1997

K964703

SUMMARY OF SAFETY AND EFFECTIVENESS
For the
Bayer Immuno 1™ CA 15-3™ Assay

This premarket notification is to add the quantitative measurement of CA 15-3™ 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 15-3™ Assay and comparison of this assay to a predicate device, the Biomira TRUQUANT® BR™ RIA has been established 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™ CA 15-3™ Assay at four 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 CA 15-3™ Assay with that of the Biomira RIA. Clinical studies were conducted at four clinical trial sites with a suitable sampling of patients to support the diagnostic claims for this device.

INDICATIONS FOR USE

The Bayer Immuno 1™ CA 15-3™ Assay is an in vitro, diagnostic, solid phase immunoassay intended to quantitatively measure CA 15-3™ Assay values in human serum on the Bayer Immuno 1 system. When used in conjunction with other clinical and diagnostic procedures, serial testing with the CA 15-3™ Assay is useful for monitoring the course of disease and therapy in metastatic breast cancer patients, and for detection of recurrence in previously treated Stage II, with greater than 2 positive lymph nodes, or Stage III breast cancer patients.

Background

The Nature of the CA 15-3™ Determinant. The tumor marker of choice for breast cancer is the CA 15-3™ assay[1]. The Immuno 1 CA 15-3™ assay measures the serum level of a mucin, the high molecular weight glycoproteinaceous product of the *MUC-1* gene [2,3]. Mucins are synthesized in two forms, secreted and membrane bound. Secreted mucins lubricate and protect the lumenal surfaces of the digestive, respiratory and reproductive epithelia. The structure of the membrane mucins have been deduced from nucleotide sequences of the *MUC-1* gene [2, 3]. Mucins contain a peptide backbone with very high serine and threonine content which serves as a scaffold for the attachment of a high density of O-linked oligosacharride side chains [4-6]. The N-terminus of the *MUC-1* mucin (known as episialin, polymorphic epithelial mucin, or epithelial membrane antigen) is followed by a mucinous domain containing a large number of repetitive sequences, tandem repeats, each of which contains 20 amino acids with a large number of serine, threonine, and proline residues. The number of tandem repeats varies from 30 to 60 due to genetic polymorphism. Homology in the tandem repeat domains between various membrane-associated mucins is low suggesting that these regions serve solely as a scaffold for the attachment of O-linked oligosaccharide. Following the tandem repeat domain is a transmembrane segment and an intracellular domain. Immediately following synthesis in the endoplasmic reticulum, the *MUC-1* gene product is cleaved such that the extracellular mucinous domain is separated from the transmembrane and intracellular domains [7]. The fragments remain associated, however, through non-covalent interactions.

The function of the membrane associated mucins is not well established, but since they inhibit cellular aggregation, they may play a role as antagonists of cellular adhesion [8]. The large size of episialin suggests that it extends approximately 500 nm above the cell surface, greatly in excess of the 10 nm extent of the glycocalyx [2]. This anti-adhesion function may maintain lumenal structure by inhibiting the adhesion of the lumenal and apical surfaces. The adhesion modulating properties of membrane bound mucins may also promote tumor cell metastasis [9].

The structure of membrane bound mucins also varies between normal and tumor cells. The number of O-linked residues and the length of the oligosaccharide chains are reduced in tumor cells compared with mucins from normal tissues. This has led to the development of monoclonal antibodies (MAbs) which show differential binding to tumor and normal cells. The MAb DF3 was derived from mice immunized with a membrane enriched fraction of a metastatic human breast carcinoma, and has been shown to bind to the heptapeptide sequence TRPAPGS [10, 11]. Binding of the DF3 MAb is reduced by pretreatment of episialin with neuraminidase [12], suggesting that the oligosaccharide side chain is critical for the binding of the DF3 antibody.

The DF3 MAb has been paired with an antibody of similar specificity, 115D8, in the tumor marker assay CA 15-3™. Both antibodies react with different epitopes which are expressed on certain high molecular weight mucinous glycoproteins. It has been reported that the breast cancer associated antigen contains epitopes for both DF3 and 115D8, thus permitting the detection of antigen by double antibody determinant assays [2-4, 10, 11].

CA 15-3™ Serum Levels in Breast Cancer. Measurement of episialin in serum using the CA 15-3™ Assay has shown considerable promise in the management of breast cancer patients. The overall sensitivity of serum CA 15-3™ measurement is low for Stage I and II breast cancer, but increases to approximately 60-80% in late stage patients with metastatic disease [13-15]. Accordingly, the CA 15-3™ assay has not been found useful for early detection of breast cancer in the asymptomatic population, but has shown considerable utility in management of patients with breast cancer diagnosed by other means. For example, longitudinal elevations of serum CA 15-3™ in breast cancer patients with no clinical evidence of disease has been shown to coincide with cancer recurrence in 37-77% of these patients [15-20]. In one study, serial increases in serum CA 15-3™ values were shown to occur prior to clinical detection in 67% of patients with breast cancer recurrence, with a lead time of 4-48 months [20]. In a similar study, CA 15-3™ was found to detect breast cancer recurrence with a median lead time of 6 months [21]. It has also been shown that changes in serum concentrations of episialin correlate with changes in the clinical course of disease in patients with active breast cancer [16,17,18,20]. In fact, in one particular study, serum CA 15-3™ values correlated with clinical status in 21/22 (95%) of breast cancer

patients [15]. In another study, CA 15-3™ values increased in 19/21 (90%) of patients with progressive cancer, and decreased in 7/9 (78%) of patients with disease regression [20]. Taken together, these results indicate that longitudinal measurement of CA 15-3™ in serum may be helpful in monitoring breast cancer patients, particularly those with metastatic disease; and may also be of value in monitoring for the detection of cancer recurrence in previously treated breast cancer patients with no clinical evidence of disease.

CA 15-3™ Serum Levels in Non-Breast Malignancies. Some non-breast malignant diseases are associated with elevated levels of CA 15-3™ assay values [1, 22, 23]. Studies examining this issue have demonstrated that the occurrence of elevated circulating levels of CA 15-3™ assay values is not specific for breast cancer. Elevated serum CA 15-3™ assay values have been demonstrated in patients with advanced ovarian, cervical, and endometrial cancer [1, 22-24].

CA 15-3™ Serum Levels in Healthy Controls and Benign Disease. Recent studies have defined the specificity of the CA 15-3™ assay. Patients with benign breast lesions had CA 15-3™ levels that were not significantly different from those of healthy controls [10, 13, 15, 17]. While third trimester pregnancy is often associated with serum CA 15-3™ concentrations of up to 50 U/mL, lactation had no detectable effect on CA 15-3™ levels [1]. The upper limit of normal values of CA 15-3™ assay values, as determined in different studies employing different assay systems, ranged from 15 to 50 U/mL.

Elevated serum CA 15-3™ levels have been reported to be associated with several non-malignant conditions. Like CEA, inflammatory liver disease and liver cirrhosis result in low, but significant elevations in certain patients [1]. Additionally, several studies have demonstrated the detection of slightly elevated CA 15-3™ assay values in the circulation of individuals with generalized autoimmune disease [1].

DEVICE DESCRIPTION

Indicated Use. The Bayer Immuno 1™ CA 15-3™ Assay is an *in vitro* device indicated for the quantitative measurement of CA 15-3™ assay values in human serum. When used in conjunction with other clinical and diagnostic procedures, serial testing with the CA 15-3™ Assay is useful for monitoring the course of disease and therapy in metastatic breast cancer patients, and for detection of recurrence in previously treated Stage II, with greater than two positive lymph nodes, or Stage III breast cancer patients. 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 Assay. The Bayer Immuno 1™ CA 15-3™ Assay is a sandwich immunoassay in which one monoclonal antibody (115D8) is conjugated to fluorescein (R1) and a second monoclonal antibody (DF3) is conjugated to alkaline phosphatase (R2). Immuno 1 magnetic particles 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, which binds to the immobilized CA 15-3™ 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 15-3™ assay values in the sample. A cubic-through-zero curve fitting algorithm is used to generate standard curves. A schematic representation of the magnetic separation sandwich assay technique is presented in Figure 1 below.

The assay has a range of 0.2 to 200 U/mL. The assay uses six calibrators with CA 15-3™ concentrations of 0, 12.5, 25, 50, 100, and 200 U/mL. A typical standard curve for the assay is presented in Figure 2.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The Immuno 1 CA 15-3™ 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 15-3™ 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 breast 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 CA 15-3™ assay values be used in conjunction with the results of the patient's overall clinical assessment.

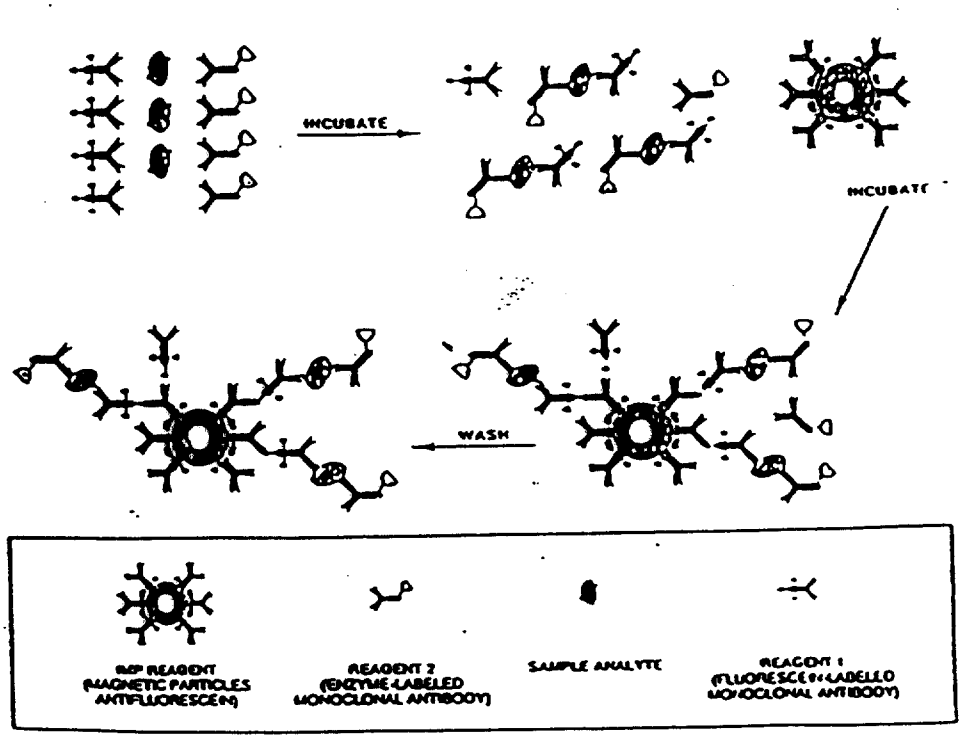
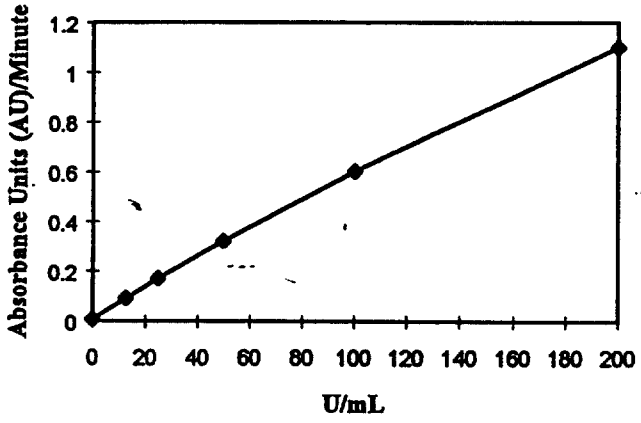


Figure 1. Schematic Representation of the Magnetic Separation Sandwich Immunoassay of the Bayer Immuno 1™ System.



U/mL	AU/Min
0	0.0073
12.5	0.0925
25	0.1700
50	0.3198
100	0.6031
200	1.1002

Figure 2. Standard Curve for the Bayer Immuno 1™ CA 15-3™ Assay.

PRECAUTIONS AND WARNINGS

This device is not indicated for breast cancer screening, as a predictor of stage of disease, or as a sole diagnostic tool to confirm the presence or absence of malignant breast disease. CA 15-3™ assay values should be used for the management of breast cancer patients in conjunction with the information from a complete clinical evaluation including physical exam and other diagnostic tests.

Confirmed breast carcinoma patients, in particular patients with Stage I or II disease, can have CA 15-3™ assay values that are frequently in the same range as healthy individuals [13-15]. Additionally, patients with certain non-malignant conditions (inflammatory liver disease, liver cirrhosis, generalized autoimmune disease, and third trimester pregnancy), and patients with certain non-breast malignancies (advanced ovarian, cervical, or endometrial carcinoma) can exhibit elevations in CA 15-3™ assay levels [1, 22-24]. As such, serum CA 15-3™ assay levels should not be interpreted as absolute evidence of the presence or absence of malignant disease.

The CA 15-3™ concentration 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 15-3™ assay used. Values obtained with different CA 15-3™ assays cannot be used interchangeably. If in the course of monitoring the patient, the assay method used for determining serial CA 15-3™ levels is changed, additional sequential testing should be carried out to confirm baseline values.

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. In addition, accuracy was evaluated by comparison of the Immuno 1 CA 15-3™ Assay with the FDA-approved Biomira TRUQUANT® BR™ RIA, and Centocor CA 15-3™ RIA in a method concordance evaluation. Using a panel of 501 serum samples, this concordance analysis

evaluated the relationship between CA 15-3™ assay values determined by the Immuno 1 CA 15-3™ Assay and the Biomira TRUQUANT® BR™ RIA and Centocor CA 15-3™ RIA .

NONCLINICAL STUDIES

Characterization of the Antigen. The antigen used in the Immuno 1 CA 15-3™ assay calibrators is the DF3 reactive determinant (designated DF3 antigen) isolated from the supernatant fluid of an *in vitro* culture of ZR75-1 Anchorage Dependent Cells. The antigen is manufactured by Centocor, Inc., and is supplied to Bayer in a partially purified form. Antigenic preparations, analyzed by non-reducing SDS-PAGE, and immunoblotted with alkaline phosphatase conjugated DF3 MAb revealed two high molecular weight bands of 290 and 490 kDa. This electrophoretic analysis demonstrated that the antigenic calibrator preparation used in the assay is consistent with previous descriptions of the CA 15-3™ glycoprotein.

Immunoreactivity of the Antibodies. Monoclonal antibody preparations DF3 and 115D8 are used in the Immuno 1 CA 15-3™ Assay. Both antibodies are manufactured by Centocor, Inc., and are supplied to Bayer in partially purified form.

The DF3 and 115D8 antibodies were characterized in a series of experiments. Isotype analysis demonstrated that the MAbs DF3 and 115D8 were of the murine IgG1 and IgG2b subclasses respectively. Relative affinity analysis revealed that both antibodies bound to CA 15-3™ antigen in a similar and saturable manner, and bound to "native" CA 15-3™ antigen with an apparent higher relative affinity than to desialyated CA 15-3™ antigen. Additionally, the binding of MAb 115D8 to native antigen was found to be more sensitive to changes in pH than was MAb DF3. 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 DF3 and 115D8 MAbs bind to the CA 15-3™ antigen quantitatively and specifically, and display biophysical properties expected of mouse monoclonal antibodies.

Specificity And Interfering Substances. The recovery of CA 15-3™ assay values from the Medical Decision Pool, an internal serum-based control containing a CA 15-3™ concentration of approximately 31 U/mL, was studied before and after spiking with the potentially interfering substances listed below. Each potential interferant was tested in duplicate, at five equally spaced concentration levels, on one system, using one lot of assay reagent.

Endogenous Interferants. The Immuno 1 CA 15-3™ Assay was performed on the Medical Decision Pool to which was added various concentrations of either triglycerides, immunoglobulin, hemoglobin, heparin, bilirubin, or albumin. The highest concentration of each potential interferant used was greater than that normally observed during routine clinical testing. The highest concentration of each potential endogenous interferant tested and the maximum effect on the observed CA 15-3™ recovery are summarized in Table 1. None of the potential endogenous interferants demonstrated any significant interfering effects on CA 15-3™ assay recovery.

Anti-neoplastic Drug Interferants. Because of the possibility that serum CA 15-3™ measurements might be performed while patients are undergoing a regimen of chemotherapy, CA 15-3™ assay values were measured in the Medical Decision Pool after spiking the pool with 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 drug cocktail are presented in Table 2.

Interferant	Highest Concentration Tested	Maximum Effect
Triglycerides	900 mg/dL	-0.2%
Immunoglobulin	5.3 g/dL	0.5%
Hemoglobin	1.0 g/dL	1.6%
Heparin	0.15 mg/mL	1.3%
Bilirubin	25 mg/dL	1.3%
Albumin	6.5 g/dL	-1.5%

Table 1. Endogenous Interference

Pool Number	Drug	Highest Concentration Tested (1X)
Drug Pool #1	Cyclosphosphamide (Cytoxan)	700 ug/mL
	Doxorubicin (Adriamycin)	51.8 ug/mL
	5-Fluorouracil	346 ug/mL
	Methotrexate	30 ug/mL
	Mitomycin C	13.8 ug/mL
Drug Pool # 2	Diethylstelbestrol	23 ug/mL
	Tamoxifen Free Base	60 ug/mL
	Tamoxifen Citrate (Novadex)	60 ug/mL
	Vincristine	1.4 ug/mL
Drug Pool # 3	Aminoglutethemide (Cytadren)	398 ug/mL
	Cis-Platin	173 ug/mL
	Megace	243 ug/mL

Table 2. Chemotherapeutic Drugs Used For Interference Testing

Inhibition of CA 15-3™ recovery (-21.3%) by the chemotherapeutic Drug Pool # 1 was noted (Table 3). The five individual drugs which comprised Drug Pool #1 were evaluated individually. Only methotrexate exhibited any change in percent recovery with increasing drug concentration. Methotrexate, at a concentration of 300 ug/mL inhibited CA 15-3™ recovery by approximately 65% as shown in Figure 3.

High dose methotrexate chemotherapy with autologous stem cell rescue is presently used in the treatment of primary breast carcinoma [25]. The use of high doses of methotrexate leading to plasma concentrations of 10^{-4} to 10^{-3} M (45.4-454 ug/mL) is sufficient to allow passive entry of methotrexate into tumor cells, overcome drug resistance, and prolong

drug action so that more tumor cells can be exposed to the drug during DNA synthesis (26). Methotrexate exhibits a "triphasic" pattern of clearance from the circulation, with the highest serum levels of the drug (10^{-4} to 10^{-3} M), exhibiting a terminal clearance half-life of 8-15 hours (average 10.4 hours) [26,27,28]. Therefore, a patient with a high therapeutic serum level of methotrexate would clear the drug from the circulation (assuming the slowest rate of elimination) to a level which will not significantly effect Immuno 1 CA 15-3™ Assay quantitation after four days.

The observed methotrexate effect is not likely to result in patient mismanagement for several reasons. First, Stage II or Stage III patients, clinically free of disease, and being monitored for the early detection of breast cancer recurrence, are not likely to be receiving methotrexate therapy at the time of testing. Secondly, for patients who may be receiving methotrexate treatment, testbleeds for serum tumor marker immunoassay analysis would commonly be drawn between cycles of chemotherapy, just prior to the initiation of a new round of treatment. Given standard chemotherapeutic regimens, these serum specimens would be collected well after the last dose of drug was received, and after sufficient time to allow the clearance of methotrexate to a level which would not significantly effect the assay.

Interferant	Maximum Effect
Therapeutic Drug Pool 1	-21.3%
Therapeutic Drug Pool 2	0%
Therapeutic Drug Pool 3	2.0%

Table 3. Drug Pool Interference

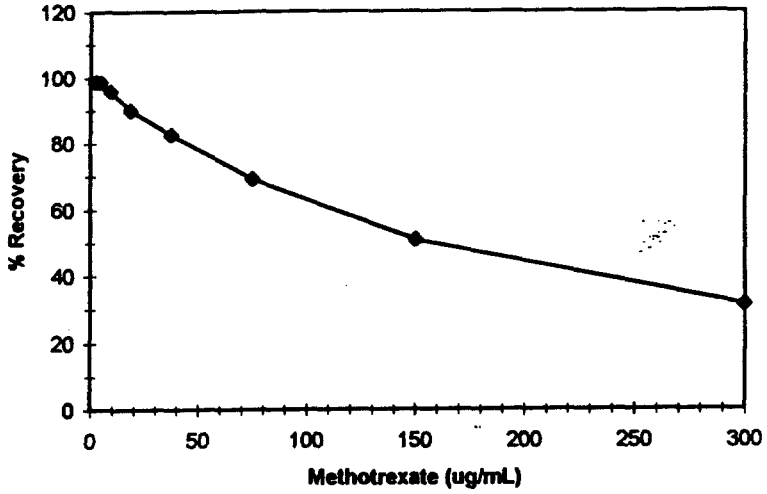


Figure 3. Methotrexate Interference

Common "Over the Counter" Drugs and Vitamin Supplements. The potential interference of common vitamin supplements, "Over the Counter" (OTC) drugs, caffeine, and codeine in the Immuno 1 CA 15-3™ Assay was investigated. Each of the substances were added individually to a patient sample serum pool with CA 15-3™ assay concentration of approximately 100 U/mL. The concentrations of drugs added were greater than that normally observed during routine clinical testing. The concentration of each potential interferant tested and the maximum effect on the observed Immuno 1 CA 15-3™ Assay recovery are summarized in Table 4. None of the vitamins, OTC drugs, caffeine, or codeine demonstrated any significant interfering effects on CA 15-3™ assay recovery.

Interferant	Highest Concentration Tested	Maximum Effect
Vitamin A	10 IU/mL	0.2%
Thiamin (B1)	3.0 µg/mL	0.3%
Riboflavin (B2)	3.4 µg/mL	0.7%
Vitamin B6	4.0 µg/mL	0.0%
Vitamin B12	0.012 µg/mL	-2.7%
Vitamin C	30 µg/mL	-1.5%
Vitamin D2	0.8 IU/mL	-0.3%
Vitamin E	0.6 IU/mL	1.9%
Niacin	0.4 mg/mL	2.1%
Folic Acid	0.8 µg/mL	-3.5%
Caffeine	100 µg/mL	1.1%
Codeine	240 µg/mL	-2.1%
Acetaminophen	200 µg/mL	-2.7%
Ibuprofen	400 µg/mL	-1.5%
Aspirin	500 µg/mL	0.6%

Table 4. Common vitamins, OTC Drugs and Codeine Interference

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 six samples with high human anti-mouse antibody titers were assayed with two lots of reagents. All samples were tested both undiluted and at a 50% dilution using the Level 1 Immuno 1 CA 15-3™ calibrator (containing no CA 15-3™ antigen). All samples recovered CA 15-3™ assay values linearly when diluted 50% with the Level 1 CA 15-3™ calibrator. The percent recovery for all samples ranged from 83.9% to 92.6%. This observed linear CA 15-3™ recovery indicates a lack of significant heterophilic interference in the assay and demonstrates the effectiveness of the reagent formulation in minimizing these interferences.

Linearity. To determine if CA 15-3™ assay recoveries are linear over the entire calibration range, three clinical sample pools containing a high concentration of CA 15-3™ assay values were diluted with normal serum (low CA 15-3™ assay values) to final concentrations of 100%

(undiluted) 75%, 50%, 25%, and 0% (low CA 15-3™ serum only). Each pool was assayed with two lots of Immuno 1 CA 15-3™ reagent.

The three pools of sera with CA 15-3™ assay values of approximately >180 U/mL diluted linearly. Recoveries of the intermediate dilutions were all between 98.2% and 105.2% of the expected value. These results clearly demonstrate the linearity of CA 15-3™ assay recoveries over the entire calibration range.

Hook Effect. Extremely high concentrations of CA 15-3™ assay values 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 15-3™ antigen, isolated from human ascites and purchased from BioDesign (Kennebunk, ME), was value-assigned by testing on the Immuno 1, and diluted in Level 1 calibrator at concentrations of 28500, 14250, 7125, 3562, 1781, 890, 445, 223, 111, 56, 28, 14, and 7 U/ml. This collection of samples was tested in the assay using two lots of reagents. 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 (28.5 kU/mL). These results demonstrate the lack of a hook effect in the Immuno 1 CA 15-3™ Assay at CA 15-3™ assay values $\leq$ 28,500 U/mL.

Spiked Recovery. To determine how well CA 15-3™ antigen, when spiked into a patient sample, is recovered by the Immuno 1 CA 15-3™ Assay, antigen was spiked into seven patients at two different CA 15-3™ concentrations (30 and 120 U/mL). Each sample was assayed in quadruplicate with one lot of Immuno 1 CA 15-3™ reagent.

Recoveries of assay values for all samples ranged from 92.9 to 122.9%. No significant deviation was noted with regard to expected versus observed assay values. These results demonstrate the accurate quantitation of spiked and recovered CA 15-3™ assay values using the Immuno 1 assay.

Parallelism. To confirm the use of the Immuno 1 CA 15-3™ Level 1 calibrator (0.0 U/mL) as a sample diluent and to further verify assay linearity, four patient serum sample pools containing a high level of CA 15-3™ 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. Linear regression analysis for the determination of deviations from linearity for each of these clinical samples showed no deviation from linearity. The recovery of CA 15-3™ assay values ranged from 88.2-116.9%. The accurate recovery of CA 15-3™ assay values in diluted patient samples further illustrates the linearity of the Immuno 1 CA 15-3™ Assay throughout the entire calibration range and confirms the use of the Immuno 1 CA 15-3™ Level 1 calibrator as a sample diluent.

Minimum Detectable Concentration. Analytical sensitivity of the Immuno 1 CA 15-3™ assay was evaluated by determination of the Minimum Detectable Concentration (MDC). The MDC is defined as the minimum concentration of CA 15-3™ 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 15-3™ assay was determined as the CA 15-3™ concentration corresponding to an absorbance value equal to two within-run standard deviations above the mean absorbance value of the zero calibrator.

An MDC of 0.13 U/mL was observed, based on 1336 determinations of the Level 1 calibrator (0 U/mL), at four clinical trial sites, using three lots of calibrators and three lots of reagents. This level of analytical sensitivity is acceptable for an assay of this type with an upper limit of normal at approximately 35 U/mL. This level of sensitivity exceeds the Immuno 1 CA 15-3™ Method Sheet claim of 0.2 U/mL.

Imprecision. Intra- and interassay reproducibility were evaluated for two levels of commercial controls, three lots of Technicon SETpoint™ CA 15-3™ calibrators, and an internal human serum control material at the medical decision level. These materials were assayed with three lots of reagent over a period of 10 days in two runs per day at four

clinical trial sites. Imprecision estimates pooled across sites, reagent lots, and calibrator lots are shown in Table 5.

Table 5

Immuno 1 CA 15-3™.
Pooled Across Clinical Trial Sites, Reagent and Calibrator Lots

Product /Levels	Mean (U/mL)	Total Number of Observations	Within-Run SD (U/mL)	Within-Run CV (%)	Total SD (U/mL)	Total CV (%)
CONTROLS: BioRad Lot 44010, Immuno 1 CA 15-3 Medical Decision Pool Lot						
BioRad 1	13.5	445	0.46	3.4	0.54	4.0
BioRad 2	35.0	445	0.84	2.4	1.09	3.1
MDP	33.2	446	0.53	1.6	1.09	3.3
Technicon SETpoint CA 15-3 Calibrators: Pooled Across Lots						
Level 1	0.1	774	0.05	-	0.06	-
Level 2	13.3	1325	0.23	1.8	0.47	3.5
Level 3	26.0	1338	0.55	2.1	0.96	3.7
Level 4	50.7	1340	1.22	2.4	1.85	3.7
Level 5	102.3	1334	1.89	1.9	3.52	3.4
Level 6	190.9	301	2.47	1.3	5.73	3.0

Within-run CVs ranged from 1.3% to 3.4%, while total CVs ranged from 3.0% to 4.0%. Excellent precision was obtained at all analyte concentrations tested covering the range of the assay (0.0 to 200 U/mL). These results show that the recovery of Immuno 1 CA 15-3™ assay values are highly reproducible over time, using different lots of reagent, when tested in different laboratories.

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™ CA 15-3™ Assay with those obtained using the Biomira TRUQUANT® BR™ RIA and the Centocor CA 15-3™ RIA. Patient sample CA 15-3™ or BR 27.29 values generated by the three methods were compared by correlation analysis and a determination of normal range cutoff.

Human serum samples from approximately 501 patients obtained from BioClinical Partners, Inc. (Sharon, MA), and our in-house specimen collection (Bayer Corporation, Business Group Diagnostics; Tarrytown, NY), were analyzed using the Immuno 1 CA 15-3™ Assay, the Biomira TRUQUANT® BR™ RIA and the Centocor CA 15-3™ RIA. The samples were analyzed at two investigational sites. The Immuno 1 testing and Centocor RIA analysis was performed in the Research and Development Laboratories at Bayer Corporation, Tarrytown, N.Y. The Biomira RIA analysis on all patient samples was performed by Dianon Systems, Inc. in their laboratories in Stratford, CT. Samples above the upper limit of the Immuno 1, Biomira, or Centocor standard curves were diluted and re-assayed. The number, source, and clinical classification of the patient samples used in this concordance study are summarized in Table 6.

CLINICAL CLASSIFICATION	SOURCE	NUMBER OF SAMPLES
Normal (Premenopausal)	BioClinical Partners	100
Normal (Postmenopausal)	BioClinical Partners	100
Breast Cancer (Single Point)	Bayer Diagnostics	150
Lung Cancer	BioClinical Partners	35
Ovarian Cancer	BioClinical Partners	35
Colorectal Cancer	BioClinical Partners	35
Benign Breast Disease	BioClinical Partners	46
Total		501

Table 6. Summary of Patient Samples Used In The Clinical Concordance Evaluation.

Normal Range. The range of values for normal specimens was determined for all methods by calculation of the mean, median, standard deviation, range, mean + 1.96 x SD, and 97.5th percentile of CA 15-3™ or BR 27.29 assay values in 199 normal healthy females (100 premenopausal and 99 post-menopausal).

The results of the normal range analysis are present in Table 7 and Figure 4. The mean serum CA 15-3™ or BR 27.29 values for all samples analyzed by the Immuno 1 CA 15-3™ Assay, the Biomira TRUQUANT® BR™ RIA and the Centocor CA 15-3™ RIA were 17.1 U/mL, 17.6 U/mL and 18.6 U/mL respectively. The mean value + 1.96 x SD gave an upper range of 32.9

U/mL for the Immuno 1 CA 15-3™ Assay, 30 U/mL for the Biomira TRUQUANT® BR™ RIA and an upper range of 33.5 U/mL for the CA 15-3™ Centocor RIA. The 97.5th percentile normal range cut-off for the Immuno 1 was 35.9 U/mL, 31.3 U/mL for the Biomira RIA and 35.6 U/mL for the Centocor RIA. As shown in Table 7 and Figure 4, there were no significant differences between the three assays in the CA 15-3™ or BR 27.29 mean, median, or cutoff ranges for the entire collection of specimens. Notably, all three methods denoted modest differences in the mean, median and cutoff ranges for the pre- versus the post-menopausal samples (Table 7). Overall, the means and ranges of the Biormira RIA assay values (Figure 4) were very similar to those generated by the Immuno 1 CA 15-3™ assay and, thereby demonstrate the concordance of the two methods in *MUC -1* gene product quantitation.

	PRE-MENOPAUSAL			POST-MENOPAUSAL			TOTAL		
	IMMUNO 1	CENTOCOR	BIOMIRA	IMMUNO 1	CENTOCOR	BIOMIRA	IMMUNO 1	CENTOCOR	BIOMIRA
n	100	100	100	99	99	99	199	199	199
Mean	14.1	15.5	15.7	20	21.7	19.6	17.1	18.6	17.6
Median	13.9	14.5	15	19.2	21	18.7	14.9	16.8	16.4
Range	4.2-32.4	4.4-31.7	6.6-34.7	5.9-43.6	8.1-43.2	8.9-36	4.2-43.6	4.4-43.2	6.6-36
Mean +1.96 SD	25.1	25.7	25.9	37.8	38.2	32.3	32.9	33.5	30
97.5th Percentile	27	27.1	25.7	38.9	41.5	33.2	35.9	35.6	31.3

Table 7. Normal Range Cut-off Analysis Results (U/mL)

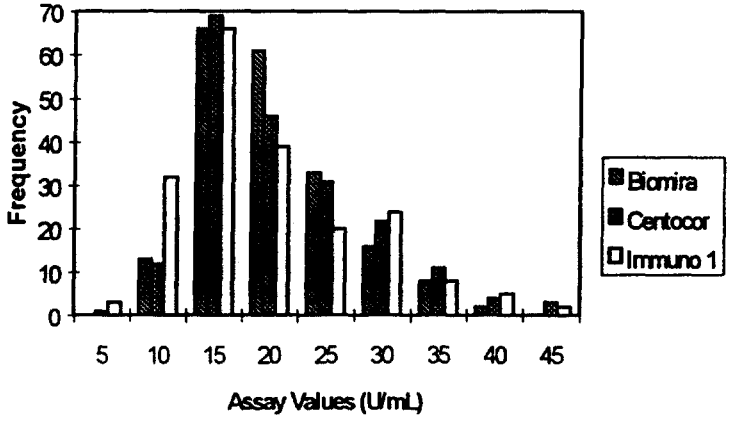


Figure 4. Distribution of Normals

Method Concordance. In order to compare the values obtained from serum samples analyzed by the Immuno 1 CA 15-3™ Assay with Biomira TRUQUANT® BR™ and Centocor CA 15-3™ RIA derived values, a correlation study using 501 female serum samples was performed. The 501 serum samples consisted of approximately 199 normal samples (pre- and post-menopausal), 150 single point breast cancer specimens, 35 lung cancer specimens, 35 ovarian cancer specimens, 35 colorectal cancer specimens, and 46 benign breast disease samples, and one unknown disease sample with elevated an CA 15-3™ and BR 27.29 value. Ordinary least squares linear regression statistics were used to compare the assay values obtained.

The calculated statistics for sample results that were within the linear range (i.e. standard curve range) of the Immuno 1 Assay, Biomira RIA and the Centocor RIA are presented in Table 8. These results demonstrate that the quantitation of CA 15-3™ and CA 27.29 assay values by the methods is concordant and equivalent.

	CA 15-3™ CORRELATION (STANDARD CURVE RANGE SAMPLES ONLY)				
	ORDINARY LINEAR LEAST SQUARES REGRESSION				
	SLOPE	INTERCEPT	N	R	$S_{Y.X}$
Immuno 1 vs. Biomira	0.852	3.48	498	0.93	11.40
Immuno 1 vs. Centocor	0.791	3.23	499	0.96	9.26

Table 8. Correlation of All Methods (Standard Curve Range Samples Only)

Conclusions from Method Comparison Studies. The results of this comparative clinical analysis of serum assay values in a panel of healthy subjects, and patients with non-malignant and malignant diseases clearly demonstrates the concordance of the Immuno 1 CA 15-3™ Assay with the Biomira TRUQUANT® BR™ RIA results. Normal reference ranges were essentially equivalent, and correlation statistics showed good concordance between the Immuno 1 and the predicate device.

REAGENT STABILITY TESTING

Shelf-Life Stability Testing. Reagents were subjected to temperature stress 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 tested. At each timepoint, the sensitivity of the calibrators, as well as the recovery of controls and the Medical Decision Pool (a serum based control material manufactured at Bayer Corporation and used internally for routine quality control) were monitored.

A shelf-life of 18 months is recommended for CA 15-3™ reagents. The data for the four lots (Experimental 1 & 2 ; Trials 1 & 2) indicated that the product will still be well within the product stability specifications at 18 months. In all cases, at the latest time point tested, calibrator sensitivity (2-8°C) and control recoveries were within acceptable limits.

On-System Stability Testing. Testing of the Experimental lots was performed over a 32 day period every 26 weeks for the entire shelf life of the reagent. Five packs of reagents were opened at the beginning of each 32 day study. One reagent pack was tested at each time point (days 0, 7, 14, 21, and 32) for each study performed at weeks 0, 26, 52, 78 and 104. During the testing of the Trial reagent lots, one reagent pack remained on the system for the duration of the 32 day study and was performance tested at the defined time points (Days 0, 7, 14, 21, and 32). Additionally, a fresh reagent pack, maintained at 2-8°C was run at each time point in parallel with the test reagent pack as a control. At each timepoint, the sensitivity of the calibrators, as well as the recovery of controls and the Medical Decision Pool were monitored against both the day 0 and individual time point calibration curves (Day 7, 14, 21, and 32). On-system stability studies on four lots of Immuno 1 CA 15-3™ Assay reagents support an on-system stability recommendation of 30 days. The specifications for minimum calibrator sensitivity and control recovery were met throughout all studies.

Shipping Stabilty Testing. Three lots of reagents were subjected to 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 time points. The sensitivity of the calibrators and the recovery of controls and the Medical Decision Pool 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 CA 15-3™ assay reagents support the requirement of refrigerated (2-8°C) shipping of these reagents.

Labeling. Reagent stability is summarized in the Immuno 1 CA 15-3™ method insert sheet. Expiration dates are also indicated on the labels of each reagent kit.

CALIBRATOR STABILITY TESTING

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 <-10°C were tested in parallel with the stressed calibrators at all timepoints. At each testpoint, recovery of the BioRad controls and the Medical Decision Pool 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. Concentrations were determined for calibrators stored at elevated temperatures using a calibration curve generated with calibrators stored at -80°C. Two lots have completed 52 weeks of testing. The third lot, Trial 3, has completed 26 weeks of testing. For all lots tested, recoveries of calibrators stored at the elevated temperatures exceeded the specification of ± 13%, while recoveries of control materials remained within specifications for the duration of the testing. These results support the conclusion that the Immuno 1 CA 15-3™ calibrators are stable for 12 months when stored at -10°C or below.

Open Vial Stability Testing. The open vial stability testing protocol specifies the use of one set [or pool if necessary] of opened calibrators throughout the 5 week study. After opening, the 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 the Medical Decision Pool, run as unknowns at 0, 1, 3 and 5 week 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.

Two lots of calibrators were tested for open vial stability. A single five week study has been completed on each lot. Recoveries of the Medical Decision Pool were determined at week 0, 1, 3, and 5 using both the week 0 and timepoint calibration curves. At all timepoints tested, using either calibration curve, control recoveries remained within specifications. These results support the claim that opened Immuno 1 CA 15-3™ assay calibrators are stable for 30 days when stored at 2-8°C.

Shipping Studies The three lots of calibrators were subjected to three freeze/thaw (-20°C /2-8°C) cycles, and then were tested versus the -80°C stored 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 until tested.

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. The recoveries of calibrators subjected to cyclical freezing and thawing did not exceed the percent difference specification following one, two, or three freeze-thaws. These results support the claim that the Immuno 1 CA 15-3™ assay calibrators are stable for up to three cycles of freezing and thawing.

Labeling. Calibrator stability is summarized in the Immuno 1 CA 15-3™ method insert sheet. Expiration dates are also indicated on the labels of each reagent kit.

MULTI-SITE CLINICAL STUDIES

Introduction. To assess the safety and effectiveness of the Bayer Immuno 1™ CA 15-3™ Assay, multi-site clinical studies were performed with the following objectives:

1. To evaluate the Bayer Immuno 1 CA 15-3™ Assay as a quantitative measure of episialin (DF3 determinant) in human serum during the course of disease and therapy for use as an adjunctive test in the management of breast cancer patients with metastatic disease. The null hypotheses, H_0, tested is: Changes in Immuno 1 CA 15-3™ Assay values over time are consistent with changes in clinical condition in < 60% of breast cancer patients with metastatic disease monitored longitudinally with the Immuno 1 CA 15-3™ Assay.

2. To evaluate the Bayer Immuno 1 CA 15-3™ Assay as a quantitative measure of episialin in human serum for use as an adjunctive test to indicate local or distant metastasis in previously treated Stage 2 or Stage 3 breast cancer patients who have no evidence of disease following therapy. The null hypotheses, H_0, tested is: Longitudinal Immuno 1 CA 15-3™ Assay values will show statistically significant increases prior to or in conjunction with clinical evidence of breast cancer recurrence in < 40% of those patients that recur.

3. To estimate the upper limit of normal of episialin in premenopausal and postmenopausal healthy females as measured by the Immuno 1 CA 15-3™ Assay.

4. To estimate the maximum level of serial variability in Immuno 1 CA 15-3™ assay values in the normal healthy female population.

5. To estimate the clinical sensitivity of the Immuno 1 CA 15-3™ Assay in patients with breast cancer Stages 1-4.

6. To estimate the clinical specificity of the Immuno 1 CA 15-3™ Assay in patients with benign breast diseases, other non-malignant diseases, non-breast cancers, in pregnant women, and in normal healthy individuals.

The principal investigators and the investigational sites that conducted these studies were:

Dr. Roy Beveridge, Fairfax - Prince William Hematology Oncology Associates, 3289 Woodburn Road #230, Annandale, Virginia 22003

Dr. Daniel W. Chan, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, Maryland 21287-7065

Dr. Herbert A. Fritsche, M .D. Anderson Cancer Center, 1515 Holcome Boulvard, Houston, Texas 77030

Dr. Morton K. Schwartz, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Dr. Alan H. B. Wu, Hartford Hospital, 80 Seymour Street, Hartford, Connecticut 06102

Management Value of the Bayer Immuno 1™ CA 15-3™ Assay for Monitoring the Disease Course of Metastatic Breast Cancer Patients. The clinical utility of the Immuno 1 CA 15-3™ Assay as an adjunctive test for monitoring the course of disease and therapy in patients with metastatic breast cancer was evaluated. The Immuno 1 CA 15-3™ Assay was used to determine CA 15-3™ reactive determinant values in serial serum specimens collected from 158 metastatic breast cancer patients enrolled at four clinical trial sites. At least four serial specimens were evaluated for each patient including one specimen prior to a treatment cycle and ≥ 3 specimens during or following treatment. The monitoring periods ranged from 6 months to one year. The medical history was collected for each enrolled patient and included age, smoking history,

therapeutic interventions, physician evaluations, and diagnostic testing as guides for changes in clinical status.

The use of the Immuno 1 CA 15-3™ Assay for monitoring the course of disease and therapy in metastatic breast cancer patients was demonstrated in this study. Serial CA 15-3™ Assay testing demonstrated sensitivity for detecting changes in clinical status in 64% (98/152) of the patients studied. The positive predictive value of a change in serial assay values of greater than or equal to 25% was 95%. Table 9 compares changes in clinical status of the metastatic breast cancer patients to changes in Immuno 1 CA15-3™ Assay values.

TABLE 9. EVALUATION OF METASTATIC BREAST CANCER PATIENTS MONITORED FOR CHANGES IN DISEASE STATUS

SERIAL CHANGES IN IMMUNO 1 CA15-3™ ASSAY RESULTS COMPARED TO DISEASE STATUS

		CLINICAL STATUS CHANGE DURING THE PROFILE PERIOD		
		YES	NO	TOTAL
Increase ≥ 25% with clinical progression or decrease ≥ 25% with clinical response	YES	98	5	103
	NO	54	1	55
	TOTAL	152	6	158

Sensitivity: 64% (98/152)

Predictive value of a 25% or greater change in assay values: 95% (98/103)

These results demonstrate the reliability of serial Immuno 1 CA 15-3™ Assay determinations as an indicator of clinical course in metastatic breast cancer patients and support the clinical utility of

the Immuno 1 CA 15-3™ Assay for use in monitoring metastatic breast cancer patients during the course of disease and therapy.

Management Value of the Bayer Immuno 1™ CA 15-3™ Assay for Early Detection of Recurrence in Previously Treated Stage II or Stage III Breast Cancer Patients who are Clinically Free of Disease. The clinical utility of the Immuno 1 CA 15-3™ Assay as an adjunctive test to indicate local or distant metastasis in previously treated Stage 2 and Stage 3 breast cancer patients with no clinical evidence of disease following therapy was evaluated. Objective measurement of recurrence (CT scans, MRI, X-rays, bone scans, liver scans, physical examination) was used as an endpoint for patient monitoring. A total of 182 Stage 2 and Stage 3 breast cancer patients, previously treated for their disease, were enrolled into this study during a period of no clinical evidence of disease. Seventeen patients demonstrated recurrence to metastatic disease. The ability of longitudinal Immuno 1 CA 15-3™ assay results to accurately reflect the clinical course of disease for these 17 patients was evaluated and compared to the Biomira TRUQUANT® BR™ RIA test results for 15 of these patients. In addition, the ability of longitudinal Immuno 1 CA 15-3™ Assay results to accurately reflect the clinical status of 120 patients (with at least 4 specimen results) that remained disease free during the profile period (as determined by CT scans, MRI, X-rays, bone scans, liver scans, physical examination) was evaluated and compared to the Biomira TRUQUANT® BR™ RIA test results for 103 of these patients. These analyses used 25% as the minimum level of increase (above a disease free baseline mean) indicative of recurrence for Immuno 1 CA 15-3™ Assay results and 45% for Biomira results. These percentages were determined in this study from analysis of the variance of serial assay results for healthy women. Longitudinal sensitivity and specificity for both assays considering both patients that recurred and those that remained disease free is presented in Table 10 for the Immuno 1 CA 15-3™ Assay and in Table 11 for the Biomira assay.

TABLE 10. BREAST CANCER PATIENTS MONITORED FOR RECURRENCE

SERIAL TRENDS OF IMMUNO 1 CA 15-3™ ASSAY RESULTS COMPARED TO DISEASE STATUS

		BREAST CANCER RECURRENCE		
		YES	NO	TOTAL
Increase ≥ 25% with a second specimen confirming the ≥ 25% increase	YES	9	5	14
	NO	8	115	123
	TOTAL	17	120	137

Sensitivity:	53%
Specificity:	96%
Predictive Value of Positive:	64%
Predictive Value of Negative:	93%

TABLE 11. BREAST CANCER PATIENTS MONITORED FOR RECURRENCE

SERIAL TRENDS OF BIOMIRA ASSAY RESULTS COMPARED TO DISEASE STATUS

		BREAST CANCER RECURRENCE		
		YES	NO	TOTAL
Increase ≥ 45% with a second specimen confirming the ≥ 45% increase	YES	8	9	17
	NO	7	94	101
	TOTAL	15	103	118

Sensitivity:	53%
Specificity:	91%
Predictive Value of Positive:	47%
Predictive Value of Negative:	93%

Eight patients in this study did not demonstrate rising Immuno 1 CA 15-3™ Assay results prior to or at the time of clinical detection of recurrence. This is also consistent with the previous study reported in the Biomira product labeling where there were 11 false negatives among 26 patients that recurred.

The Immuno 1 CA 15-3™ Assay demonstrates equivalent longitudinal sensitivity (53%) to the Biomira RIA Assay and improved longitudinal specificity of 96% compared to 91% for Biomira. Immuno 1 serial trends agreed with Biomira serial trends for approximately 90% of patients monitored for recurrence. Agreement was found for 14/15 (93%) of patients that recurred and 91/103 (88%) of patients that remained disease free.

Distribution of Immuno 1 CA 15-3™ Assay Results and Concordance with Clinical Condition. The Immuno 1 CA 15-3™ Assay was used to determine the distribution of CA 15-3™ assay concentrations in 216 premenopausal and 202 postmenopausal healthy women, 81 pregnant women, 114 patients with benign breast disease, 180 patients with other benign diseases, 318 patients with breast cancer in various stages of disease, and 210 patients with non-breast cancer. The distribution of Immuno 1 CA 15-3™ Assay values is presented in Table 12.

The reference interval, defined as the lowest concentration which exceeds the values for 95% of the serum CA 15-3™ assay measurements in healthy women, was determined from Immuno 1 CA 15-3™ Assay values from 216 premenopausal and 202 postmenopausal women. The age distribution was from 18 to 97 years. For the total population of 418 women, the mean CA 15-3™ assay value was 18.3 U/mL and the 95th percentile was 34.8 U/mL. An upper limit of normal of 35 U/mL for the Immuno 1 CA 15-3™ Assay is consistent with the previous literature and with the upper limit of normal reported for the predicate device.

Table 12. Distribution of Immuno 1 CA 15-3™ Assay Concentrations

PATIENT POPULATION	N	0 to 35 U/mL N (%)	>35 to 100 U/mL N (%)	>100 to 200 U/mL N (%)	>200 U/mL N (%)	Median U/mL
HEALTHY						
Premenopausal	216	216 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	15.1
Postmenopausal	202	182 (90.0%)	20 (10%)	0 (0.0%)	0 (0.0%)	18.9
TOTAL HEALTHY	418	398 (95.2%)	20 (4.8%)	0 (0.0%)	0 (0.0%)	16.3
PREGNANT	81	78 (96.3%)	3 (3.7%)	0 (0.0%)	0 (0.0%)	18.3
BENIGN BREAST	114	109 (95.6%)	5 (4.4%)	0 (0.0%)	0 (0.0%)	20.4
NON-BREAST BENIGN						
Autoimmune/Inflamm.	55	45 (81.8%)	10 (18.2%)	0 (0.0%)	0 (0.0%)	22.6
GI/Pancreas/Gallbladder	51	51 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	16.7
Gynecological	13	12 (92.3%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	15.9
Urinary	7	5 (71.4%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	15.6
Cardiopulmonary	26	21 (80.8%)	5 (19.2%)	0 (0.0%)	0 (0.0%)	16.0
Liver	21	19 (90.5%)	2 (9.5%)	0 (0.0%)	0 (0.0%)	15.8
Benign Other	7	6 (85.7%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	14.9
TOTAL non-breast benign	180	159 (88.3%)	21 (11.7%)	0 (0.0%)	0 (0.0%)	—
NON-BREAST CANCER						
Bladder/Urinary	22	21 (95.5%)	0 (0.0%)	0 (0.0%)	1 (4.5%)	21.1
Cervix	18	15 (83.3%)	3 (16.7%)	0 (0.0%)	0 (0.0%)	18.1
Colorectal/Stomach	43	39 (90.7%)	4 (9.3%)	0 (0.0%)	0 (0.0%)	19.5
Liver	10	9 (90.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	19.7
Lung	32	23 (71.9%)	6 (18.8%)	0 (0.0%)	3 (9.4%)	25.2
Ovary	45	27 (60.0%)	12 (26.7%)	3 (6.7%)	3 (6.7%)	29.8
Pancreas	18	12 (66.7%)	2 (11.1%)	4 (22.2%)	0 (0.0%)	22.0
Uterus	13	9 (69.2%)	3 (23.1%)	0 (0.0%)	1 (7.7%)	24.7
Other Ca.	9	7 (77.8%)	2 (22.2%)	0 (0.0%)	0 (0.0%)	24.9
TOTAL non-breast cancer	210	162 (77.1%)	33 (15.7%)	7 (3.3%)	8 (3.8%)	23.3
BREAST CANCER						
Stage 1	71	55 (77.5%)	11 (15.5%)	3 (4.2%)	2 (2.8%)	23.0
Stage 2	79	65 (82.3%)	8 (10.1%)	0 (0.0%)	6 (7.6%)	23.2
Stage 3	68	42 (61.8%)	18 (26.5%)	4 (5.9%)	4 (5.9%)	26.6
Stage 4	98	29 (29.6%)	33 (33.7%)	17 (17.3%)	19 19.4%)	56.7

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 15-3™ 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 serial Immuno 1 CA 15-3™ Assay values for monitoring the course of disease and therapy in metastatic breast cancer patients, and for early detection of recurrence in previously treated Stage II or Stage III breast cancer patients who are clinically free of disease. The correlations between Immuno 1 CA 15-3™ Assay values and disease course demonstrate that this assay may be used in conjunction with other clinical indicators to monitor success or failure of therapy for metastatic disease and to signal possible recurrence to malignant disease.

Substantial Equivalence. The method concordance studies confirm the substantial equivalence of the Immuno 1 CA 15-3™ Assay with the Biomira TRUQUANT® BR™ RIA predicate device. There is a high degree of correlation between the Immuno 1 and Biomira TRUQUANT® BR™ specimen values. Multi-site clinical studies demonstrated longitudinal sensitivity and specificity for predicting recurrence of disease are equivalent for the two tests. Therefore, based upon the analytical and clinical concordance established in these studies, the Bayer Immuno 1 CA 15-3™ Assay and the Biomira TRUQUANT® RIA are equivalent with respect to method performance, clinical utility and device safety and effectiveness.

Cited References

1. Moghadam AF, and Steiber P. *Sensible Use of Tumor Markers.* Jurgen Hartmann Verlag GMBH. 1993.

2. Siddiqui J, Abe M, Hayes D, Shani E, Yunis E, Kufe D. Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma antigen. *Proc Natl Acad Sci. 85:* 2320-2323 (1988).

3. Gendler SJ, Lancaster CA, Taylor-Papadimitriou, Duhig T, *et a/.* Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem. 265:*15285-15293 (1990).

4. Hilkens J, Ligtenberg MJL, Vos HL, Litvinov SV. Cell membrane associated mucins and their adhesion-modulating property. *TIBS 17:* 359-363 (1992).

5. Gendler SJ, Spicer AP, Lalani E-N, *et al.* Structure and biology of a carcinoma-associated mucin, *MUC- 1. Amer Rev Resp Dis. 144:* S42-47 (1991).

6. Ho JJL, Kim YS. Serological pancreatic tumor markers and the MUC-1 apomucin. *Pancreas 9:* 674-691 (1994).

7. Ligtenberg MJL, Kruijshaar L, Buijs F, van Meijer M, Litvinov SV, Hilkens J. Cell-associated episialin is a complex containing two proteins derived from a common precursor. *J Biol Chem. 267:* 6171-6177 (1992).

8. Ligtenberg MJL, Buijs F, Vos HL, Hilkens J. Suppression Of Cellular Aggregation by High Levels of Episialin. *Cancer Res. 52:* 2318-2324

9. Ho JJL, Kim YS. Do mucins promote tumor cell metastasis? *Int J Oncol. 1995.* In Press.

10. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma 3:* 223 (1983).

11. Perey L, Hayes DF, Maimonis P, Abe M, O'Hara C, Kufe DW. Tumor selective reactivity of a monoclonal antibody prepared against a recombinant peptide derived from the DF3 human breast carcinoma-associated antigen. *Cancer Res. 52:* 2563-2568 (1992).

12. Sekine H, Ohno T, Kufe DW. Purification and characterization of a high molecular weight glycoprotein detectable in human milk and breast carcinomas. *J Immunol 135:* 3610-3615 (1985).

13. van Dalen A. Tumour markers in breast cancer. *Ann Chirurgiae Gynecol. 78:* 54-64 (1989).

14. Stieber P, Diergarten K, Eiermann W, Albiez M, Fateh-Moghadam A. CA 15-3: Evaluation and clinical value in breast carcinomas compared with CEA and TPA. in *New Tumor Markers And Their Monoclonal Antibodies.* Klapdor R, ed. Thieme Verla.g, Stuttgart. 1981. pages 46-51.

15. Dnistrian AM, Schwartz MK, Greenberg EJ, Smith CA, Schwartz DC. CA 15-3 and carcinoembryonic antigen in the clinical evaluation of breast cancer. *Clin Chem Acta 200:* 81-94 (1991).

16. Tondini C, Hayes DF, Gelman R, Henderson IC, Kufe DW. Comparison of CA 15-3 and carcinoembryonic antigen in monitoring the clinical course of patients with metastatic breast cancer. *Cancer Res. 48:* 4107-4112 (1988).

17. Safi F, Kohler I, Rottinger E, Beger H-G. The value of the tumor marker CA 15-3 in diagnosing and monitoring breast cancer. *Cancer 68:* 574-582 (1991).

18. Hayes DF, Zurawski VR, Kufe DW. Comparison of circulating CA 15-3 and carcinoembryonic antigen levels in patients with breast cancer. *J Clin Oncol. 4:* 1542-1550 (1986).

19. O'Dwyer PJ, Duffy MJ, O'Sullivan F, McDermott E, Losty P, O'Higgins, NJ. CEA and CA 15-3 in primary and recurrent breast cancer. *World J Surg. 14:*562-566 (1990).

20. Bombadieri E, Pizzichetta M, Veronisi P, et al. CA 15-3 determination in patients with breast cancer: clinical utility for the detection of distant metastases. *Eur J Cancer 29A:* 144-146 (1993).

21. Allard WJ, Neaman IE, Very DL, Boyer C, Daly L, O'Briant KS, Yeung KK, Bast RC Jr. Nonspecific cross-reacting antigen 50/90 as a member of a cancer biomarker panel detects breast cancer recurrence with a lead time of 5-6 months. *Cancer Res. 36*: 211 (1995).

22. Bast RJ, Knauf S, Epenetos A, Dhokia B, Daly L, Tanner M, Soper J, *et al.* Coordinate elevation of serum markers in ovarian cancer but not in benign disease. *Cancer 68:* 1758-1763 (1991).

23. Jacobs IJ, Rivera H, Oram DH, and Bast RJ. Differential diagnosis of ovarian cancer with tumor markers CA 125, CA 15-3, and TAG 72.3. *Br. J. Obstet. Gynaecol. 100:* 1120-1124 (1993).

24. Lan MS, Bast RJ, Colnaghi MI, Knapp RC, Colcher D, Schlom J, and Metzgar RS. Co-expression of human cancer-associated epitopes on mucin molecules.

Int. J. Cancer 39: 68-72 (1987).

25. Tannock IF, Boyd NF, Debou G, et al. A randomized Trial of Two Dose Levels of Cyclophosphamide, Methotrexate, and Fluorouracil Chemotherapy For Patients with Metastatic Breast Cancer. *J. Clin Oncol. 6:* 1377 (1988).

26. Salivert J, Cowan K, et al. The Pharmacology and Clinical Use of Methotrexate. *N. Eng J Med 309:* 1094, (1983).

27. Medical Economics Staff, Physicians' Desk Reference, 50th Ed., 1996, Montvale, N.J.: Medical Economics Co.

28. Olsen, EA. The Pharmacology of Methotrexate. *J Am Acad Dermatol 25:* 306 (1991).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

DEC - 1 1997

Gabriel J. Muraca, Jr.
Manager, Regulatory Affairs
BAYER CORPORATION
511 Benedict Avenue
Tarrytown, NY 10591-5097

Re: K964703
Trade Name: Bayer Immuno 1 TM CA 15-3TM Assay
Regulatory Class: II
Product Code: MOI, 82
Dated: November 21, 1996
Received: November 22, 1996

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 requirements, 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

000156

510(k) Number (if known): K964703

Device Name: Bayer Immuno 1™ CA 15-3™ Assay

Indications For Use:

The Bayer Immuno 1™ CA 15-3™ Assay is an in vitro, diagnostic, solid phase immunoassay intended to quantitatively measure CA 15-3 Assay values in human serum on the Bayer Immuno 1 system. When used in conjunction with other clinical and diagnostic procedures, serial testing with the CA 15-3 Assay is useful for monitoring the course of disease and therapy in metastatic breast cancer patients, and for detection of recurrence in previously treated Stage II, with greater than 2 positive lymph nodes, or Stage III breast cancer patients.

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

Concurrence of CDRH, ~~Office~~ of Device Evaluation (ODE)

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K964703

Prescription Use ✓ OR Over-The-Counter Use ____
(Per 21 CFR 801.109)

Optional Format 1-2-96)