria

Quality control of the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64 (Max Intercept - Max MUP intercept) each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2, for further information on this parameter.

RESULTS

AxSYM AFP utilizes a point-to-point data reduction method to generate a Standard Calibration curve.

Alternate Result Units

When selecting the alternate result unit, IU/mL, the conversion factor used by the AxSYM System is 0.83.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Section 2.

LIMITATIONS OF THE PROCEDURE

AxSYM AFP is a valuable aid in the management of nonseminomatous testicular cancer patients when used in conjunction with information available from the clinical evaluation and other diagnostic procedures. Increased serum AFP concentrations have also been observed in ataxia telangiectasia, hereditary tyrosinemia, primary hepatocellular carcinoma, teratocarcinoma, gastrointestinal tract cancers with and without liver metastases, and in benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis, and cirrhosis.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[44,45] These specimens should not be assayed with the AxSYM AFP assay.

Valid measurements of AFP in maternal serum or plasma CANNOT be made after amniocentesis; therefore, maternal serum or plasma specimens MUST be drawn PRIOR to amniocentesis. For further information, refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this assay package insert.

The reliability of MSAFP evaluation in prenatal testing is dependent upon the accurate determination of gestational age. An overestimated gestational age may result in the misinterpretation of MSAFP values, and thus, underestimation of risk of NTD. An underestimated gestational age may also result in the misinterpretation of MSAFP values, that is, the overestimation of risk of NTD. Both situations may lead to unnecessary additional testing. When gestational age is uncertain, a reliable ultrasound examination is important.

While elevated levels of MSAFP indicate increased risk of NTD, they are not diagnostic. Increased serum AFP concentrations have been seen in some cancers and some nonmalignant diseases as described above and, thus, may be indicative of maternal conditions. Other conditions including placental malformations, open fetal malformations such as omphalocele or gastroschisis (ventral wall defects), fetal kidney abnormalities, threatened or imminent abortion, and fetal demise are associated with elevated levels of MSAFP. Elevated MSAFP levels have also been associated with premature deliveries and low birth weights and have been seen in multiple births. Rarely, singleton, viable, and unaffected pregnancies may exhibit elevated MSAFP levels. Confirmatory testing, such as amniocentesis for AFAFP evaluation, high resolution ultrasonography or amniography, is an essential part of the AFP testing process.

This kit is not intended to be used for the risk evaluation of Trisomy 21.

EXPECTED VALUES

The distribution of AFP values determined in 1,029 specimens from normal individuals and patients with nonmalignant or malignant diseases is shown in the following table.

Distribution of AFP Values (%)

	Number of Subjects	0-15.0 ng/mL	15.1-20.0 ng/mL	20.1-100.0 ng/mL	100.1-350.0 ng/mL	> 350 ng/mL
Normals	400	99.2	0.2	0.5	0.0	0.0
Carcinoma						
Testicular:						
Seminoma	65	92.3	1.5	6.2	0.0	0.0
Nonseminoma	150	46.7	3.3	10.7	7.3	32.0
Primary						
Hepatocellular	58	32.8	3.4	10.3	10.3	43.1
Pancreatic	51	96.1	0.0	2.0	0.0	2.0
Gastrointestinal	83	98.8	0.0	0.0	0.0	1.2
Nonmalignant Disease						
Pancreatitis	38	100.0	0.0	0.0	0.0	0.0
Genitourinary	42	100.0	0.0	0.0	0.0	0.0
Cirrhosis	87	89.7	2.3	5.7	1.1	1.1
Hepatitis	55	85.5	0.0	7.3	5.5	1.8

In this study, 99% of the healthy subjects had AFP levels less than 10.9 ng/mL. It is recommended that each laboratory establish its own reference range for the population of interest.

AFP Values in Maternal Serum and Amniotic Fluid

It is important for each laboratory to establish its own reference ranges for maternal serum and amniotic fluid AFP. Due to potential variation in testing at different laboratories, it has proven useful to express AFP values as the median and multiples of the median (MoM)* for each gestational week. Each laboratory should gather 100 or more values for each gestational week in order to arrive at median values and then utilize a Cutoff Value (MoM) which most closely suits its needs for sensitivity and specificity.

$$* \text{ MoM} = \frac{\text{AFP Specimen Concentration}}{\text{Median AFP Concentration for Gestational Week}}$$

AFP Values in Maternal Serum

Maternal serum AFP values from 1,277 unaffected, singleton pregnancies expressed as the regressed medians and multiples of the regressed medians (MoM) for gestational weeks 15 to 21 are shown in the following table. These results may serve as a guide until the laboratory has gathered sufficient data of its own.

Maternal Serum AFP by AxSYM AFP Assay Results of Clinical Evaluations

Gest. Week	Number of Specimens	Regressed* Medians (ng/mL)	Multiples of Regressed Medians (ng/mL) 2.0	2.5	3.0
15	197	33.2	66.5	83.1	99.7
16	202	38.6	77.3	96.6	115.9
17	198	45.0	89.9	112.4	134.9
18	201	52.3	104.6	130.7	156.9
19	200	60.8	121.6	152.1	182.5
20	159	70.7	141.5	176.9	212.2
21	120	82.3	164.6	205.7	246.9

AFP Values for Amniotic Fluid

Amniotic fluid AFP values from 1,066 unaffected, singleton pregnancies expressed as regressed medians and multiples of the regressed medians (MoM) for gestational weeks 15 to 21 are displayed in the following table. These results may serve as a guide until the laboratory has gathered sufficient data of its own.

Amniotic Fluid AFP by AxSYM AFP Assay Results of Clinical Evaluations

Gest. Week	Number of Specimens	Regressed* Medians (µg/mL)	Multiples of Regressed Medians (µg/mL)		
			2.0	2.5	3.0
15	132	16.0	31.9	39.9	47.8
16	197	13.5	27.0	33.8	40.5
17	199	11.5	22.9	28.6	34.3
18	190	9.7	19.4	24.3	29.1
19	136	8.2	16.5	20.6	24.7
20	131	7.0	13.9	17.4	20.9
21	81	5.9	11.8	14.8	17.7

Alpha-fetoprotein values have been assigned on the basis of COMPLETED gestational weeks. For example, a specimen obtained on gestational week 18 day 6 is assigned to week 18.

* The regressed median values were determined using a weighted log-linear regression analysis.[18]

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Precision was determined as described in Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-T2.[46] A six member serum based panel was assayed at three laboratories in replicates of two at two separate times per day for twenty days (n=80 for each sample) using a single lot of reagents and a single calibration. Data from this study are summarized in the following table.

Reproducibility of AxSYM AFP

	Lab	Mean AFP (ng/mL)	Within Run (%) CV	Between Run (%) CV	Between Day (%) CV	Total (%) CV
Panel 1	1	2.6	3.0	3.9	3.3	6.0
	2	2.6	2.7	2.9	3.4	5.2
	3	2.6	3.1	2.7	4.2	5.9
Panel 2	1	14.1	3.1	4.4	1.8	5.7
	2	14.8	3.1	2.7	3.6	5.4
	3	14.7	2.8	3.8	2.6	5.4
Panel 3	1	25.5	2.9	4.3	4.1	6.6
	2	26.2	3.4	2.6	3.7	5.6
	3	26.1	3.4	2.3	4.5	6.1
Panel 4	1	68.0	3.1	5.6	1.3	6.5
	2	70.3	3.1	3.4	2.9	5.5
	3	71.0	3.2	3.4	4.9	6.8
Panel 5	1	128.6	3.5	5.7	2.3	7.1
	2	133.6	3.6	2.6	3.8	5.8
	3	134.5	3.4	3.4	4.5	6.5
Panel 6	1	224.0	4.6	8.1	2.7	9.7
	2	232.6	4.6	2.8	6.0	8.1
	3	233.1	4.0	3.7	4.7	7.2

The standard deviation may be calculated by multiplying the mean AFP concentration by the percent CV and dividing by 100.

$$SD = \frac{\text{Mean (ng/mL)} \times \text{(\%) CV}}{100}$$

Recovery

Known amounts of AFP were added to human serum and plasma. The concentration of AFP was determined using the AxSYM AFP assay and the resulting percent recovery was calculated.

Recovery

Specimen Type	Endogenous AFP Level (ng/mL)	AFP Added (ng/mL)	Value Obtained (ng/mL)	(%) Recovery*
Serum				
A	0.8	85.4	81.9	95.0
		160.5	166.0	102.9
		226.0	236.8	104.4
B	1.2	85.4	87.7	101.3
		160.5	168.3	104.1
		226.0	238.3	104.9
EDTA				
A	0.8	85.4	87.3	101.3
		160.5	156.2	96.8
		226.0	243.2	107.3
B	1.3	85.4	87.6	101.1
		160.5	152.9	94.5
		226.0	238.1	104.8
HEPARIN				
A	0.9	85.4	84.4	97.8
		160.5	175.2	108.6
		226.0	255.7	112.7
B	1.4	85.4	85.6	98.6
		160.5	171.0	105.7
		226.0	228.2	100.4
CITRATE				
A	0.8	85.4	85.9	99.6
		160.5	178.9	111.0
		226.0	243.8	107.5
B	1.1	85.4	87.6	101.3
		160.5	164.8	102.0
		226.0	245.4	108.1

Known amounts of AFP were added to amniotic fluid which had been diluted 101-fold in AxSYM AFP Specimen Diluent. The concentration of AFP was determined using the AxSYM AFP assay and the resulting percent recovery was calculated.

Recovery in Amniotic Fluid

Endogenous Level (ng/mL)	AFP added to Diluted Amniotic Fluid (ng/mL)	Value Obtained (ng/mL)	(%) Recovery*
71.24	36.80	108.28	101
	137.35	206.33	98

$$\text{* \% Recovery} = \frac{\text{AFP Value Obtained (ng/mL)} - \text{Endogenous Level (ng/mL)}}{\text{AFP Added (ng/mL)}} \times 100$$

Sensitivity

The sensitivity of the AxSYM AFP assay was calculated to be better than 0.4 ng AFP/mL. This sensitivity is defined as the concentration at two standard deviations above the AFP Standard Calibrator A (0 ng AFP/mL) and represents the lowest measurable concentration of AFP that can be distinguished from zero.

Specificity

The specificity of the AxSYM AFP assay was analyzed by testing sera containing the compounds listed in the following table. These compounds did not show interference in the AxSYM AFP assay at the levels indicated. Maternal vitamins were also tested and did not show any interference with assay performance.

Test Compound	Test Concentration
Acetaminophen	6.5 mg/mL
Albumin	160 mg/mL
Alpha-I-Acid Glycoprotein	2 mg/mL
Alpha-I-Antitrypsin	5 mg/mL
Alpha-Globulins	32 mg/mL
Aspirin	10 mg/mL
Bilirubin	50 mg/dL
Bleomycin	1000 µU/mL
Ceruloplasmin	2.5 mg/mL
Cisplatin	1000 µg/mL
Chorionic Gonadotropin	1000 IU/mL
Fetal Hemoglobin	6.75 mg/mL
Gamma-Globulins	30 mg/mL
Hemoglobin	1000 mg/dL
Placental Lactogen	100 µg/mL
Pregnancy Associated Glycoproteins (SP-1)	500 µg/mL
Prolactin	500 ng/mL
Red Blood Cells	1 %
Total Protein	3-14 g/dL
Triglycerides	3000 mg/dL
Transferrin	25 mg/mL
Vinblastine	500 µg/mL

Linearity

One serum with an AFP concentration about 400 ng/mL has been serially diluted with the AxSYM AFP Specimen Diluent to achieve the following dilutions: 1:2; 1:4; 1:8; 1:16; 1:32; 1:64. The neat serum and the dilutions have been tested with AxSYM AFP. The results are the following:

Dilutions	Observed values ng/mL	Expected values ng/mL	Recovery %
neat	> 350	400	
1:2	204	200	102
1:4	101	100	101
1:8	44.7	50	89.4
1:16	23.0	25	92
1:32	11.42	12.5	91.4
1:64	5.65	6.3	89.7

Hook Effect

Studies demonstrate that the AxSYM AFP method does not exhibit a high dose hook effect at AFP concentrations up to 2,057,600 ng/mL.

Carryover

No significant carryover (less than 0.001%) was detected when a sample containing 64,000 ng AFP/mL was assayed.

BIBLIOGRAPHY

1. Bergstrand CG, Czar B. Demonstration of a New Protein Fraction in Serum from the Human Fetus. *Scand J Clin Lab Invest* 1956;8:174.
2. Ruoslahti E, Engvall E, Kessler MJ. Chemical Properties of Alpha-Fetoprotein. In: Herberman RB, McIntire KR, editors. Immunodiagnosis of Cancer. New York: Marcel Dekker, Inc., 1979:101-17.
3. Ruoslahti E, Seppala M. Studies of Carcinofetal Proteins: Physical and Chemical Properties of Human Alpha-Fetoprotein. *Int J Cancer* 1971;7:218-25.
4. Tatarinov YS. Detection of Embryospecific Alpha-Globulin in the Blood Sera of Patients with Primary Liver Tumor. *Vopr Med Khim* 1964;10:90-1.
5. Silver HKB, Gold P, Feder S, Freedman SO, Shuster J. Radioimmunoassay for Human Alpha-Fetoprotein. *Proc Natl Acad Sci* USA 1973;70: 526-30.
6. Waldmann TA, McIntire KR. The Use of a Radioimmunoassay for Alpha-Fetoprotein in the Diagnosis of Malignancy. *Cancer* 1974;34:1510-5.
7. Kohn J, Orr AH, McElwain TJ, Bentall M, Peckham MJ. Serum Alpha-Fetoprotein in Patients with Testicular Tumors. *Lancet* 1976;2: 433-6.
8. Abelev GI. Alpha-Fetoprotein in Ontogenesis and Its Association with Malignant Tumors. *Adv Cancer Res* 1971;14:295-358.
9. Scardino PT, Cox HD, Waldmann TA, McIntire KR, Mittemeyer B, Javadpour N. The Value of Serum Tumor Markers in the Staging and Prognosis of Germ Cell Tumors of the Testis. *J Urol* 1977;118: 994-9.
10. Bosl GJ, Lange PH, Fraley EE, Goldman A, Nochomovitz LE, Rosai J, *et al.* Human Chorionic Gonadotropin and Alpha-Fetoprotein in the Staging of Nonseminomatous Testicular Cancer. *Cancer* 1981;47: 328-32.
11. Lange PH, McIntire KR, Waldmann TA, Hakala TR, Fraley EE. Serum Alpha-Fetoprotein and Human Chorionic Gonadotropin in the Diagnosis and Management of Nonseminomatous Germ-Cell Testicular Cancer. *N Engl J Med* 1976;295:1237-40.
12. Javadpour N, McIntire KR, Waldmann TA. Human Chorionic Gonadotropin (hCG) and Alpha-Fetoprotein (AFP) in Sera and Tumor Cells of Patients with Testicular Seminoma. *Cancer* 1978;42: 2768-72.
13. Report from the Medical Research Council Working Party on Testicular Tumors 1985. Prognostic Factors in Advanced Nonseminomatous Germ-Cell Testicular Tumors: Results of a Multicenter Study. *Lancet i* 1985:8-11.
14. Perlin E, Engeler JE, Edson M, Karp D, McIntire KR, Waldmann TA. The Value of Serial Measurement of Both Human Chorionic Gonadotropin and Alpha-Fetoprotein for Monitoring Germinal Cell Tumors. *Cancer* 1976;37:215-9.
15. Wepsic HT. Alpha-Fetoprotein: Its Quantitation and Relationship to Neoplastic Disease. In: Kirkpatrick A, Nakamura R, editors. Alpha-Fetoprotein, Laboratory Procedures and Clinical Applications. New York: Masson Publishing, 1981:115-29.
16. McIntire KR, Waldmann TA, Moertel C, Go VLW. Serum Alpha-Fetoprotein in Patients with Neoplasms of the Gastrointestinal Tract. *Cancer Res* 1975;35:991-6.
17. National Committee for Clinical Laboratory Standards. Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline - Second Edition. NCCLS Documents M29-A2. Wayne, PA: NCCLS, 2001.
18. Report of U.K. Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects. Maternal Serum Alpha-Fetoprotein Measurement in Antenatal Screening for Anencephaly and Spina Bifida in Early Pregnancy. *Lancet i* 1977:1323-32.
19. Second Report of U.K. Collaborative Study Alpha-Fetoprotein in Relation to Neural Tube Defects. Amniotic Fluid Alpha-Fetoprotein Measurement in Antenatal Diagnosis of Anencephaly and Open Spina Bifida in Early Pregnancy. *Lancet ii* 1979:651-61.
20. Haddow JE, Kloza EM, Smith DE, Knight GJ. Data from an Alpha-Fetoprotein Pilot Screening Program in Maine. *Obstet Gynecol* 1983;62:556-60.
21. Main DM, Mennuti MT. Neural Tube Defects: Issues in Prenatal Diagnosis and Counselling. Obstet Gynecol 1986;76(1):1-16.
22. Adams MJ, Windham GC, Greenberg LMJ, *et al.* Clinical Interpretation of Maternal Serum Alpha-Fetoprotein Concentrations. Am J Obstet Gynecol 1984;148(3):241-54.
23. American Society of Human Genetics Policy Statement for Maternal Serum Alpha-Fetoprotein Screening Programs and Quality Control for Laboratories Performing Maternal Serum and Amniotic Fluid Alpha-Fetoprotein Assays. Am J Hum Genet 1987;40:75-82.
24. Cuckle HS, Nanchahal K, Wald NJ. Maternal Serum Alpha-Fetoprotein and Ethnic Origin. Br J Obstet Gynaecol 1987;94:1111-2.
25. Crandall BF, Lebherz TB, Schroth PC, Matsumoto M. Alpha-Fetoprotein Concentrations in Maternal Serum: Relation to Race and Body Weight. *Clin Chem* 1983;29(3):531-3.
26. Milunsky A, Alpert E, Kitzmiller JL, Younger MD, Neff RK. Prenatal Diagnosis of Neural Tube Defects. VIII. The Importance of Serum Alpha-Fetoprotein Screening in Diabetic Pregnant Women. *Am J Obstet Gynecol* 1982;142(8):1030-2.

27. Baumgarten A, Robinson J. Prospective Study of an Inverse Relationship Between Maternal Glycosylated Hemoglobin and Serum Alpha-Fetoprotein Concentrations in Pregnant Women with Diabetes. *Am J Obstet Gynecol* 1988;159(1):77-81.
28. Palomaki GE, Knight GJ, Kloza EM, Haddow JE. Maternal Weight Adjustment and Low Serum Alpha-Fetoprotein Values. *Lancet i* 1985:468.
29. Wald NJ, Cuckle H, Boreham J, Stirrat GM, Turnbull AC. Maternal Serum Alpha-Fetoprotein and Diabetes Mellitus. *Br J Obstet Gynaecol* 1979;86:101-5.
30. Crandall BF. Second Trimester Maternal Serum Screening to Identify Neural Tube Defects. In: Kirkpatrick AM, Nakamura RM, editors. Alpha-Fetoprotein, Laboratory Procedures and Clinical Applications. New York: Masson Publishing, 1981:93-105.
31. Seppala M, Rapola J, Huttunen NP, Aula P, Karjalainen O, Ruoslahti E. Congenital Nephrotic Syndrome: Prenatal Diagnosis and Genetic Counselling by Estimation of Amniotic Fluid and Maternal Serum Alpha-Fetoprotein. *Lancet ii* 1976:123-4.
32. Seppala M. Increased Alpha-Fetoprotein in Amniotic Fluid Associated with Congenital Esophageal Atresia. *Obstet Gynecol* 1973;42:613-4.
33. Palomaki GE, Hill LE, Knight GJ, Haddow JE, Carpenter M. Second Trimester Maternal Serum Alpha-Fetoprotein Levels in Pregnancies Associated with Gastroschisis and Omphalocele. *Obstet Gynecol* 1988;71:906-8.
34. Wald N, Barker S, Cuckle H, Brock DJH, Stirrat SM. Maternal Serum Alpha-Fetoprotein and Spontaneous Abortion. *Br J Obstet Gynaecol* 1977;84:357-62.
35. Brock DJH, Barron L, Duncan P, Scrimgeour JB, Watt M. Significance of Elevated Mid-Trimester Maternal Plasma Alpha-Fetoprotein Values. *Lancet i* 1979:1281-2.
36. Nelson LH, Bensen J, Burton BK. Outcome in Patients with Unusually High Maternal Serum Alpha-Fetoprotein Levels. *Am J Obstet Gynecol* 1987;157(3):572-5.
37. Redford DH, Whitfield CR. Maternal Serum Alpha-Fetoprotein in Twin Pregnancies Uncomplicated by Neural Tube Defect. *Am J Obstet Gynecol* 1985;152(5):550-3.
38. Davenport DM, Macri JN. The Clinical Significance of Low Maternal Serum Alpha-Fetoprotein. *Am J Obstet Gynecol*, 1983;146:657-61.
39. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
40. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* 5th ed. Washington, DC: US Government Printing Office; January 2007.
41. World Health Organization. *Laboratory Biosafety Manual.* 3rd ed. Geneva: World Health Organization; 2004.
42. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition.* CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
43. Horacek KL, Peperall RJ, Hay DL, Barrie JV, Battery BW. Detection of Feto-Maternal Hemorrhage by Measurement of Maternal Serum AFP. *Lancet ii* 1976:200.
44. Primus FJ, Kelley EA, Hansen HJ, Goldenberg DM. "Sandwich"-Type Immunoassay of Carcinoembryonic Antigen in Patients Receiving Murine Monoclonal Antibodies for Diagnosis and Therapy. *Clin Chem* 1988;34:261.
45. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC Jr. Human Anti-Murine Immunoglobulin Responses in Patients Receiving Monoclonal Antibody Therapy. *Cancer Res* 1985;45:879-85.
46. National Committee for Clinical Laboratory Standards. Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition; Tentative Guideline. NCCLS Document EP5-T2, March 1992.

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