

000192

SUMMARY OF SAFETY AND EFFECTIVENESS

For the
Bayer Immuno 1™ AFP Assay

K972462
SEP 22 1997

This premarket notification is to add the quantitative measurement of AFP in human serum to the intended use of the Bayer Immuno 1™ Immunoassay System. The performance of the Bayer Immuno 1™ AFP Assay has been established by comparison to a predicate device, the Abbott IMx AFP Assay, in accordance with Section VI. (A) of the "Guidance Document For Submission of Tumor Associated Antigen Premarket Notifications, 510(k), to the FDA." Clinical evaluations of the Bayer Immuno 1™ AFP Assay at two US clinical trial sites demonstrated clinical safety and effectiveness and substantial equivalence to the predicate device in accordance with Section VI.(B) of the Guidance Document. The information presented in this Summary of Safety And Effectiveness was derived from nonclinical performance and clinical evaluation studies comparing the performance of the Immuno 1 AFP Assay with that of the FDA-approved Abbott IMx AFP Assay. Clinical studies were conducted at two clinical sites with a suitable sampling of patients to support the diagnostic claims for this device.

INDICATIONS FOR USE

The Bayer Immuno 1™ AFP Assay is an *in vitro* diagnostic assay intended to quantitatively measure AFP in human serum on the Bayer Immuno 1™ System as an aid in the management of nonseminomatous testicular cancer. AFP values obtained using the Bayer Immuno 1™ AFP Assay must be interpreted in conjunction with all other available clinical and laboratory data before a medical decision is determined. The assay is designed to run on the Bayer Immuno 1™ Immunoassay System, a fully automated random-access analyzer which performs both homogeneous and heterogeneous immunoassays.

Background

General. Alpha-fetoprotein (AFP), a major serum protein of the developing fetus, is a monomeric, heterogeneous, glycoprotein of approximately 70 kilodaltons (kDa) (1). The molecular heterogeneity of AFP, which can be observed either electrophoretically or chromatographically, is thought to be due to either differences in peptide sequence and/or glycosylation among the various isoforms detectable in serum (2-4). AFP consists of a single polypeptide chain and is 4.3% carbohydrate (1). Produced by the fetal yolk sac, liver, and gastrointestinal tract (5), serum levels of AFP peak at about 14 weeks gestation and gradually decline until birth. The AFP concentration is approximately 50 ug/mL at birth and is present in a normal adult at a low basal range of 0.5 to 10 ng/mL (6). AFP is thought to function as a natural ligand for fatty acids and estrogens, and possesses physiochemical properties and an amino acid sequence which are very similar to albumin (reviewed in 4). AFP from different species all reversibly bind fatty acids, particularly polyunsaturated fatty acids. It has been

postulated that the transport and cell delivery of these acids could be the major biological role of AFP (7).

AFP Serum Levels in Testicular Cancer. Elevated AFP levels are seen in patients with nonseminomatous testicular cancer. More than 95% of testicular cancers belong to a heterogeneous group called germ-cell tumors because it is widely believed that they arise in primordial germ cells (8). Germ cell tumors (GCTs) are classified either as seminomatous or as nonseminomatous (NSGCTs). The latter can be further classified as embryonal carcinoma, teratoma, or choriocarcinoma. The seminoma histologic subtype can be found in 40% of all GCTs while the nonseminoma histologic subtype can be found in 60% of GCTs (9). The different histologic types of germ-cell tumors may occur singly or in various combinations. Elevated AFP levels have been observed in patients diagnosed as having seminomatous testicular cancer with nonseminomatous elements, but not in patients with pure seminoma (10-15).

Both AFP and HCG are measured in testicular cancer. Approximately 40% of patients with NSGCTs have elevation of only one marker (16). During the clinical course of the disease, the levels of the two markers do not always parallel each other.

A direct relationship has been observed between the incidence of elevated AFP levels in nonseminomatous testicular cancer, and the stage of disease (10-12). Elevation of AFP (>10 IU/L or 12.1 ng/mL) occurs in 80% of metastatic and in 57% of stage 1 nonseminomatous germ cell tumors (16). In Clinical Stage 2B or higher, AFP and/or HCG are elevated in 65-80% of the cases with increasing frequency according to the bulk of the disease (18).

The usefulness of AFP measurements in the management of nonseminomatous testicular cancer patients undergoing cancer therapy has been well established (10, 12, 19). Current management of the testicular germ cell tumors (GCTs) relies upon the use of serum tumor markers which can indicate the presence of small foci of active tumor that cannot be detected by currently available imaging techniques (16). Markers augment and complement information obtained from radiographic and other staging procedures (20). Also, the short half-lives of tumor markers facilitate their use in assessing tumor burden during therapy. AFP has a serum half-life of 3.5-6 days (21). AFP and/or HCG levels are elevated before orchiectomy in about 60% of all Clinical Stage I patients but follow a normal decline after the testicle is removed (18). It is not unusual to have AFP levels at 1000 ng/mL at orchiectomy, which means 5-6 weeks before normalization according to expected half-life values.

For patients in clinical remission following treatment, AFP levels generally decrease (12). Post-operative AFP levels which fail to return to normal strongly suggest the presence of residual tumor (10, 12, 22). Following successful resection of primary or metastatic disease, AFP and HCG decline at a rate proportional to their respective half-lives (21). An elevated actual half-life (AHL) of serum markers following orchiectomy or retroperitoneal lymph node dissection (RPLND) may indicate the presence of occult, persistent disease (20). Residual disease is the rule if such a decline does not ensue.

As recently as the 1970s, NSGCTs were often fatal. Due to advances in chemotherapy, most patients are cured, even those with disseminated disease (8). The clinical use of AFP and HCG measurements has been essential to this success. Many patients have a marker surge during the first week of chemotherapy, presumably secondary to tumor lysis. AFP may increase from 20% to 200% over pretreatment levels (20). Chemotherapeutic responses are accompanied by a decline in marker levels. Persistent marker elevation is usually the result of residual malignancy. Rising marker values may occur before or after clinical recurrence and one marker may rise in discordance with the other (21). Tumor recurrence is often accompanied by a rise in serum AFP values prior to clinical evidence of progressive disease (10-11).

AFP Serum Levels in Non-Testicular Malignancies. Elevated serum levels of AFP are also associated with some non-testicular cancers. Increased serum concentrations of AFP were first observed in human subjects with primary hepatocellular carcinoma (17). Subsequently, elevated serum AFP values have been associated with other malignant diseases such as teratocarcinoma (with yolk sac components) of the ovary, endodermal sinus tumors, certain gastrointestinal tumors (with and without liver metastasis), and tumors of other tissues (18-19, 22-26). A study performed at the National Institutes of Health and the Mayo Clinic demonstrated elevated AFP values in patients with pancreatic, gastric, colon, and lung cancer (20). In additional studies, AFP was elevated in 60-80% of patients with hepatocellular cancer, in 23% of patients with gastrointestinal cancer and in 10% of patients with liver metastasis from various tumor types (18). However, a normalization of markers does not mean that all viable tumor has been eliminated (20).

AFP Serum Levels in Healthy Controls and Benign Disease. Several studies have defined the specificity of the AFP assay. In the NIH study (20), 210 control patients (blood donors) were tested for AFP by RIA. All controls had serum values below 40 ng/mL. AFP assay values for healthy adults, as determined in additional studies employing different assay systems, ranged from 1 to 25 ng/mL (18, 27-28).

Notably however, elevated serum AFP concentrations have also been reported in patients with noncancerous diseases such as ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, cirrhosis, and other benign hepatic conditions (20, 22, 29-34). AFP is modestly elevated (up to 100 ng/mL) in 20% of patients with non-malignant liver disease (18). In the NIH study, most benign liver diseases were associated with levels below 500 ng/mL (20). Elevated serum AFP concentrations are also associated with pregnancy (35-36). Due to its lack of specificity for malignant conditions, AFP testing is not recommended as a screening procedure to detect cancer in the general population.

DEVICE DESCRIPTION

Indicated Use. The Bayer Immuno 1™ AFP Assay is an *in vitro* diagnostic assay intended to quantitatively measure AFP in human serum on the Bayer Immuno 1™ System as an aid in the management of nonseminomatous testicular cancer. AFP values obtained using the Bayer

Immuno 1™ AFP Assay must be interpreted in conjunction with all other available clinical and laboratory data before a medical decision is determined. The assay is designed to run on the Bayer Immuno 1™ Immunoassay System, a fully automated random-access analyzer which performs both homogeneous and heterogeneous immunoassays.

Description of the Method. The Bayer Immuno 1™ AFP Assay utilizes a well-established immunoassay technology in which one monoclonal AFP antibody is conjugated to fluorescein (designated Reagent 1, or R1) and a second monoclonal AFP antibody is conjugated to alkaline phosphatase (Reagent 2, or R2). The R1 and R2 conjugates are reacted with patient sample, calibrator, or control and are incubated at 37°C on the system. An Immuno 1 Magnetic Particle coated with an anti-fluorescein antibody is then added and a second incubation occurs during which the antibody complex is bound. The magnetic particles complexed with the immunological sandwich are then washed to separate unbound molecules, and a colorimetric substrate is added. The rate of conversion of substrate to a compound with absorbance at 405 and 450 nm is measured and the measured rate is proportional to the concentration of AFP antigen in the sample. A cubic-through-zero curve fitting algorithm is used to generate standard curves. A schematic representation of the magnetic separation sandwich immunoassay technique used by the Bayer Immuno 1™ Analyzer is depicted in Figure 1 below.

The assay has a range of 0.1 to 400 ng/mL. The assay uses six calibrators with AFP concentrations of 0, 5, 25, 100, 200, and 400 ng/mL. A typical standard curve for the assay is presented in Figure 2.

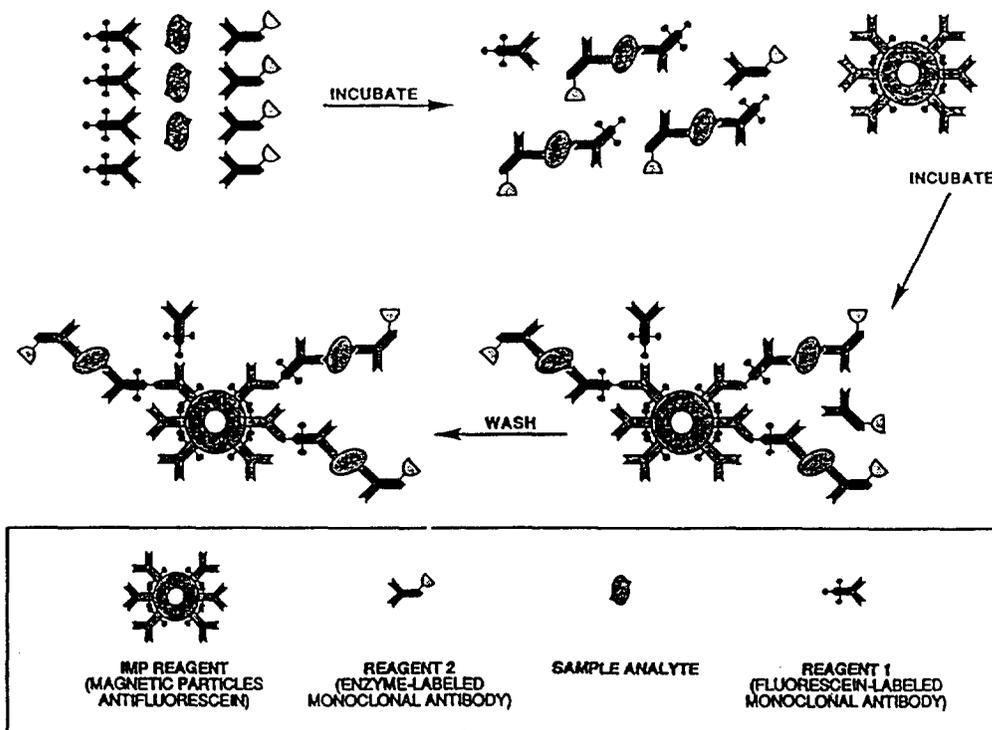


Figure.1 Schematic Representation of the Magnetic Separation Sandwich Immunoassay on the Bayer Immuno 1™ System.

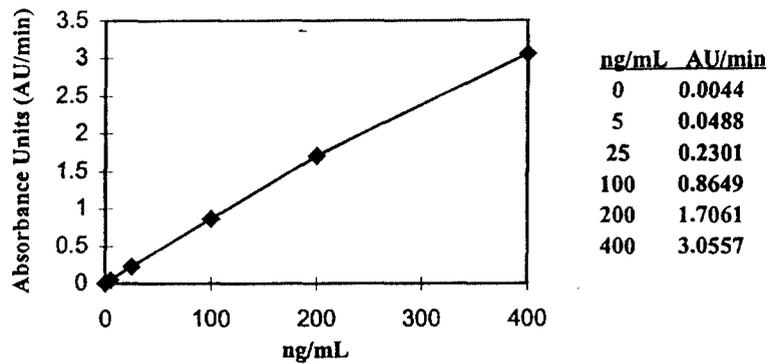


Figure 2 Standard Curve for the Bayer Immuno 1™ AFP Assay.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The Immuno 1 AFP Assay is intended for *in vitro* diagnostic use only. There are no known potential adverse effects on the health of clinically managed patients when this device is used as indicated. It is imperative that the physician use the Immuno 1 AFP test results in conjunction with the patient's overall clinical assessment and other diagnostic tests. False test results could affect physician decisions regarding treatment. If falsely low, treatment may be delayed in cases of recurring testicular cancer. If falsely high, new therapy may be instituted unnecessarily. These false positive and false negative values should not lead to patient mismanagement as it is indicated that AFP assay values be used in conjunction with the results of the patient's overall clinical assessment.

PRECAUTIONS AND WARNINGS

This device is not indicated for testicular cancer screening or as a sole diagnostic tool to confirm the presence or absence of malignant testicular disease. AFP assay values should be used for the management of nonseminomatous testicular cancer patients in conjunction with the information from a complete clinical evaluation including a physical exam and other diagnostic tests.

In some cases, confirmed testicular carcinoma patients may show declining AFP levels while tumor masses continue to enlarge (16). Additionally, patients with certain non-malignant conditions (pregnancy, hepatitis, and cirrhosis) and other benign hepatic conditions (6, 20-24) and patients with certain non-testicular malignancies (hepatocellular carcinoma, teratocarcinoma of the ovary, endodermal sinus tumors, certain gastrointestinal tumors, and tumors of other tissues) can exhibit elevations in AFP assay levels (8, 16-18, 22-25, 31-36). As such, serum AFP assay levels should not be interpreted as absolute evidence of the presence or absence of malignant disease.

The concentration of AFP in a given specimen determined with assays from different manufacturers can vary due to differences in assay methodology 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 AFP assays cannot be used interchangeably. If in the course of monitoring the patient, the assay method used for determining serial AFP levels is changed, additional sequential testing should be carried out to confirm baseline values. At the same time, a range of normal values for the new assay should be determined based on normal sera.

SUMMARY OF STUDIES

Nonclinical studies were performed to evaluate assay specificity and interfering substances, minimum detectable concentration, imprecision, linear range, hook effect, parallelism, and spiked recovery.

The clinical evaluation of the Bayer Immuno 1™ AFP Assay, as an aid in the management of patients with nonseminomatous testicular cancer, was performed at two clinical trial sites. Cross-sectional sensitivity and specificity, reference range, and longitudinal monitoring were evaluated. Immuno 1 AFP values were compared to Abbott IMx AFP values in a method concordance study using a panel of 863 cross-sectional samples and 56 longitudinal monitored patients.

NONCLINICAL STUDIES

Characterization of the Antigen. The antigen used in the Immuno 1 AFP Assay is purified from human fetal cord serum. The AFP antigen is manufactured by Scripps Laboratories (San Diego, CA) and is supplied to Bayer. Antigen preparations, analyzed by reducing SDS-PAGE, and immunoblotted with rabbit anti-human alpha-fetoprotein followed by horseradish peroxidase conjugated goat anti-rabbit IgG, showed that the immunoreactive material migrated with a molecular weight of approximately 72 kDa. This electrophoretic analysis demonstrated that the antigen preparation used in the assay is consistent with previous descriptions of AFP. Isoelectric focusing of the AFP antigen resolved a pI of 4.8 which is consistent with literature references which show a pI of 4.75 for AFP (2).

Immunoreactivity of the Antibodies. Two monoclonal antibody preparations are used in the Immuno 1 AFP Assay. Both antibodies are manufactured by Medix and are supplied to Bayer following purification by protein A chromatography.

The monoclonal antibodies were characterized in a series of experiments. Isotype analysis demonstrated that the monoclonal antibodies are both of the murine IgG1 subclass. Relative affinity analysis revealed that both antibodies bound to AFP antigen in a similar and saturable manner. Biochemical analysis of MAb preparations, under conditions of reducing or non-reducing SDS-PAGE and isoelectric focusing, revealed bands characteristic of murine immunoglobulin molecules of the IgG isotype. These results demonstrate that the monoclonal antibodies bind to the AFP antigen quantitatively and specifically, and display biophysical properties expected of mouse monoclonal antibodies.

Specificity And Interfering Substances. The recovery of AFP values from human serum was studied before and after spiking with the potentially interfering substances listed below. Each potential interferent was tested at a maximum concentration. Several potential interferents were tested at five equally spaced concentration levels (including no interferent and the maximum concentration of interferent). All testing was performed on one Immuno 1 system, using one lot of assay reagent.

Endogenous Interferents. The Immuno 1 AFP Assay was performed on serum samples or pools of serum to which was added various concentrations of either triglycerides, immunoglobulin, hemoglobin, heparin, bilirubin, albumin, or carcinoembryonic antigen (CEA). The highest concentration of each potential interferent used was greater than that normally observed during routine clinical testing. The highest concentration of each potential endogenous interferent tested and the maximum effect on the observed AFP recovery are summarized in Table 1. None of the potential endogenous interferents demonstrated any significant interfering effects on AFP recovery.

Exogenous Interferents. Because of the possibility that serum AFP measurements might be performed while patients are undergoing a regimen of chemotherapy, AFP values were measured in serum samples after spiking with either an individual drug or a cocktail of drugs commonly used to treat cancer. The identities of the individual chemotherapeutic drugs and their final concentrations in the test are presented in Tables 2 and 3. Because of the possibility that serum AFP measurements might be performed while patients are taking "Over the Counter" (OTC) drugs or dietary supplements, AFP values were also measured in serum samples after spiking with either an individual OTC drug or vitamin. The identities of the individual OTC drugs/dietary supplements and their final concentrations in the test are presented in Table 4. None of the potential exogenous interferents demonstrated any significant interfering effects on AFP recovery.

Interferent	Highest Concentration Tested	% Recovery
Triglycerides	900 mg/dL	99.9
IgG	0.053 g/mL	98.2
Hemoglobin	10.0 mg/mL	102.1
Heparin	0.15 mg/mL	101.5
Bilirubin	0.25 mg/mL	99.7
Albumin	0.065 g/mL	104.0
CEA	10,000 ng/mL	99.9

Table 1. Endogenous Interference

Pool Number	Drug Cocktail	Highest Concentration Tested	% Recovery
Drug Cocktail #1	Cyclophosphamide (Cytosan)	800ug/mL	99.7%
	Doxorubicin (Adriamycin)	51.8 ug/mL	
	Bleomycin	0.16 U/mL	
Drug Cocktail #2	Cis-platin	173 ug/mL	98.7%
	Etoposide	415.2 ug/mL	
	Dactinomycin	16 ug/mL	

Table 2. Chemotherapeutic Drugs Used For Interference Testing Analyzed in a Drug Cocktail

Drug	Concentration Tested (1X)	% Recovery
Mitomycin C	73 ug/mL	99.2
Vinblastine	5.11 ug/mL	97.8
Vincristine	13.5 ug/mL	100.8
5-Fluorouracil	1600 ug/mL	101.3
Amethopterin (Methotrexate)	450 ug/mL	95.2

Table 3. Chemotherapeutic Drugs Used For Interference Testing Analyzed Individually

OTC Drug or Dietary Supplement	Concentration Tested	% Recovery
Acetaminophen	200 ug/mL	104.8
Aspirin	500 ug/mL	99.4
Ibuprofen	400 ug/mL	102.8
Caffeine	100 ug/mL	101.1
Vitamin A	10 IU/mL	101.8
Vitamin B ₁ (Thiamin)	3 ug/mL	102.6
Vitamin B ₂ (Riboflavin)	3.4 ug/mL	100.5
Vitamin B ₆	4 ug/mL	104.8
Vitamin B ₁₂	12 ng/mL	99.8
Vitamin C (Ascorbic Acid)	30 ug/mL	100.6
Vitamin D ₂	0.8 IU/mL	100.5
Vitamin E	0.6 IU/mL	101.3
Folic Acid	0.8 ug/mL	99.9
Niacin	40 ug/mL	100.6

Table 4. OTC Drugs and Dietary Supplements Used For Interference Testing

Heterophilic Antibodies. To investigate the effectiveness of the assay's reagent formulation in minimizing heterophilic antibody interferences, ten samples with high rheumatoid factor titers and nine samples with high human anti-mouse antibody titers were spiked with approximately 200 ng/mL of AFP antigen. Each sample was assayed in triplicate with one lot of Immuno 1 reagent. The percent recovery range for samples high in rheumatoid factor was 96.4-103.2% while the percent recovery range for samples high in HAMA was 93.5-102.4%. The observed AFP recoveries indicate a lack of significant heterophilic interference in the assay and demonstrate the effectiveness of the reagent formulation in minimizing these interferences.

Minimum Detectable Concentration. Analytical sensitivity of the Immuno 1 AFP assay was evaluated by determination of the Minimum Detectable Concentration (MDC). The MDC is defined as the minimum concentration of AFP which can be statistically distinguished from the concentration of the lowest standard as calculated from a typical standard curve. The estimate of the minimum detectable concentration of the assay is obtained from the imprecision of Level 1 calibrator's reaction rates. Specifically, the MDC of the Immuno 1 AFP assay is the AFP concentration corresponding to an absorbance value two within-run standard deviations above the reaction rate of the zero calibrator.

The minimum detectable concentration (MDC) of the Immuno 1 AFP Assay was based on multiple determinations of the Level 1 calibrator using two lots of reagents and two lots of calibrators. The study was performed on two systems at Tarrytown and on one system each at Memorial Sloan-Kettering Cancer Center and Diagnostic Oncology CRO (DOCRO). The MDC's from each of the sites are shown in Table 5.

SITE	N	LEVEL 1 WITHIN-RUN SD (mA)	MDC (ng/mL)
Tarrytown	320	0.23	0.05
Memorial Sloan-Kettering	160	0.18	0.04
DOCRO	144	0.33	0.09
TOTAL	624	0.25	0.06

Table 5. Minimum Detectable Concentration at all Clinical Trial Sites

The Minimum Detectable Concentrations from three clinical trial sites ranged from 0.04 ng/mL to 0.09 ng/mL. These levels of analytical sensitivities are acceptable for an assay of this type and support a method sheet claim of 0.1 ng/mL.

Imprecision. Within-run and total assay imprecision were evaluated by testing five levels of Immuno 1 AFP Assay Calibrators and BioRad Tumor Marker Controls in a minimum of 18-20 assay qualification runs performed over a minimum period of ten days. The study was performed on two systems at Site 1 and on one system each at Sites 2 and 3. Typical imprecision obtained at a clinical trial site is presented in Table 6. The total imprecision was not greater than 3.6%.

SAMPLE	MEAN CONCENTRATION (ng/mL)	OBSERVATIONS	WITHIN-RUN		TOTAL	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
BioRad Level 1	31.0	160	0.65	2.1	0.88	2.8
BioRad Level 2	129.9	160	4.50	3.5	4.69	3.6
BioRad Level 3	258.0	160	8.17	3.2	8.86	3.4
Calibrator 2	5.0	320	0.09	1.7	0.13	2.5
Calibrator 3	25.4	320	0.35	1.4	0.53	2.1
Calibrator 4	98.3	320	1.51	1.5	2.17	2.2
Calibrator 5	199.9	320	6.44	3.2	7.26	3.6
Calibrator 6	398.2	320	6.26	1.6	10.54	2.6

Table 6. Imprecision at a Clinical Site

These results demonstrate that the recovery of Immuno 1 AFP assay values are highly reproducible over time.

Linearity. To determine if AFP recoveries are linear over the entire calibration range, three clinical sample pools containing a high concentration of AFP values were diluted with normal serum (low AFP values) to final concentrations of 100% (undiluted), 75%, 50%, 25%, and 0% (low AFP serum only). Each pool was assayed with two lots of Immuno 1 AFP reagent.

The three pools of sera with AFP values of approximately 275-375 ng/mL were diluted. Recoveries of the intermediate dilutions were all between 95.7% and 102.8% of the expected value. These results clearly demonstrate the linearity of AFP recoveries over the entire calibration range.

Hook Effect. Extremely high concentrations of AFP seen in some malignant conditions may cause a "hook effect" in an assay. An excess of analyte saturates both label and capture antibody and causes the reported concentration to "hook" back into the assay range rather than be flagged as above range. AFP antigen, isolated from human fetal cord serum and purchased from Scripps Laboratories (San Diego, CA), was value-assigned by testing on the Immuno 1, and was diluted in Level 1 Calibrator at concentrations of 32.0 ng/mL to 204,268 ng/mL. This collection of samples was tested in the assay using two lots of reagents. The reaction rate did not "hook" back into the assay range (Calibrator Level 6 reaction rate) until a concentration of 204,268 ng/mL AFP was reached. These results clearly demonstrate the lack of a hook effect in the Immuno 1 AFP Assay at AFP assay values $\leq 160,000$ ng/mL.

Spiked Recovery. To determine how well AFP antigen, when spiked into a patient sample, is recovered by the Immuno 1 AFP Assay, antigen was spiked into five patient samples at an AFP concentration of approximately 300 ng/mL. Each sample was assayed in triplicate with one lot of Immuno 1 reagent.

Recoveries of assay values for all samples ranged from 96.7 to 99.3%. No significant deviation was noted with regard to expected versus observed assay values. These results demonstrate the accurate quantitation of spiked and recovered AFP values using the Immuno 1 assay.

Parallelism. As a further verification of assay linearity and to determine the acceptability of 0 ng/mL Calibrator as a diluent, four patient serum sample pools containing a high level of AFP values were diluted with Level 1 calibrator to final concentrations of 100% (undiluted), 75%, 50%, 25%, and 0% (Level 1 calibrator only). Each dilution of each sample pool was assayed with two lots of reagents. Two serum pools were tested on one Immuno 1 system while the other two serum pools were tested on a second Immuno 1 system. Linear regression analysis for the determination of deviations from linearity for each of these clinical samples showed no deviation from linearity. The recovery of AFP assay values ranged from 92.7-111.0%. The accurate recovery of AFP values in diluted patient samples further illustrates the linearity of the Immuno 1 AFP Assay throughout the entire calibration range and the acceptability of 0 ng/mL Calibrator as diluent.

CLINICAL STUDIES AND METHOD COMPARISON STUDIES

Introduction. The objective of the method comparison studies was to examine the concordance of sample assay values obtained using the Bayer Immuno 1™ AFP Assay with those obtained using the Abbott IMx AFP Assay. Patient sample AFP values generated by the two methods were compared by correlation analysis, a determination of normal range cutoff, an analysis of clinical sensitivity and specificity, and an analysis of longitudinal sample AFP values.

This retrospective study was performed at two investigational sites. Both the Immuno 1 testing and Abbott IMx analysis on patient specimens was performed in the laboratories at Diagnostic Oncology CRO (“DOCRO”) in Stratford, CT and at Memorial Sloan-Kettering Cancer Center (“MSKCC”) in New York, NY. Human serum samples from 545 patients obtained from Western States Plasma Company (Fallbrook, CA) were tested at DOCRO and a total of 318 samples from MSKCC’s own in-house specimen collection were analyzed for method correlation using the Immuno 1 AFP Assay and the Abbott IMx AFP Assay. The number, source, and clinical classification of the single point patient samples used in this comparison study are summarized in Tables 7 and 8.

Clinical Classification	Source	Number of Samples
Normal (Male)	Western States Plasma	150
Normal (Female)	Western States Plasma	100
Testicular Cancer (Mixed Germ Cell Tumor)	Western States Plasma	5
Testicular Cancer (Seminomatous Germ Cell Tumor)	Western States Plasma	1
Hepatocellular Carcinoma	Western States Plasma	52
Genito-Urinary Cancer	Western States Plasma	33
Gastrointestinal, Lung & Pancreatic Cancer	Western States Plasma	48
Hepatitis	Western States Plasma	50
Cirrhosis	Western States Plasma	50
Benign Genito-Urinary Diseases	Western States Plasma	19
Other non-malignant Diseases	Western States Plasma	37
Total	Western States Plasma	545

Table 7. Summary of Single Point Patient Samples Used In Comparison Study At DOCRO Site.

Clinical Classification	Source	Number of Samples
Normal Serum	Memorial Sloan-Kettering	100
Testicular Cancer (Nonseminomatous Germ Cell Tumor)	Memorial Sloan-Kettering	74
Testicular Cancer (Mixed Germ Cell Tumor)	Memorial Sloan-Kettering	22
Testicular Cancer (Seminomatous Germ Cell Tumor)	Memorial Sloan-Kettering	6
Prostate Cancer	Memorial Sloan-Kettering	10
Liver Cancer	Memorial Sloan-Kettering	10
Colon Cancer	Memorial Sloan-Kettering	10
Breast Cancer	Memorial Sloan-Kettering	10
Lung Cancer	Memorial Sloan-Kettering	10
Benign GU Disease	Memorial Sloan-Kettering	10
Randomly-Selected Testicular Cancer Longitudinal Patients	Memorial Sloan-Kettering	51
Randomly-Selected Hepatocellular Cancer Longitudinal Patients	Memorial Sloan-Kettering	5
Total	Memorial Sloan-Kettering	318

Table 8. Summary of Single Point Patient Samples Used In Comparison Study At MSKCC Site.

Longitudinal patient samples were also tested at MSKCC as part of the method correlation analysis. A total of 314 longitudinal samples from 51 testicular cancer patients and 5 hepatocellular cancer patients were tested by Immuno 1 and IMx assays and compared. A summary of the longitudinal patients and sample number is presented in Table 9.

Cancer Type	# Patients	# Samples per Patient			
		5	6	7	8
Testicular	51	31	9	8	3
Hepatocellular	5	5	0	0	0
Total	56	36	9	8	3
Number of Total Samples		180	54	56	24

Table 9. Summary of Longitudinal Patient Samples Used In Comparison Study At MSKCC Site

Normal Range. The normal range cut-off for normal specimens was determined by calculation of the 97.5th percentile of AFP values in 150 normal healthy male patients. The distribution of

Immuno 1 and Abbott IMx values for healthy subjects used in this study are shown in Tables 10 and 11 respectively. Male subjects ranged in age from 17 to 60.

The results of the normal range analysis show the mean serum AFP values for all samples analyzed by the Immuno 1 AFP Assay and the Abbott IMx AFP Assay were 3.02 ng/mL and 2.61 ng/mL respectively. Females were included in this analysis only for comparative purposes. To determine the upper range of normal for the Immuno1 AFP Assay, data derived only from males was used. The greatest 97.5th percentile normal range cut-off found at any one site for the Immuno 1 was 8.9 ng/mL compared to 8.1 ng/mL for Abbott IMx AFP Assay. These results are consistent with the manufacturer's suggested normal range cutoff ranges for the Abbott IMx AFP Assay which states that 99% of the healthy subjects had AFP levels less than 8.9 ng/ml. Overall, the means and normal range cut-off of the AFP assay values calculated for the normal samples using these two methods were similar, and demonstrate the concordance of assay values determined by these methods. As the 97.5th percentile values ranged between 4.6 and 8.9 ng/mL, a cut-off of 8.9 ng/ml was determined to be appropriate for the Immuno 1 AFP Assay.

Normal, Healthy, Subjects	N	Distribution of IMMUNO 1 AFP Assay Results (ng/mL) FOR NORMAL SUBJECTS					Mean (SD) AFP ng / mL	Non-Parametric 97.5 th % (ile) ng / mL
		0-8.9 (N%)	>8.9-15 (N%)	>15-20 (N%)	>20-30 (N%)	>30 (N%)		
MSKCC (males)	100	100 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2.48 (1.03)	4.6
DOCRO (males)	150	146 (97.3%)	2 (1.3%)	2 (1.3%)	0 (0%)	0 (0%)	3.38 (2.46)	8.9
DOCRO (females)	100	98 (98%)	0 (0%)	1 (1%)	1 (1%)	0 (0%)	3.02 (3.33)	8.2
TOTAL	350	344 (98.3%)	2 (0.6%)	3 (0.9%)	1 (0.3%)	0 (0%)	3.02 (2.48)	8.0

Table 10. Distribution of Immuno1 AFP Assay Results for Normal Subjects

Normal, Healthy, Subjects	N	Distribution of ABBOTT IMX 1 AFP Assay Results (ng/mL) FOR NORMAL SUBJECTS					Mean (SD) AFP ng / mL	Non-Para-metric 97.5 th % (ile) ng / mL
		0-8.9 (N%)	>8.9-15 (N%)	>15-20 (N%)	>20-30 (N%)	>30 (N%)		
MSKCC (males)	100	100 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2.30 (0.91)	4.5
DOCRO (males)	150	147 (98%)	2 (1.3%)	1 (0.7%)	0 (0%)	0 (0%)	2.86 (2.27)	8.10
DOCRO (females)	100	98 (98%)	0 (0%)	1 (1%)	1 (1%)	0 (0%)	2.53 (2.83)	7.80
TOTAL	350	345 (98.6%)	2 (0.6%)	2 (0.6%)	1 (0.3%)	0 (0%)	2.61 (2.18)	7.0

Table 11. Distribution of Abbott IMx AFP Assay Results for Normal Subjects

Method Comparison. In order to compare the values obtained from serum samples analyzed by the Immuno 1 AFP Assay with the Abbott IMx AFP assay values, a correlation study was performed using data collected at two clinical sites. A summary of the patient populations used for these studies at Diagnostic Oncology CRO and Memorial Sloan-Kettering Cancer Center is shown in Tables 12 and 13 respectively. This study included the analysis of 545 single point serum samples at DOCRO and 318 single point serum samples at MSKCC.

Passing-Bablok and ordinary least squares linear regression analyses were performed to compare the Immuno 1 and Abbott IMx AFP assay values. Sample values were obtained in singlicate determinations for both the Immuno 1 and Abbott IMx AFP assays.

The data for 0-400 ng/mL and >400 AFP ranges are summarized in Tables 12 and 13 for both clinical sites. Results include 95% confidence intervals for the slopes and intercepts. An excellent comparison is demonstrated by slopes near 1.0 and correlation coefficients (r) greater than 0.99. Irrespective of range, site of analysis or regression analysis algorithm, the correlation between the two methods is excellent.

Range of Analyte Conc. ng/mL	N	r	Slope	95% Confidence Interval (slope)	Intercept	95% Confidence Interval (intercept)	Regression Method
0-400	516	0.993	1.047	1.036 to 1.058	0.350	-0.040 to 0.741	Ordinary Least Squares
>400	29	0.992	1.046	0.992 to 1.099	-165.8	-1452.8 to 1121.2	Ordinary Least Squares
0-400	516	0.993	1.109	1.075 to 1.141	0.258	0.183 to 0.341	Passing-Bablok
>400	29	0.992	0.985	0.922 to 1.072	-1.843	-63.9 to 51.7	Passing-Bablok

Table 12. Method Comparison at DOCRO

Range of Analyte Conc. ng/mL	N	r	Slope	95% Confidence Interval (slope)	Intercept	95% Confidence Interval (intercept)	Regression Method
0-400	302	0.997	0.931	0.923 to 0.939	0.932	0.390 to 1.473	Ordinary Least Squares
>400	16	0.999	0.939	0.914 to 0.965	26.462	-129.139 to 182.064	Ordinary Least Squares
0-400	302	0.997	1.024	1.000 to 1.049	0.089	0.002 to 0.201	Passing-Bablok
>400	16	0.999	0.931	0.878 to 0.967	21.238	-12.968 to 127.8	Passing-Bablok

Table 13. Method Comparison at MSKCC

Clinical Sensitivity and Specificity. The clinical sensitivity of the Immuno 1 and Abbott IMx AFP assays was determined by measurement of AFP values in serum samples from 213 patients with either active testicular or liver cancer. The testicular cancer samples used in this analysis were obtained from patients with either nonseminomatous germ cell tumor (GCT) or mixed germ cell tumor by DOCRO and MSKCC. The clinical specificity of the Immuno 1 AFP Assay and Abbott IMx assays was determined by measurement of AFP values in 641 serum samples from patients without nonseminomatous testicular cancer. These included

healthy individuals, or patients with seminomatous testicular cancer, non-testicular cancers, or non-malignant diseases.

The frequency distribution of AFP values from patients in all categories are presented in Table 14 for Immuno 1 values and in Table 15 for Abbott IMx values. The upper limit of the reference interval that was used was 8.9 ng/mL AFP for both assays.

PATIENT POPULATION	N	BAYER IMMUNO 1 SYSTEM AFP ASSAY VALUES (ng/mL)				MEDIAN
		0-8.9 (%)	>8.9-100 (%)	>100-400 (%)	>400 (%)	
Healthy Subjects	350	98.3	1.7	0.0	0.0	2.5
Testicular Ca.- nonseminomatous	100	36.0	40.0	14.0	10.0	20.2
Testicular Ca.- Mixed Germ Cell Tumor	46	43.5	45.7	6.5	4.3	11.6
Testicular Ca.- seminomatous	8	75.0	25.0	0.0	0.0	2.5
Prostate Ca./ Bladder Ca.	40	97.5	2.5	0.0	0.0	3.4
Lung Ca.	29	96.6	0.0	0.0	3.4	3.8
Colorectal Ca.	38	89.5	10.5	0.0	0.0	4.5
Liver Ca.	67	0.0	31.3	20.9	47.8	310.9
Breast Ca.	10	80.0	20.0	0.0	0.0	4.8
Cirrhosis	50	88.0	12.0	0.0	0.0	4.0
Hepatitis	50	88.0	12.0	0.0	0.0	3.9
Benign Genito- Urinary Disease	29	93.1	6.9	0.0	0.0	3.1
Other Non- malignant	37	100.0	0.0	0.0	0.0	2.3

Table 14. Clinical Sensitivity and Specificity with Immuno 1 AFP Values

PATIENT POPULATION	N	ABBOTT IMx AFP ASSAY VALUES (ng/mL)				MEDIAN
		0-8.9 (%)	>8.9-100 (%)	>100-400 (%)	>400 (%)	
Healthy Subjects	350	98.6	1.4	0.0	0.0	2.1
Testicular Ca.- nonseminomatous	100	36.0	38.0	16.0	10.0	17.9
Testicular Ca.- Mixed Germ Cell Tumor	46	45.7	41.3	8.7	4.3	10.8
Testicular Ca.- seminomatous	8	75.0	25.0	0.0	0.0	2.5
Prostate Ca./ Bladder Ca.	40	97.5	2.5	0.0	0.0	2.8
Lung Ca.	29	96.6	0.0	0.0	3.4	2.8
Colorectal Ca.	38	92.1	7.9	0.0	0.0	3.4
Liver Ca.	67	0.0	31.3	20.9	47.8	306.7
Breast Ca.	10	80.0	20.0	0.0	0.0	4.3
Cirrhosis	50	88.0	12.0	0.0	0.0	4.0
Hepatitis	50	80.0	20.0	0.0	0.0	3.8
Benign Genito- Urinary Disease	29	100.0	0.0	0.0	0.0	2.6
Other Non- malignant	37	97.3	2.7	0.0	0.0	1.9

Table 15. Clinical Sensitivity and Specificity with IMx AFP Values

The clinical sensitivity of both the Immuno 1 and IMx AFP assays for nonseminomatous testicular cancer was 64.0%. The sensitivity for mixed germ cell tumor testicular cancer (containing both nonseminomatous and seminomatous tumors) was 56.5 % for the Immuno 1 assay and 54.3% for the IMx assays. This is comparable to previous studies reporting 50-75% of AFP values above normal for nonseminomatous and mixed GCT (8-10, 12, 15, 18).

The clinical specificity of the Immuno 1 AFP Assay for healthy individuals was 98.3% while the specificity of the IMx assay was 98.6%. Both are consistent with results generally seen with the normal population (28, 30). Specificity across all other benign diseases (cirrhosis, hepatitis, benign genito-urinary disease, and other non-malignant disease) ranged from 88%-100% for both the Immuno 1 and IMx assays. Elevated Immuno 1 and Abbott IMx AFP Assay results were observed in 12% of cirrhosis and hepatitis patients. These observations are consistent with previous literature reports of AFP elevations in cirrhosis, hepatitis and other benign conditions (20, 28, 30, 33-34).

Elevated Immuno 1 observations were also observed in 2.5% prostate or bladder cancer patients. Of the 40 patients tested in this group, only one patient with bladder cancer possessed an elevated AFP level. Additionally, elevated Immuno 1 AFP values were observed in 3.4% of lung cancer specimens, 10.5 % of colorectal cancer specimens, and 20% of breast cancer specimens. Most notably, virtually identical results were obtained using the IMx assay. These results are consistent with previous published studies which also noted that elevations of AFP is not limited to malignant disease of the testes (8, 16, 22, 26, 28). Interestingly, elevated AFP levels were seen in all patients (N=67) with liver cancer. This observation correlates with the published observations that elevated AFP was first noted in patients with hepatocellular cancer (17, 24) and that elevated AFP values can be found in 60-90% of these patients (18, 21-22, 29-30).

The data presented in Tables 14 and Table 15 demonstrate the concordance of AFP assay values determined by the Immuno 1 and IMx assays. Percent sensitivity and percent specificity values are comparable. The data in these tables also confirm the conclusion that AFP is not recommended as a screening procedure to detect cancer in the general population. Not all nonseminomatous testicular cancers produce AFP while at the same time, elevated AFP levels may be found in other conditions, both malignant and non-malignant (29).

Analysis of Longitudinal Sample AFP Values The principal clinical utility of AFP testing is in the evaluation of longitudinal or serial serum samples from testicular cancer patients. The level of AFP can be used to assess the effectiveness of chemotherapy, the prognosis of the disease, and the possible recurrence of the cancer (37-39). Tumor markers for testicular cancer are measured regularly in patients on surveillance for stage I disease, on chemotherapy, and on follow up after surgery (37). The regression of serum tumor marker levels such as AFP help to predict treatment outcome (38). Patients with an appropriate serum tumor marker decline have a longer event-free survival. Decreasing levels indicate regression even though a return to normal levels does not mean the eradication of all tumor cells (39).

In this part of the study, the clinical utility of the Bayer Immuno 1™ AFP Assay as an adjunctive test in the management of nonseminomatous testicular cancer patients was evaluated.

Longitudinal samples from specimen banks at Memorial Sloan-Kettering Cancer Center included serum samples from 47 testicular cancer patients and for comparison purposes only, 5 hepatocellular cancer patients. Each patient had ≥ 5 specimens which met inclusion criteria, spanning the longitudinal profile. Each patient's age, documented medical, diagnostic, and course of therapy information was available. In the course of the evaluation, 4 testicular cancer patients were eliminated from the analysis because there was insufficient accompanying clinical information to allow complete evaluation of their longitudinal AFP profiles.

All patient samples were tested using both the Immuno 1 AFP Assay and the Abbott IMX AFP Assay. Each patient's serial Immuno 1 AFP results were graphically compared to the patient's

course of disease using diagnostic testing, therapeutic interventions, and physician evaluations as guides to describe the clinical picture.

The ability of the Immuno 1 AFP Assay to accurately reflect each patient's course of disease was evaluated by Bayer scientists and the profile was classified into one of the following categories as listed in Tables 16-18.

Table 16 presents the comparison of serial Immuno 1 AFP results with the clinical course of disease for all of the patients evaluated (47 testicular and 5 hepatocellular cancer patients). Table 17 presents the comparison of serial Immuno 1 AFP results with the clinical course of disease for testicular cancer patients and Table 18 presents results for hepatocellular cancer patients.

IMMUNO 1 AFP LONGITUDINAL PATIENT EVALUATION RESULTS ALL PATIENTS		
Correspondence (Parallels Clinical Course)	N	Percentage
Increasing AFP with Progression	2	3.8
Decreasing AFP with Response	17	32.7
Increasing and Decreasing AFP with Progression and Response	19	36.5
Elevated AFP with Active Disease	0	0
Normal AFP with No Evidence of Disease	1	1.9
Stable AFP with Stable Disease	2	3.8
Total Paralleling Clinical Course	41	78.8
No Correspondence (Does Not Parallel Clinical Course)	11	21.2

Table 16. Evaluation of All Longitudinal Patients

IMMUNO 1 AFP LONGITUDINAL PATIENT EVALUATION RESULTS TESTICULAR CANCER PATIENTS ONLY		
Correspondence (Parallels Clinical Course)	N	Percentage
Increasing AFP with Progression	0	0
Decreasing AFP with Response	17	36.2
Increasing and Decreasing AFP with Progression and Response	17	36.2
Elevated AFP with Active Disease	0	0
Normal AFP with No Evidence of Disease	1	2.1
Stable AFP with Stable Disease	1	2.1
Total Paralleling Clinical Course	36	76.6
No Correspondence (Does Not Parallel Clinical Course) Total	11	23.4

Table 17. Evaluation of Testicular Cancer Longitudinal Patients

IMMUNO 1 AFP LONGITUDINAL PATIENT EVALUATION RESULTS HEPATOCELLULAR CANCER PATIENTS ONLY		
Correspondence (Parallels Clinical Course)	N	Percentage
Increasing AFP with Progression	2	40
Decreasing AFP with Response	0	0
Increasing and Decreasing AFP with Progression and Response	2	40
Elevated AFP with Active Disease	0	0
Normal AFP with No Evidence of Disease	0	0
Stable AFP with Stable Disease	1	20
Total Paralleling Clinical Course	5	100
No Correspondence (Does Not Parallel Clinical Course) Total	0	0

Table 18. Evaluation of Hepatocellular Cancer Longitudinal Patients

The percent of patients whose changes in longitudinal AFP values reflected changes in the clinical profile was calculated. The Bayer Immuno 1™ AFP Assay demonstrated clinical utility for 78.8% (41/52) of the all patients evaluated longitudinally and 76.6% (36/47) of testicular cancer patients evaluated. Among the patients where Immuno 1 AFP accurately reflected disease course, 2 patients demonstrated increasing AFP concentrations with progressive cancer, 17 patients demonstrated decreasing AFP concentrations in response to therapy and 19 patients demonstrated both increasing and decreasing AFP concentrations corresponding to periods of both disease progression and response to therapy. For 1 patient with no evidence of disease, AFP values were consistently normal throughout the monitoring period. For 1 patient under therapy with stable disease, AFP levels were consistently normal and stable throughout the monitoring period. For 1 hepatocellular cancer patient, AFP concentrations were consistently stable at high concentrations.

One testicular cancer patient demonstrated only seminomatous germ cell tumor on histology which usually does not show elevated AFP levels. At the point of increasing AFP levels, metastasis to the liver was discovered. The literature supports increasing AFP levels in seminomatous GCT when metastasis to the liver is present (24). The data from the 5 hepatocellular cancer patients also demonstrates the correspondence of AFP values with the

clinical course of the disease. The AFP values paralleled the clinical course of the disease in all of these patients.

The data presented in Table 17 demonstrate the utility of AFP testing in the management of nonseminomatous testicular cancer. The level of AFP can be used to assess the effectiveness of chemotherapy, the prognosis of the disease, and the possible recurrence of the cancer (37-39). Abbott IMx results were directly compared to Immuno 1 AFP values and the correlation between the two methods was excellent. There were clear rising trends with both methods in correspondence to disease progression and declining trends in response to therapy. The trends agreed in 100% of the graphs when comparing the Immuno 1 and IMx AFP values.

The usefulness of AFP measurements in the management of nonseminomatous testicular cancer in patients undergoing cancer therapy has been well established (10, 12, 19). Current management of the testicular germ cell tumors (GCTs) relies upon the use of serum tumor markers which can indicate the presence of small foci of active tumor that cannot be detected by currently available imaging techniques (16). Markers augment and complement information obtained from radiographic and other staging procedures (20). Also, the short half-lives of tumor markers facilitate their use in assessing tumor burden during therapy. AFP has a serum half-life of 3.5-6 days (21). AFP and/or HCG levels are elevated before orchiectomy in about 60% of all Clinical Stage I patients but follow a normal decline after the testicle is removed (18). It is not unusual to have AFP levels at 1000 ng/mL at orchiectomy, which means 5-6 weeks before normalization according to expected half-life values. At the same time, in patients recently diagnosed with testicular cancer, a highly elevated AFP value indicates a poor prognosis (39).

For patients in clinical remission following treatment, AFP levels generally decrease (12). Post-operative AFP levels which fail to return to normal strongly suggest the presence of residual tumor (10, 12, 22). Following successful resection of primary or metastatic disease, AFP declines at a rate proportional to its respective half-life (21). An elevated actual half-life (AHL) of serum markers following orchiectomy or retroperitoneal lymph node dissection (RPLND) may indicate the presence of occult, persistent disease (20). Residual disease is the rule if such a decline does not ensue. AFP values may also rise before there is other evidence of disease progression (10) and is sometimes used as a first indicator of relapse (16).

The results of this study demonstrates the clinical utility of the Bayer Immuno 1™ AFP Assay as an aid in the management of testicular cancer patients. Identical trends in longitudinal results were observed with both the Immuno 1 and IMx AFP assays. This demonstrates equivalent clinical utility for the two methods.

Conclusions from Clinical Studies and Method Comparison Studies. The results of these clinical studies and comparative analysis of serum AFP assay values in the determination of AFP in normal, malignant, and non-malignant patient serum, clearly demonstrates the concordance of the Immuno 1 AFP Assay with the predicate device, the Abbott IMx AFP Assay. Normal reference ranges, clinical sensitivity and specificity, and analysis of longitudinal

serum samples were essentially equivalent. Correlation statistics showed good concordance between the Immuno 1 and the IMx assays. Longitudinal monitoring data demonstrated the utility of the Immuno 1 AFP Assay in the management of patients with nonseminomatous testicular cancer.

REAGENT STABILITY TESTING

Reagent Shelf-Life Stability Testing. Reagents were subjected to temperature stress at 25°C, 30°C, and 40°C and tested at selected timepoints. Additional reagent stored at 2-8°C was tested in parallel with the stressed reagents at all timepoints tested. At each timepoint, the sensitivity of the calibrators, as well as the recovery of controls were monitored. Data obtained using three lots of reagents support a shelf life stability claim of 12 months.

Reagent On-System Stability Testing. The On-System stability of Immuno 1 AFP assay reagents was evaluated using three lots of reagent. At all timepoints tested, all control recoveries, calculated from either “week 0” or timepoint calibration curves, were within specifications. On-system stability studies on the three lots of Immuno 1 AFP reagents support an on-system stability recommendation of 21 days.

Reagent Shipping Stability Testing. Three lots of reagents were subjected to three freeze/thaw cycles (three days at -80°C, and then three days at 2-8°C) and three heat/chill cycles (three days at 40°C, then three days at 2-8°C). Following these cycles, the reagents were stored at 2-8°C, and performance was evaluated at selected timepoints. Additional reagents were stored at 2-8°C and run alongside the stressed reagents at all timepoints. The sensitivity of the calibrators and the recovery of controls were monitored at each timepoint.

No significant change in control recovery, as compared to the non-stressed control reagent, was noted throughout the duration of testing. Recoveries for the cycled reagent remained well within specification. Shipping, temperature stress, and temperature cycling stability data on three lots of Immuno 1 AFP assay reagents support the requirement of refrigerated (2-8°C) shipping of these reagents.

Labeling. Reagent stability is summarized in the Immuno 1 AFP method insert sheet. Expiration dates are also indicated on the labels of each reagent kit.

CALIBRATOR STABILITY TESTING

Calibrator Shelf-Life Stability Testing. The calibrator lots were stressed at 2-8°C, 25°C, 30°C and 40°C and tested at selected timepoints. Additional calibrators stored at 2-8°C and at <-80°C were tested in parallel with the stressed calibrators at all timepoints. At each testpoint, recovery of controls was determined. Additionally, the recovery of the temperature stressed calibrators (analyzed as unknowns) using the -80°C stored calibrators for a calibration curve, was also determined.

Three lots of calibrators were tested for shelf-life stability. For all lots tested, recoveries of calibrators stored at 2-8°C were within specifications. Recoveries of calibrators stored at the elevated temperatures exceeded the specification of $\pm 13\%$. Recoveries of control materials remained within specifications for the duration of the testing. These results support the conclusion that the Immuno 1 AFP calibrators are stable for 9 months when stored at 2-8°C.

Calibrator Open Vial Stability Testing. After opening, calibrators were stored at 2-8°C and analyzed at selected weekly timepoints. In addition, at each timepoint a fresh (control) set of calibrators was run. Recovery of the calibrators and controls, run as unknowns at Day 0, 1 week, 2 weeks, 3 weeks, and 52 day timepoints were determined. Analyte values were derived using a calibration curve generated at that timepoint using the calibrators opened and stored at 2-8°C.

Three lots of calibrators were tested for open vial stability. At all timepoints tested, control recoveries remained within specifications. These results support the claim that opened Immuno 1 AFP calibrators are stable for 30 days when stored at 2-8°C.

Calibrator Shipping Studies. Shipping stability studies were conducted on three lots of calibrators. Recoveries were determined for calibrators subjected to three cycles of freezing and thawing using a calibration curve generated with control calibrators stored at -80°C. Based on these studies, it is recommended that assay calibrators be shipped in a refrigerated package (2-8°C).

Labeling. Calibrator stability is summarized in the Immuno 1 AFP method insert sheet. Expiration dates are also indicated on the labels of each reagent kit.

CONCLUSIONS DRAWN FROM ALL THE STUDIES

Valid Scientific Evidence. The conclusions drawn from these studies are based upon valid scientific evidence. Data were gathered following a well designed protocol, in a research laboratory operating under the principles of Good Laboratory Practices.

Method Performance. Immuno 1 AFP Assay nonclinical performance, including analytical sensitivity (minimum detectable concentration), imprecision, parallelism, linear range, hook effect, and spiked recovery met accepted specifications for an assay of this type.

Safety and Effectiveness. The clinical studies demonstrate the safety and effectiveness of measuring AFP in human serum on the Bayer Immuno 1™ system to aid in the management of nonseminomatous testicular cancer. The correlations between the Immuno 1 AFP Assay values and disease course demonstrate that this assay may be used in conjunction with other available clinical and laboratory data to manage nonseminomatous testicular cancer patients.

Substantial Equivalence. The method concordance studies confirm the substantial clinical equivalence of the Immuno 1 AFP Assay with the Abbott IMx AFP Assay predicate device. There is a high degree of correlation between the Immuno 1 and IMx AFP specimen values. Multi-site clinical studies demonstrate cross-sectional and longitudinal specimen results that are equivalent for the two tests. Therefore, based upon the analytical and clinical concordance established in these studies, the Bayer Immuno 1™ AFP Assay and the Abbott IMx AFP Assay are equivalent with respect to method performance, clinical utility, and device safety and effectiveness.

Cited References

1. Ruoslahti E, Seppala M. Studies of Carcino-fetal Proteins: Physical and Chemical Properties of Human Alpha-Fetoprotein. *Int. J. Cancer* 7: 218-225 (1971).
2. Hirai H. Alpha-Fetoprotein. In *Biochemical Markers for Cancer*. Ming Chu T (ed.) New York, Dekker. 1982. pages 25-59.
3. Deutsch HF. Chemistry and Biology of Alpha-Fetoprotein. *Adv. Cancer Res.* 56: 253 (1991).
4. Nunez EA. Biological Role of Alpha-Fetoprotein in the Endocrinological Field: Data and Hypothesis. *Tumor Biol.* 15: 63-72 (1994).
5. Gitlin D, Perricelli A, Gitlin GM. Synthesis of Alpha-Fetoprotein by Liver, Yolk Sac, and Gastrointestinal Tract of the Human Conceptus. *Cancer Res.* 32: 979-982 (1972)..
6. Sundram s., Goldstein PJ, Saravanan M, et al. Alpha-Fetoprotein and Screening Markers of Congenital Disease. *Clin. Lab. Med.* 12: 481-493 (1992).
7. Esteban C., Trojan A., Mishal Z., et al. Activation of an Alpha-Fetoprotein/Receptor Pathway in Human Normal and Malignant Peripheral Blood Mononuclear Cells. *Leukemia.* 7: 1807-1816 (1993).
8. Lange PH. Testicular Cancer Markers. in *Human Cancer Markers*. Sell S and Wahren B (ed.) Clifton, Humana. 1985. pages 259-273.
9. Small EJ, Torti, FM. Testes. in *Clinical Oncology*. New York, Churchill Livingstone. 1995. pages 1493-1526.
10. Kohn J, Orr AH, McElwain TJ, et al. Serum Alpha-Fetoprotein in Patients with Testicular Tumours. *Lancet* 2: 433-436, (1976).
11. Scardino PT, Cox HD, Waldmann TA, et al. The Value of Serum Tumor Markers in the Staging and Prognosis of Germ Cell Tumors of the Testis. *J. Urol.* 118: 994 (1977).
12. Lange PH, McIntire KR, Waldmann TA, et al. Serum Alpha-Fetoprotein and Human Chorionic Gonadotropin in the Diagnosis and Management of Nonseminomatous Germ Cell Testicular Cancer. *Medical Intelligence* 295: 1237 (1976).
13. Javadpour N, McIntire KR, Waldmann TA. Human Chorionic Gonadotropin (HCG) and Alpha-Fetoprotein (AFP) in Sera and Tumor Cells of Patients with Testicular Seminoma A Prospective Study. *Cancer* 42: 2768-2772 (1978).

14. Lange PH, Nochomovitz LE, Rosai J, et al. Serum Alpha-Fetoprotein and Human Chorionic Gonadotropin in Patients with Seminoma. *J. Urol.* 124: 472-478 (1980).
15. Jacobsen GK. Alpha-Fetoprotein (AFP) and Human Chorionic Gonadotropin (HCG) in Testicular Germ Cell Tumors. *Acta. Path. Microbiol. Immunol. Scand.* 91: 183-190 (1983).
16. Doherty AP, Bower M, Christmas TJ. The Role of Tumour Markers in the Diagnosis and Treatment of Testicular Germ Cell Cancers. *Brit. J. Urol.* 79: 247-252 (1997).
17. Tatarinov YS. Finding of an Embryonic Alpha Globulin in the Blood Stream in a Patient with Primary Hepatic Cancer. *Vopr. Med. Khim.* 10: 90 (1964).
18. Klepp O. Serum Tumour Markers in Testicular and Extragonadal Germ Cell Malignancies. *Scand. J. Clin. Lab. Invest. Suppl.* 51: 28-41 (1991).
19. Perlin E, Engeler JE, Edson M, et al. The Value of Serial Measurement of Both Human Chorionic Gonadotropin and Alpha-Fetoprotein for Monitoring Germinal Cell Tumors. *Cancer* 37: 215-219 (1976).
20. Bartlett NL, Freiha FF, Torti FM. Serum Markers in Germ Cell Neoplasms. *Hem./Onc. Clinics of N.A.* 5: 1245-1260 (1991).
21. Jacobs EL, Haskell CM. Clinical Use of Tumor Markers in Oncology. in *Current Problems in Cancer*. Littleton, Mosby-Year Book. 1991. Pages 299-359.
22. Waldmann TA, McIntire KR. The Use of a Radioimmunoassay for Alpha-Fetoprotein in the Diagnosis of Malignancy. *Cancer* 34: 1510-1515 (1974).
23. Silver HKB, Gold P, Feder S, et al. Radioimmunoassay for Human Alpha-Fetoprotein. *Proc. Nat. Acad. Sci. USA* 70: 526-530 (1973).
24. Abelev GI. Alpha-Fetoprotein in Ontogenesis and Its Association With Malignant Tumors. *Adv. Cancer Res.* 14: 295, 1971.
25. Maeyama M, Tayama C, Inoue S, et al. Serial Serum Determination on Alpha-Fetoprotein as a Marker of the Effect of Postoperative Chemotherapy in Ovarian Endodermal Sinus Tumor. *Gynecol. Oncol.* 17: 104-116 91984 0.
26. Yasunami R, Hashimoto Z, Ogura T, et al. Primary Lung Cancer Producing Alpha-Fetoprotein: A Case Report. *Cancer* 47: 926-929, 91981 0.
27. D'Costa M, Feld R, Laxdal V., et al. A Multicenter Evaluation of the Boehringer Mannheim ES 300 Immunoassay System. *Clin. Biochem.* 26: 51-57 (1993).

28. Cattini R., Cooksey M, Robinson D, et al. Measurement of Alpha-Fetoprotein, Carcinoembryonic Antigen and Prostate-Specific Antigen in Serum and Heparinised Plasma by Enzyme Immunoassay on the Fully Automated Serono SR1™ Analyzer. *Eur. J. Clin. Chem. Clin. Biochem.* 31: 517-524 (1993).
29. Wepsic HT. Alpha-Fetoprotein: Its Quantitation and Relationship to Neoplastic Disease. in *Alpha-Fetoprotein, Laboratory Procedures and Clinical Applications*.
30. Chen DS, Sung JL. Relationship of Hepatitis B Surface Antigen to Serum Alpha-Fetoprotein in Non-Malignant Diseases of the Liver. *Cancer* 44: 984-992 (1979).
31. Waldmann TA, McIntire KR. Serum Alpha-Fetoprotein Levels In Patients with Ataxia Telangiectasia. *Lancet* 2: 1112-1115 (1972).
32. Belanger L. Tyrosinémie Héritaire et Alpha-Foetoprotéine. II. Recherche Tissulaire Comparée de L'Alpha-Foetoprotéine dans Deux Cas de Tyrosinémie Héritaire. Considérations sur L'Ontogénèse de la Foetoprotéine Humaine. *Pathol. Biol.* 21: 457-462 (1973)
33. Kew MC, Purves LR, Bersohn I. Serum Alpha-Fetoprotein Levels in Acute Viral Hepatitis. *Gut* 14: 939-942 (1973).
34. Endo Y, Kanai K, Oda T, et al. Clinical Significance of Alpha-Fetoprotein in Hepatitis and Liver Cirrhosis. *Ann. NY Acad. Sci.* 259: 234-238 (1975).
35. Purves LR, Purves M. Serum Alpha-Fetoprotein. VI. The Radioimmunoassay Evidence for the Presence of AFP in the Serum of Normal People and During Pregnancy. *S. Afr. Med. J.* 46: 1290 (1972).
36. Seppala M, Ruoslahti E. Alpha-Fetoprotein: Physiology and Pathology During Pregnancy and Application to Antenatal Diagnosis. *J. Perinat. Med.* 1: 104 (1973).
37. Seckl MJ, Rustin GJS, Bagshawe KD. Frequency of Serum Tumour Marker Monitoring in Patients with Non-Seminomatous Germ Cell Tumours. *Br. J. Cancer.* 61: 916 (1990).
38. Murphy BA, Motzer RJ, Mazumdar M, et al. Serum Tumor Marker Decline is an Early Predictor of Treatment Outcome in Germ Cell Tumor Patients Treated with Cisplatin and Ifosfamide Salvage Chemotherapy. *Cancer.* 73: 2520 (1994).
39. Motawy MS, Szymendera JJ, Al-Jazzaf H, et al. Serum AFP, hCG and CEA in the Management of Patients with Testicular, Ovarian and Extragonadal Germ Cell Tumors. *Intl. J. Biol. Markers.* 7: 80 (1992).



SEP 22 1997

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Mr. Gabriel J. Muraca, Jr.
Manager, Regulatory Affairs
Bayer Corporation
511 Benedict Avenue
Tarrytown, New York 10591-5097

Re: K972462
Trade Name: AFP Assay for the Bayer Immuno 1 System
Regulatory Class: II
Product Code: LOJ
Dated: June 30, 1997
Received: July 1, 1997

Dear Mr. Muraca:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Pre-market Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the current Good Manufacturing Practice requirement, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic (QS) inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal Laws or Regulations.

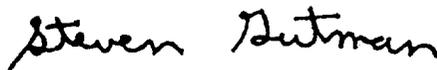
Page 2

Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770)488-7655.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll free number (800) 638-2041 or at (301) 443-6597 or at its internet address "<http://www.fda.gov/cdrh/dsmamain.html>"

Sincerely yours,



Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical
Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

