K960098

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SUMMARY OF SAFETY AND EFFECTIVENESS
For the
Bayer Immuno 1™ CA 125 II™ Assay

This premarket notification is to add the quantitative measurement of CA 125 II™ assay values in human serum to the intended use of the Bayer Immuno 1™ Immunoassay System. The performance characteristics of the Bayer Immuno 1™ CA 125 II™ Assay, substantial equivalence of this assay to the predicate device, the Centocor CA 125 II™ RIA, and evidence of safety and effectiveness in the target population has been established in accordance with Section VI. of the "Guidance Document For Submission of Tumor Associated Antigen Premarket Notifications, 510(k), to the FDA." The information presented in this Summary of Safety and Effectiveness was derived from nonclinical performance and clinical evaluation studies comparing the Immuno 1 CA 125™ Assay to the Centocor CA 125 II™ RIA.

INDICATIONS FOR USE

The Bayer Immuno 1™ CA 125 II™ Assay, hereafter referred to as Immuno 1 CA 125 II™ Assay, is an *in vitro* device indicated for the quantitative measurement of OC 125 reactive determinants associated with a high molecular weight glycoprotein in serum of women with primary epithelial invasive ovarian cancer. The CA 125 II Assay is indicated as a one time test for use as an aid in the detection of residual ovarian carcinoma in patients who have undergone first-line therapy and would be considered for diagnostic second-look procedures. An assay value of greater than 35 U/mL is indicative of residual disease, provided that alternative causes of an elevated CA 125 II assay value can be excluded (see under Limitations of the Procedure). It is recommended that the assessment and treatment of patients with ovarian cancer and the use of the Bayer Immuno 1 CA 125 II Assay be under the order of a physician trained and experienced in the management of gynecological cancers.

BACKGROUND

General. CA 125 is an antigenic determinant on a high-molecular-weight glycoprotein historically recognized by the monoclonal antibody OC 125, which was raised using an ovarian cancer cell line as an immunogen. The CA 125 determinant is expressed by epithelial ovarian tumors as well as by other tissues. The function of the glycoprotein expressing CA 125 is unknown, and, because of its complex nature, information about the physical and immunological nature of this antigen is limited.

Serum levels of CA 125 were initially reported to be elevated in > 80% of patients with ovarian cancer and to reflect the clinical course of the disease. Subsequently, a great deal of research and clinical interest has concentrated on the role of this antigen in several aspects of the management of ovarian malignancy including detection of residual carcinoma following completion of first line therapy.

In addition to ovarian cancer, serum levels of CA 125 are elevated in a number of other pathological and physiological states. Of particular interest in the area of human reproduction is the evidence from studies of tissue distribution that the CA 125 determinant is a normal product of endometrial tissue. Serum CA 125 levels are elevated in some individuals at the time of menstruation, in early pregnancy and in endometriosis. Recent studies suggest that serum CA 125 measurement may be also be of value in the management of endometriosis.

The Nature of the CA 125 Determinant. Column chromatography and SDS-PAGE electrophoresis followed by Western blotting indicate that CA 125 activity in ovarian cancer serum, amniotic fluid, human milk and supernatant from an ovarian cancer cell line is associated with a moiety of > 1,000,000 daltons and a lower-molecular-weight moiety of 200,000 - 400,000 daltons. Although the precise nature of the CA 125 determinant remains unclear, there is agreement that the molecule with which it is associated is a glycoprotein. Using antigen purified from an ovarian cancer cell line, a

carbohydrate content of 24% was reported on the basis of carbohydrate compositional analysis and buoyant density. This is a lower carbohydrate content than is typical for mucins, such as the other epithelial tumor antigens recognized by monoclonal antibodies (e.g., 19-9, B72.3, DU-PAN-2 and F36/22). Differences were noted in the buoyant density of the CA 125 antigen isolated from human milk, seminal plasma and an ovarian cancer cell line suggesting slight variability in protein or carbohydrate content.

CA 125 Serum Levels in Healthy Controls and Benign Disease. Expression of CA 125 is neither specific for ovarian cancer, nor for cancerous tissue in general. The CA 125 antigen has been demonstrated in cyst fluids of benign and malignant tumors, in benign and malignant pleural effusions, in ascites of benign and malignant origin, in human milk, in seminal plasma, in amniotic fluid and in cervical mucus. CA 125 is probably a secretory product of many normal human epithelia and shedding of the antigen occurs from the cell surface, possibly by an active shedding mechanism.

The cutoff level of the CA 125 assay was originally set at 35 U/mL which was the 99th percentile of a large population of blood donors. Many additional studies over the years have shown that, on average, 2.5 % of healthy subjects have CA 125 levels greater than 35 U/mL. A recent study has revealed that the CA 125 assay is not markedly influenced by sex, age or smoking status. However, remarkably high levels of CA 125, exceeding 65 U/mL, can be found in the first trimester of pregnancy and during menstruation. At the onset of menstruation, a sudden increase from normal values to levels exceeding 300 U/mL has been observed, possibly due to an easy access of CA 125 from the endometrial epithelial lining into the circulation during menstruation. Another explanation may be that retrograde menstruation might cause seeding of endometrial cells throughout the abdominal cavity, resulting in local inflammatory reactions and CA 125 elevation. Various authors have measured CA 125 levels in patients with benign diseases. The reported percentage of CA 125 elevations exceeding 35 U/mL ranged between 2% and 42% and those exceeding 65 U/mL ranged between 1% and 16%, depending on the population studied. Several benign diseases were found to be associated with CA 125

elevation, some of which may cause differential diagnostic problems. Patients with effusions caused by benign diseases such as congestive heart failure, tuberculosis or liver cirrhosis can be highly positive, as can patients with benign gynecological diseases such as uterine myofibroma and benign ovarian tumors. Patients with endometriosis, or especially with endometriotic cysts, may have highly elevated CA 125 levels.

CA 125 Serum Levels in Non-Ovarian Malignancies. A CA 125 serum test can be positive in a wide variety of non-ovarian malignant conditions. Other gynecological carcinomas, especially those of the endometrium, fallopian tube and cervix can be positive in a substantial percentage of cases. Likewise, cancers of non-gynecological origin, such as colon and pancreas, can give rise to increased serum levels of CA 125. Also, tumors originating in organs other than the ovaries give rise to CA 125 elevations once metastasized to the ovaries. Therefore, the role of the CA 125 test in differential diagnostics between various malignancies seems to be of limited value.

CA 125 Serum Levels in Ovarian Cancer. Pretreatment levels reflect the amount of circulating tumor-associated antigen in relation to the extent of disease at the time of staging. In Stage 1 disease, 43% of all patients reported had elevated CA 125 serum levels. Consequently, in more than half of all patients with malignant disease limited to one or both ovaries (with or without malignant cells in ascites fluid or washing), CA 125 serum values were within the normal range.

Whether or not the differential grade of ovarian carcinoma correlates with CA 125 levels is not clear. Several reports have suggested that CA 125 levels increase with decreasing degree of differentiation, although this correlation was not always statistically significant or even necessarily apparent.

CA 125 elevations have been reported to precede the clinical detection of tumor progression or tumor recurrence. The lead times observed ranged between several weeks to seventeen months. After a complete clinical or pathological remission, a significant

rise in CA 125 levels preceded tumor recurrence in more than 85% of all patients. In the other patients, CA 125 remained below the cutoff level, or remained stable, or rose only after clinical detection of tumor recurrence.

In the absence of clinical signs of tumor presence, ovarian cancer patients may undergo a "second look" procedure in order to verify surgically if all tumor deposits have disappeared. In a large number of studies, patients with CA 125 levels above 35 U/mL, who appeared clinically free of tumor, were shown to have tumor at second look in 95% of cases. A more recent study of advanced ovarian cancer patients found residual disease at second-look surgery in 92% of patients with CA 125 values between 20 and 35 U/mL. However, in all studies, only half of all patients with marker levels within the normal range were surgically free of tumor, either macroscopically or microscopically. The other half still had tumor present despite normal CA 125 levels (although many reports indicated that in these cases tumor lesions did not exceed one cm in the largest diameter). Thus, the CA 125 test can be of help in the management of ovarian cancer patients. In particular, at the time of second look, surgical procedures might be deferred in patients with CA 125 levels which remain in the normal range.

DEVICE DESCRIPTION

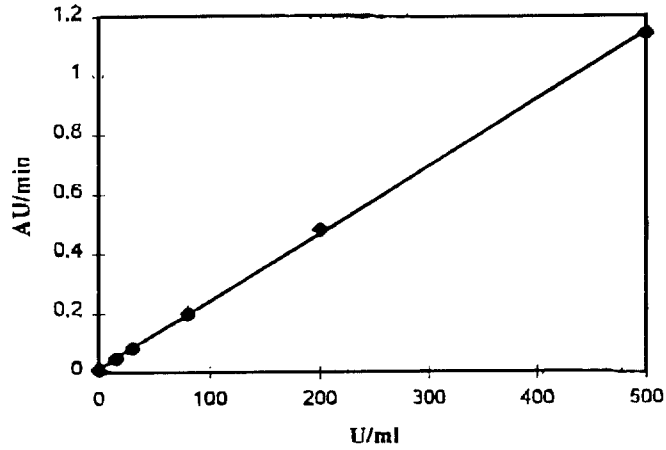
Indicated Use. The Bayer Immuno 1™ CA 125 II™ Assay is an *in vitro* device indicated for the quantitative measurement of OC 125 reactive determinants associated with a high molecular weight glycoprotein in serum of women with primary epithelial invasive ovarian cancer. The CA 125 II Assay is indicated as a one time test for use as an aid in the detection of residual ovarian carcinoma in patients who have undergone first-line therapy and would be considered for diagnostic second-look procedures. An assay value of greater than 35 U/mL is indicative of residual disease, provided that alternative causes of an elevated CA 125 II assay value can be excluded (see under Limitations of the Procedure). It is recommended that the assessment and treatment of patients with ovarian cancer and the

use of the Bayer Immuno 1 CA 125 II Assay be under the order of a physician trained and experienced in the management of gynecological cancers.

Description of the Assay. The Bayer Immuno I™ CA 125 II™ Assay is a sandwich immunoassay in which one monoclonal antibody (M11) is conjugated to fluorescein (R1) and a second monoclonal antibody (OC 125) is conjugated to alkaline phosphatase (R2). An Immuno 1 Magnetic Particle coated with anti-fluorescein antibody, the R1 conjugate, and patient sample, calibrator, or control are mixed simultaneously and incubated at 37°C on the system. The R2 conjugate is then added, and binds to the immobilized CA 125 II to form a sandwich immunocomplex on the solid phase. 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; the measured rate is proportional to the concentration of CA 125 II antigen in the sample. A cubic-through-zero curve fitting algorithm is used to generate standard curves. The assay uses six calibrators with CA 125 II concentrations of 0, 15, 30, 80, 200, and 500 U/mL.

A schematic representation of the Magnetic Separation Sandwich Immunoassay of the Bayer Immuno 1™ System is presented in Figure 1. A typical standard curve for the Immuno 1 CA 125 II™ Assay is presented in Figure 2.

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



U/ml	AU/Min
0	0.0093
15	0.0445
30	0.0795
80	0.1980
200	0.4786
500	1.1398

Figure 2. Standard Curve for the Bayer Immuno 1™ CA 125 II™ Assay.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The Immuno 1 CA 125 II™ 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 CA 125 II™ 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 or progressing ovarian cancer. If falsely high, new therapy or a change in treatment may be instituted unnecessarily. These false positive and false negative values should not lead to patient mismanagement as it is recommended that CA 125 II™ assay values be used in conjunction with the results of the patient's overall clinical assessment and under the order of a physician trained and experienced in the management of gynecological cancers.

PRECAUTIONS AND WARNINGS

This device is not indicated for ovarian cancer screening, or as a sole diagnostic tool to confirm the presence or absence of malignant ovarian disease. CA 125 II™ assay values should be used for the management of ovarian cancer patients in conjunction with the information from a complete clinical evaluation including physical exam and other diagnostic tests. CA 125 assay values greater than or equal to 35 U/mL may be found in 1-2% of healthy individuals and in patients with non-malignant conditions, such as pericarditis, cirrhosis, severe hepatic necrosis, endometriosis, first trimester pregnancy, ovarian cysts, or in patients with non-ovarian malignancies, such as uterine, hepatic, pancreatic, and lung cancers. A CA 125 II™ assay value below 35 U/mL does not insure the absence of residual ovarian cancer because patients with histological evidence of ovarian carcinoma have demonstrated CA 125 II™ assay values within the range for healthy individuals. Therefore, serum CA 125 II™ assay levels should not be interpreted as absolute evidence of the presence or absence of malignant disease.

The concentration of CA 125 antigenic determinants 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 CA 125 assay used. Values obtained with different CA 125 assays cannot be used interchangeably.

SUMMARY OF STUDIES

Nonclinical studies were performed to evaluate assay sensitivity (minimum detectable concentration), assay specificity (interfering substances), imprecision, linearity, parallelism, hook effect, and lot-to-lot variation.

The clinical evaluation of the Bayer Immuno 1™ CA 125 II™ Assay as a one time test for use an aid in the detection of residual ovarian carcinoma in patients who have undergone first-line therapy and would be considered for diagnostic second-look procedures, was performed by comparison of the Immuno 1 CA 125 II™ Assay values with second look diagnostic outcome and with the FDA-approved Centocor CA 125 II™ RIA results in a sampling of the target population. In addition, this concordance study evaluated the relationship between Immuno 1 CA 125 II™ Assay and the Centocor CA 125 II™ RIA values using a panel of 703 serum samples from healthy subjects and patients with benign diseases, ovarian cancer, and other malignant diseases.

NONCLINICAL STUDIES

Characterization of the Antigen. The antigen used in the Immuno 1 CA 125 II™ assay calibrators is the OC 125 reactive determinant (designated OC 125 antigen) isolated from the culture supernatant fluid of McDonald Anchorage Dependent Cells (OVCA 433). The antigen is produced by Centocor and is supplied to Bayer in a partially purified form.

Immunoreactivity of the Antibodies. Monoclonal antibody preparations M11 and OC 125 are used in the Immuno 1 CA 125 II™ Assay. Both antibodies are manufactured by Centocor, Inc., and are supplied to Bayer in partially purified form.

Specificity And Interfering Substances. The recovery of CA 125 II™ assay values, was studied before and after spiking with potentially interfering endogenous and exogenous substances. The potential interferents were spiked into patient samples with elevated levels of CA 125 or into the Medical Decision Pool, an internal serum-based control containing a CA 125 concentration of approximately 28 U/mL

Common Endogenous Interferents. The Immuno 1 CA 125 II™ Assay was performed on ovarian cancer patient specimens with elevated values of CA 125 or in the Medical Decision Pool to which was added various concentrations of either triglycerides, immunoglobulin, hemoglobin, glycoprotein, bilirubin, or albumin. The highest concentration of each potential endogenous interferent and the maximum effect on the observed CA 125 recovery are summarized in Table 1.

Interferent	Highest Concentration Tested	% Recovery in Patient sera 50 U/mL CA 125	% Recovery in Medical Decision Pool 28 U/mL CA 125
Triglycerides	900 mg/dL	Not Tested	102.8
Immunoglobulin	5.3 g/dL	99.9	100.6
Hemoglobin	1.0 g/dL	103.8	101.5
Glycoprotein	1 mg/mL	Not Tested	101.1
Bilirubin	25 mg/dL	Not Tested	97.3
Albumin	6.5 g/dL	107.0	106.8

Table 1. Interference

The greatest effect (6.8%-7.0%), demonstrated by Albumin, was observed in all serum media including the high patient sample and the Medical Decision Pool. Albumin was spiked at a concentration above the NCCLS recommended test level of 6 g/dL. This effect

of < 10% is likely to be insignificant given that the concentration of Albumin tested was greater than that found routinely in serum.

Exogenous Interferants. Because of the possibility that serum CA 125 II™ measurements might be performed while patients are undergoing a regimen of chemotherapy, CA 125 II™ assay values were measured in ovarian cancer patient sera with elevated CA 125 levels after spiking with a cocktail of drugs commonly used to treat cancer. The individual chemotherapeutic drugs, the concentrations tested in sera, and their effects on the base serum sample are presented in Table 2.

Drug	Concentration Tested	% Recovery in patient sera 40-60 U/mL CA 125
5-Flurouracil	1600 ug/mL	99.55
Aminoglutethamide	398 ug/mL	99.12
Amethopterin(Methotrexate)	450 ug/mL	99.3
Cis-Platin	173 ug/mL	104.8
Cyclosphosphamide (Cytoxan)	800 ug/mL	100.14
Diethylstilbesterol	23 ug/mL	100.36
Doxorubicin (Adriamycin)	51.8 ug/mL	99.0
Etoposide	415.2 ug/mL	99.56
Flutamide	10 ug/mL	99.61
Mitomycin C	73 ug/mL	99.01
Tamoxifen	60 ug/mL	99.66
Vinblastine	5.11 ug/mL	99.39
Vincristine	13.5 ug/mL	99.88

Table 2. Chemotherapeutic Drugs Used For Interference Testing

There was no significant interference from any of the chemotherapeutic agent tested. The maximum effect (4.8%) was observed with the drug Cis-Platin in a patient specimen with an elevated level of CA 125. This effect is likely to be insignificant given that the concentration of the drug tested was well in excess of the normal therapeutic dose.

"Over the Counter Drugs" and Dietary Supplements

"Over the Counter" drugs and dietary supplements were evaluated for possible interference in the measurement of CA 125 by the Immuno 1 CA 125 II™ Assay. All materials were tested at concentrations greater than the normal therapeutic dose or recommended dietary dose. The individual substances, the concentrations tested in sera, and their effects on the base serum sample are presented in Table 3.

Drug or Dietary Supplement	Concentration Tested	% Recovery in patient sera 40-60 U/mL CA 125
Acetaminophen	200 ug/mL	99.8
Aspirin	500 ug/mL	99.6
Ibuprofen	400 ug/mL	97.7
Caffeine	100 ug/mL	97.8
Vitamin A	10 IU/mL	100.3
Vitamin B_1 (Thiamin)	3 ug/mL	100.2
Vitamin B_2 (Riboflavin)	3.4 ug/mL	99.0
Vitamin B_6	4 ug/mL	99.2
Vitamin B_{12}	12 ug/mL	100.6
Vitamin C (Ascorbic Acid)	30 ug/mL	98.7
Vitamin D_2	0.8 IU/mL	99.2
Vitamin E	0.06 IU/mL	101.6
Folic Acid	0.8 ug/mL	99.2
Niacin	40 ug/mL	101.1

Table3. "Over the Counter" Drugs and Dietary Supplements Used For Interference Testing

None of the "Over the Counter" drugs or dietary supplements tested demonstrated significant interference in the Immuno 1 CA 125 II™ Assay. The largest observed effect was 2.3%.

Heterophilic Antibodies To investigate the effectiveness of the assay's reagent formulation in minimizing heterophilic antibody interferences, ten samples with high RF titers and six samples with high HAMA titers were assayed with two reagent lots. All samples were tested both undiluted and at a 50% dilution using the Level 1 Immuno 1 CA 125 II™ calibrator (containing no CA 125 II antigen). All samples recovered CA 125 II assay values linearly when diluted 50%. The percent recovery for all samples ranged from

92.4% to 102.2%. This observed linear CA 125 II assay value recovery indicates a lack of significant heterophilic interference in the assay and demonstrates the effectiveness of the reagent formulation in minimizing these interferences.

Minimum Detectable Concentration. Analytical sensitivity of the Immuno 1 CA 125 II™ assay was evaluated on four instruments by determination of the Minimum Detectable Concentration (MDC). The MDC is defined as the minimum concentration of CA 125 which can be statistically distinguished from the concentration of the lowest standard as calculated from a typical standard curve. Specifically, the MDC of the Immuno 1 CA 125 II™ assay was determined as the CA 125 concentration corresponding to an absorbance value two within-run standard deviations above the mean absorbance value of the zero calibrator.

An MDC of 0.24 U/mL was observed, based on multiple determinations of the Level 1 calibrator (0 U/mL), on four systems, using two lots of reagents. This level of analytical sensitivity is acceptable for an assay of this type.

Imprecision. Within-run and total assay imprecision were evaluated by testing five levels of Immuno 1 CA 125 II™ assay calibrators, BioRad Tumor Marker Controls, and an internal medical decision level serum pool (approximately 28 U/mL) over a period of twenty days of assay qualification runs. Imprecision was evaluated at three clinical trial sites. Within-run imprecision ranged from 1.4% to 3.6% CV, and total imprecision ranged from 2.2% to 3.9% CV across products and sites. These results indicate that the recovery of Immuno 1 CA 125 II™ assay values are highly reproducible over time.

Imprecision results are presented in Table 4.

PRODUCT	MEAN	OBSERVATIONS	WITHIN-RUN		TOTAL	
			SD (U/mL)	CV (%)	SD (U/mL)	CV (%)
BioRad 1	24.7	667	0.90	3.6	1.02	4.1
MD Pool	26.1	677	0.61	2.3	0.87	3.3
BioRad 2	56.8	680	2.00	3.5	2.20	3.9
Cal 1	0.1	275	0.06	-	0.08	-
Cal 2	15.1	456	0.33	2.2	0.43	2.8
Cal 3	30.2	454	0.54	1.8	0.72	2.4
Cal 4	80.6	458	1.43	1.8	2.04	2.5
Cal 5	200.2	447	3.96	2.0	5.22	2.6
Cal 6	490.7	245	6.85	1.4	10.84	2.2

Table 4. Imprecision

Linearity. To determine if CA 125 II assay value recoveries are linear over the entire calibration range, four clinical sample pools containing a high concentration of CA 125 II assay values (450-500 U/mL) were diluted with normal serum (low CA 125 II assay values) to final concentrations of 100% (undiluted) 75%, 50%, 25%, and 0% (low CA 125 II serum only). Each pool was assayed with two lots of Immuno 1 CA 125 II reagent.

All four pools of sera, with CA 125 II assay values of approximately 471 to 497 U/mL, diluted linearly. Recoveries of the intermediate dilutions were all between 98% and 106% of the expected value. These results demonstrate the linearity of CA 125 II recoveries over the entire calibration range.

Hook Effect. Extremely high concentrations of CA 125 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. CA 125 antigen, isolated from human ascites and purchased from BioDesign (Kennebunk, ME), was prepared in Level 1 calibrator at concentrations of 70, 50, 25, 10, 2, 0.5, and 0.25 kU/mL. This collection of samples was

tested in triplicate using two lots of reagent. The assay response, measured as the observed reaction rate, did not "hook" back into the assay range, even at the highest concentration of antigen tested (70 kU/mL). These results demonstrate the lack of a hook effect in the Immuno 1 CA 125 II™ Assay at CA 125 II™ assay values $\leq$ 70,000 U/mL.

Lot-To-Lot Variation. To evaluate reagent lot-to-lot variability, the results from a collection of patient samples assayed with two reagent lots were compared. A total of 309 samples were included in this analysis. This sample collection included specimens from healthy subjects and patients with malignant and non-malignant diseases.

Immuno 1 CA 125 II™ Assay results obtained with Trial 1 and Trial 2 reagents showed excellent concordance. The linear least squares regression equation for the lot-to-lot analysis was:

$$\text{Trial 2} = 1.011 \times \text{Trial 1} - 0.092;\ r = 1.000;\ S_{y,x} = 2.47$$

Parallelism. A parallelism analysis was performed to determine CA 125 II assay values in diluted patient samples. Four individual serum samples from cancer patients were each diluted with normal serum to a CA 125 II concentration of approximately 500 U/mL. Each sample was then diluted with Level 1 CA 125 II calibrator (0 U/mL CA 125) to a final concentration of 100% (undiluted), 75%, 50%, 25%, 10%, and 0%. All dilutions were analyzed using two lots of reagent. Sample recoveries ranged from 99.6% to 11.4%. Linear regression analysis for each clinical sample showed no deviation from linearity. These results demonstrate assay linearity and the acceptability of the Level 1 calibrator for dilution of high patient samples.

METHOD COMPARISON STUDIES

Introduction. The objective of the method comparison studies was to examine the concordance of sample CA 125 values obtained using the Bayer Immuno 1™ CA 125 II™ Assay with those obtained using the Centocor CA 125 II™ RIA values. Patient sample CA 125 II values generated by the two methods were compared by correlation analysis and a determination of normal range cutoff.

Human serum samples from approximately 700 patients were analyzed using both the Immuno 1 CA 125 II™ Assay and the Centocor CA 125 II™ RIA Samples above the upper limit of the Immuno 1 or Centocor standard curves were diluted and re-assayed. Any samples diluted were noted and the dilution factors recorded. The number, source, and clinical classification of the patient samples used in this concordance study are summarized in the Table 5.

CLINICAL CLASSIFICATION	SOURCE	NUMBER OF SAMPLES
Normal (Premenopausal)	BioClinical Partners	100
Normal (Postmenopausal)	BioClinical Partners	97
Ovarian Cancer (Single Point)	BioClinical Partners	240
	Bayer Diagnostics	13
Lung Cancer	BioClinical Partners	50
Breast Cancer	BioClinical Partners	52
Colorectal Cancer	BioClinical Partners	50
Other Cancers	BioClinical Partners	48
Benign Urogenital Disease	BioClinical Partners	50
Total		703

Table 5. Summary of Patient Samples Used In The Clinical Concordance Evaluation.

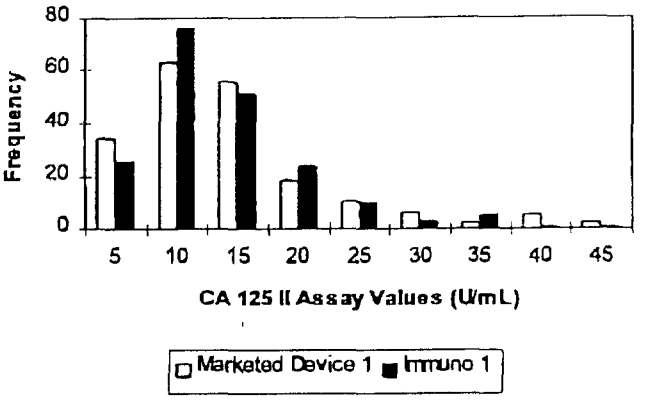
Normal Range. The range of values for normal specimens was determined for both methods by calculation of the mean, median, standard deviation, range, mean + 1.96 x SD, and 97.5th percentile of CA 125 II assay values in 197 normal healthy females (100 premenopausal and 97 post-menopausal).

The results of the normal range analysis are present in Table 6 and Figure 3. The mean serum CA 125 II assay values for all samples analyzed by the Immuno 1 CA 125 II™ Assay and the Centocor CA 125 II™ RIA was 11.6 U/mL and 12.1 U/mL respectively. The mean value + 1.96 x SD gave an upper range of 25.6 U/mL for the Immuno 1 CA 125 II™ Assay and an upper range of 28.0 U/mL for the CA 125 II™ Centocor RIA. The 97.5th percentile normal range cut-off for the Immuno 1 was 32.0 U/mL and for the RIA was 36.4 U/mL. As shown in Table 6 and Figure 3, there were no significant differences between the two assays in the CA 125 II mean, median, or cutoff ranges for the pre- or post-menopausal specimens. Overall, the means and ranges of the Centocor RIA assay values (Marketed Device 1, Figure 3) were very similar to those generated by the Immuno 1 CA 125 II™ assay, thereby demonstrating the equivalence of the two methods in CA 125 determination.

	PRE-MENOPAUSAL		POST-MENOPAUSAL		TOTAL	
	Immuno 1	**Centocor**	**Immuno 1**	**Centocor**	**Immuno 1**	**Centocor**
n	100	100	97	97	197	197
Mean	11.9	12.2	11.3	11.9	11.6	12.1
Median	9.6	9.7	9.4	10.8	9.5	10.1
Range	2.7 - 42.0	3.1 - 42.5	3.5 - 38.8	2.5 - 40.4	2.7 - 42.0	2.5 - 42.5
Mean + 1.96 SD	26.8	28.4	24.2	27.2	25.6	28.0
97.5th Percentile	32.2	36.4	29.1	35.7	32.0	36.4

Table 6. Normal Range Analysis Results(U/mL)

Figure 8.1 Distribution of Normals



Method Concordance. In order to compare the values obtained from serum samples analyzed by the Immuno 1 CA 125 II™ Assay with Centocor CA 125 II™ RIA derived values, a correlation study using 703 female serum samples was performed. The 703 serum samples consisted of 197 normal samples (pre- and post-menopausal), 253 single point ovarian cancer specimens, 50 lung cancer specimens, 52 breast cancer specimens, 50 colorectal cancer specimens, 48 samples from patients with a variety of other cancers (pancreatic, renal, liver, uterine, cervical , endometrial), 50 benign urogenital disease samples, and 3 unknown disease samples with elevated CA 125 II values. The sample CA 125 II values were obtained in duplicate determinations in the Centocor RIA assay and from a single determination on the Immuno 1. RIA results for each sample are reported as the mean of duplicate CA 125 II assay value determinations. A least squares linear regression was used to compare the assay values obtained.

The calculated statistics for all samples assayed (including dilutions) are shown in Table 7. Table 8 presents only the sample results that were within the linear range (i.e. standard curve range) of both the Immuno 1 Assay and the Centocor RIA. These results demonstrate that the quantitation of CA 125 II assay values by the two methods is concordant and equivalent.

Immuno 1 vs. Centocor CA 125 II Correlation (Total Samples; n = 703)			
Ordinary Least Squares Regression			
Slope	Intercept	N	R
0.823	20.318	703	0.985

Table 7. Immuno 1 CA 125 II™ Assay vs. Centocor CA 125 II™ RIA Correlation (Total Samples)

Immuno 1 vs. Centocor CA 125 II Correlation (Total Samples; n = 688)			
Ordinary Least Squares Regression			
Slope	Intercept	N	R
0.975	2.778	668	0.983

Table 8. Immuno 1 CA 125 II™ Assay vs. Centocor CA 125 II™ RIA Correlation (Standard Curve Range Samples Only)

Conclusions from Method Comparison Studies. The results of this comparative clinical analysis of serum CA 125 II assay values in a panel of normal and cancer patients clearly demonstrates the concordance of the Immuno 1 CA 125 II™ Assay and Centocor CA 125 II™ RIA results. Normal reference ranges were essentially equivalent, and correlation statistics demonstrated concordance between the two methods.

REAGENT STABILITY TESTING

Shelf-life Stability. The reagents were stressed at 25°C, 30°C and 40°C and tested at selected time points. Additional reagent stored at 2-8°C was tested in parallel with the stressed reagents at all time points. An extended shelf-life of 24 months is recommended for CA 125 II reagents. The data for the four lots (Experimental 1 & 2 ; Trials 1 & 2) indicate that the product will still be well within the end of shelf life specification at 24 months. In all cases, at the latest time point tested, Level 3 sensitivity (2-8°C) is at or above the minimum sensitivity specification and medical decision pool recoveries are within the end of shelf limits. Based on the rate of degradation of four reagent lots, the end of shelf life specification for Level 3 sensitivity should be met throughout the shelf-life recommendation of 24 months.

On-System Stability. Aging reagent packs on system were tested by assaying control materials and calibrators at selected time points throughout a series of 32 day periods. On system stability is defined as the duration that a single reagent cassette will continuously achieve control recoveries within ± two total standard deviations of the day zero values. Sensitivity (mA/min/U/mL) for each calibrator level was also monitored. Imprecision and sensitivity results at each time period up to 32 days were within the limits set for acceptable performance. On-system stability data on the Immuno 1 CA 125 II Assay reagents support an on-system stability recommendation of 30 days.

Shipping Stability. Shipping and shelf-life stability data on four Experimental and Trial lots of Immuno 1 CA 125 II™ assay reagents support the requirement of refrigerated (2-8°C) shipping of these reagents because of effects noted at high temperatures.

Labeling. Reagent stability is indicated in the Immuno 1 CA 125 II method insert sheet. Expiration dates are also indicated on the labels of each reagent kit.

CALIBRATOR STABILITY TESTING

Shelf Life. For the Immuno 1 CA 125 II calibrators, shelf life dating of 12 months at < -10° C has been recommended. Shelf life stability was based upon real time stability testing at -80°C and upon accelerated stability testing over a range of elevated temperatures. The calibrators were stressed at 2-8°C, 25°C, 30°C and 40°C. Medical Decision Pool recovery, precision, and recovery of the stressed calibrators as unknowns against the -80°C stored calibrators was collected. Recoveries of calibrators stored at elevated temperatures exceeded the specification of ±5%, and support the recommendation that calibrators are stable for 12 months when stored at -10°C or below.

Open Vial Stability. The open vial protocol specifies use of one set [or pool if necessary] of opened calibrators throughout the 5 week study. After opening, the calibrators are stored at 2-8°C. In addition, at each timepoint, a fresh (control) set of calibrators is run. Recovery

of the Medical Decision Pool run as unknowns at 0, 1, 3 and 5 week timepoints were collected. Analyte values were derived using a calibration curve generated at that timepoint using the calibrators opened and stored at 2-8°C. Medical decision pool recoveries calculated from the timepoint calibration remained well within specification. At the 5 week timepoint, there was less than a 1-2% increase in Medical Decision Pool recoveries from the Medical Decision value obtained at the day 0 timepoint. Based on this data, the open vial recommendation is 30 days when stored at 2-8°C.

Shipping Stability. The Calibrators were cycled three times through freeze/thaw (-20°C /2-8°C) and then tested versus the -80°C control set. Each cycle consisted of 3 days at -20°C and 3 days of thawing at 2-8°C. After all three cycles were completed, calibrators were stored at 2-8°C and evaluated at selected timepoints. After three -20/2-8°C freeze/thaw stress cycles, CA 125 II Calibrators Lot 2844CB and Lot 3004A showed a 0-2% increase in CA 125 II concentration over all levels. Based on these data, it is recommended that the calibrators be shipped in refrigerated packages. At all other times, it is recommended that the CA 125 II Calibrators be held at frozen temperature $\leq$ -10°C.

Labeling. Calibrator stability is indicated in the Immuno 1 CA 125 II method insert sheet. Expiration dates are also indicated on the labels of each calibrator kit.

CLINICAL STUDY

Introduction. The clinical safety and effectiveness of the Bayer Immuno 1™ CA 125 II™ Assay as a one time test for use as an aid in the detection of residual ovarian cancer was assessed in the target population. Specimens were collected from patients with primary epithelial invasive ovarian cancer who completed primary therapy and underwent diagnostic second look surgery. The Bayer Immuno 1 assay result for each patient was compared to the diagnostic outcome of the second look procedure and also to the assay result from the predicate device, the Centocor CA 125 II™ RIA

Management Value of the Bayer Immuno 1™ CA 125™ Assay as an aid in the detection of residual ovarian cancer in patients who have undergone first-line therapy and would be considered for diagnostic second look procedures. A total of 48 patients were evaluated; 29 patients were diagnosed with residual disease following second look surgery. Sensitivity and specificity for detection of residual ovarian cancer is presented in Table 9 for the Immuno 1 CA 125 II™ Assay and in Table 10 for the Centocor CA 125 II™ Assay. Clinical utility of the Immuno 1 CA 125 II™ Assay is demonstrated by a sensitivity of 31%, and a specificity of 95%. Positive and negative predictive values were 90% and 47%, respectively. Equivalent results were obtained for the Centocor assay.

OVARIAN CANCER RECURRENCE: Immuno 1

	YES	NO	TOTAL
> 35 U/mL	9	1	10
< 35 U/mL	20	18	38
TOTAL	29	19	48

Sensitivity:	31%
Specificity:	95%
Positive Predictive Value:	90%
Negative Predictive Value:	47%

Table 9. Ovarian Cancer Patients Undergoing Second Look Procedures; Immuno 1 CA 125 II™ Assay Results Compared to Diagnostic Outcome.

OVARIAN CANCER RECURRENCE: Centocor RIA

	YES	NO	TOTAL
> 35 U/mL	6	2	8
< 35 U/mL	23	17	40
TOTAL	29	19	48

Sensitivity:	21%
Specificity:	89%
Positive Predictive Value:	75%
Negative Predictive Value:	43%

Table 10. Ovarian Cancer Patients Undergoing Second Look Procedures; Centocor CA 125 II™ Assay Results Compared to Diagnostic Outcome.

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 research laboratories operating under the principles of Good Laboratory Practices. The patient population was well characterized and patient histories were thoroughly documented.

Method Performance. Immuno 1 CA 125 II™ Assay nonclinical performance, including analytical sensitivity (minimum detectable concentration), analytical specificity (interferences), imprecision, parallelism, linear range, and hook effect met accepted specifications for an assay of this type.

Safety and Effectiveness. Evaluation of clinical samples demonstrated the safety and effectiveness of Immuno 1 CA 125 II™ Assay values as a one time test for use as an aid in the detection of residual ovarian cancer in patients who have undergone first-line therapy and would be considered for diagnostic second look procedures. The correlation between Immuno 1 CA 125 II™ Assay values and diagnostic outcome demonstrates that this assay may be used as indicated.

Substantial Equivalence. The method concordance studies confirm the substantial clinical equivalence of the Immuno 1 CA 125 II™ Assay and the Centocor CA 125 II™ RIA. There is a high degree of correlation between the Immuno 1 and Centocor CA 125 II™ patient values.

Evaluation of clinical samples in the target population demonstrated that sensitivity and specificity for predicting residual disease are equivalent for the two tests. Therefore, based upon the analytical and clinical concordance established in these studies, the Bayer Immuno 1 CA 125 II™ Assay and the Centocor CA 125 II™ RIA are equivalent with respect to method performance, clinical utility and device safety and effectiveness.

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

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

OCT 31 1997

Re: K964098
Trade Name: Bayer Immuno I™ CA 125 II™ Assay
Regulatory Class: II
Product Code: LTK
Dated: July 31, 1997
Received: August 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 (Premarket 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.

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 Gutman

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

510(k) Number (if known): K964098

Device Name: Bayer Immuno 1™ CA 125 II™ Assay

Indications For Use:

The Bayer Immuno 1™ CA 125 II Assay is an *in vitro* device for the quantitative measurement of OC 125 reactive determinants associated with a high molecular glycoprotein in serum of women with primary epithelial invasive ovarian cancer. The CA 125 II Assay is indicated as a one-time test for use as an aid in the detection of residual ovarian carcinoma in patients who have undergone first-line therapy and would be considered for diagnostic second-look procedures. An assay value of greater than 35 U/mL is indicative of residual disease, provided that alternative causes of elevated CA 125 II Assay values can be excluded (refer to "Limitations of the Procedure" section). It is recommended that the assessment and treatment of patients with ovarian cancer and the use of the Bayer Immuno 1 CA 125 II Assay be under the order of a physician trained and experienced in the management of gynecological cancers.

(PLEASE DO NOT WRITE BELOW THIS LINE- CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Peter E. Maheux

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number _____

Prescription Use ✓ OR