SEP 2 9 2000

510(k) SUMMARY
FOR THE
ONCOGENE SCIENCE DIAGNOSTICS, INC.
MANUAL HER-2/NEU MICROTITER ELISA

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K994112

During the period since the original submission, Oncogene Science Diagnostics, Inc. was acquired by Bayer Diagnostics, Tarrytown, NY (12/1/99) and became Oncogene Science, a part of Bayer Diagnostics. Throughout this document, Oncogene Science will be referred to as Oncogene Science Diagnostics.

1. GENERAL INFORMATION

Trade Name: Oncogene Science Diagnostics, Inc. (OSDI)
Manual HER-2/neu Microtiter ELISA

Classification Name: Tumor-Associated Antigen Immunological Test Systems

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Date

In this 510(k) premarket application and notification, the performance and clinical safety and effectiveness of the Oncogene Science Diagnostics Inc. (OSDI) HER-2/neu ELISA for the management and monitoring of metastatic breast cancer patients with initial elevated HER-2/neu has been established by external clinical studies in the target population of longitudinal metastatic breast cancer patients and by comparison to accepted diagnostic procedures in accordance with the "Guidance Document For Submission of Tumor Associated Antigen Premarket Notifications, 510(k), to the FDA". Clinical evaluations of the HER-2/neu ELISA at two US clinical trial sites demonstrated clinical safety and effectiveness. Non-clinical studies indicate this assay is a stable, reproducible, highly specific and sensitive assay in which serum components and therapeutic agents do not interfere. Together these studies validated clinical performance characteristics and the comparison to accepted diagnostic procedures.

The HER-2/neu growth factor receptor encodes a full length HER-2/neu protein (p185) that is overexpressed in breast cancer cells. The extracellular domain (ECD) of this receptor (p97-115kDa) is shed into the serum of healthy women and is elevated above normal levels in women with metastatic breast cancer. Many reports indicate that monitoring the HER-2/neu serum levels has clinical utility in the management of women with metastatic breast cancer. The OSDI HER-2/neu Manual Microtiter ELISA accurately measures HER-2/neu ECD in serum.

2. INDICATIONS FOR USE

The OSDI HER-2/neu ELISA is an *in vitro* diagnostic assay intended to quantitatively measure the extracellular domain of HER-2/neu protein in human serum. HER-2/neu values obtained may be used in the follow-up and monitoring of patients with metastatic breast cancer whose initial serum level of HER-2/neu is 15 ng/ml or greater. HER-2/neu values should be used in conjunction with information available from clinical and other

diagnostic procedures in the management of breast cancer. The clinical utility of serum measurement of HER-2/neu as a prognostic indicator for early detection of recurrence and in the management of patients on immunotherapy regimens has not been fully established.

3. DEVICE DESCRIPTION

Description of the Method: The HER-2/neu ELISA is a sandwich enzyme immunoassay which utilizes two monoclonal antibodies to quantitate the extracellular domain (ECD) of the HER-2/neu protein in serum. The initial report describing the assay was published in 1991 (Carney, W. P., et al., Journal of Tumor Marker Oncology, 6(2): p. 53-72). Both the capture and detector anti-HER-2/neu monoclonal antibodies specifically bind the ECD of the HER-2/neu protein. The capture antibody (NB-3) has been immobilized on the interior surface of the microtiter plate wells. To perform the assay, an appropriate volume of serum is incubated in the coated well to allow binding of the antigen by the capture antibody. The captured HER-2/neu ECD is then reacted with a different anti-HER-2/neu antibody designated TA-1. The TA-1 antibody is biotinylated. The detection of the ECD of HER-2/neu is with a streptavidin horseradish peroxidase conjugate, which then catalyzes the conversion of the chromogenic substrate o-phenylenediamine into a colored product. The colored reaction product is quantitated by spectrophotometry (read absorbance at 490 nm) and is related to the amount of the HER-2/neu ECD in the serum sample. Six prepared HER-2/neu standards (0, 2.5, 7.5, 15, 25 and 35 ng/ml) allow construction of a standard curve for subsequent quantification of HER-2/neu in serum samples. Also available from OSDI is a set of HER-2/neu controls (10-CVX) designed to be used in conjunction with the ELISA for quality monitoring of assay performance. This control set consists of three (3) control levels at 2.9, 9.1 and 24.0 ng/mL HER-2/neu protein.

4. SUMMARY OF STUDIES

Non-clinical studies were carried out to validate the performance of the method according to the protocol entitled "Protocols for Non-Clinical Performance Evaluation of the OSDI HER-2/neu Microtiter ELISA". Protocols were performed at OSDI, Cambridge, Massachusetts. These studies included evaluation of interfering substances, cross-reactivity, heterophilic antibodies, HAMA interference, Herceptin® interference, sample linearity, parallelism (sample dilution), hook effect, reproducibility, spike and recovery, end-to-end variability, plate coating variability and reagent lot-to-lot variation.

The OSDI HER-2/neu ELISA was evaluated as an aid in the management of metastatic breast cancer patients with elevated serum HER-2/neu values during the course of disease and therapy. This clinical evaluation was conducted at two US clinical trial sites.

4.1 Characterization of the Antigen.

The standard antigen used in the OSDI HER-2/neu ELISA is the extracellular domain (ECD) of HER-2/neu, p105. It is derived from a recombinant NIH 3T3 mouse cell line designated 3-30. HER-2/neu p105 antigen is harvested from the cell culture serum-free conditioned medium and concentrated 10-fold. Western blot analysis shows a glycoprotein with a molecular weight range between 97 and 115 kd.

4.2 Characterization of the Antibodies

The NB-3 monoclonal antibody is used to capture the ECD and is therefore coated onto the surface of the microtiter well. The TA-1 monoclonal anti-HER-2/neu is used as the reagent to detect the HER-2/neu ECD. NB-3 is purified from tissue culture supernatant, produced in bioreactors, using Protein G+ affinity chromatography. The TA-1 antibody is purified from mouse ascites using Protein

A + affinity chromatography. Production and purification of antibodies utilize standard protocols and the antibodies are manufactured in Cambridge, MA. All lots are isotyped and characterized by SDS-PAGE, isoelectric focusing and Coomassie Blue staining prior to functional testing.

4.3 Assay Performance

4.3.1 Specificity: Interference

The recovery of HER-2/neu from patient samples was studied before and after spiking the serum samples with the potentially interfering substance. Each potential interferent was tested at concentrations that were higher than the recommended dietary or therapeutic doses. Endogenous compounds (common serum constituents) were tested at concentrations greater than those normally observed during routine clinical testing.

The OSDI HER-2/neu ELISA was performed on serum samples or pools of serum to which were added various concentrations of either triglycerides, hemoglobin, immunoglobulin, bilirubin, albumin, heparin or cholesterol. HER-2/neu values were also measured in serum samples after spiking with either an individual chemotherapeutic drug, "Over the Counter" (OTC) drug, vitamin or HERCEPTIN® (Trastuzumab), trademark of Genentech BioOncology, South San Francisco, CA. None of the potential endogenous or exogenous interferents demonstrated any significant interfering effects on HER-2/neu recovery.

4.3.2 Cross-Reactivity

Possible cross-reactions in the OSDI HER-2/neu ELISA were studied by assaying purified Human Epidermal Growth Factor Receptor (HER-1) spiked into Sample Diluent . The maximum effect seen with this cross-reactant was not significant (≤1%). Assays with cell line lysates have indicated that there is also no cross-reactivity with HER-3.

4.3.3 Heterophilic Antibodies and HAMA Interference

Heterophile antibodies possess a broad range of non-specific reactivities with other species of immunoglobulins. They include human anti-mouse antibodies (HAMA) and rheumatoid factors (RF). These antibodies may interfere with immunoassays and cause false positive or negative results. The OSDI HER-2/neu ELISA uses two mouse monoclonal antibodies, for capture and detection, but has Normal Mouse Serum (NMS) in the detector reagent to reduce this interference. A number of HAMA and rheumatoid factor positive serum samples were assayed in the HER-2/neu ELISA with significant interference for a small percentage of specimens without NMS in the detector diluent, but none when detector diluent contained NMS. HAMA and rheumatoid factors do not cause false negative or false positive results in the present assay format.

4.3.4 Linearity

To evaluate the linearity of recovered values, two controls containing different levels of HER-2/neu were combined in discreet proportions to produce five samples of defined concentrations for assay. When observed HER-2/neu concentration was plotted versus expected concentration, the slope and correlation coefficient of each data set were close to the optimum of 1, indicating linearity within the dynamic range of the assay.

4.3.5 Spike and Recovery

In order to determine the effect of sample matrix on the ability of the HER-2/neu ELISA to detect HER-2/neu, recovered dosages of spiked analyte in Sample Diluent (ideal matrix) were compared to recovered dosages of spiked analyte into five different patient sera. The total average percent recovery of all spiked serum samples in all three kit lots was 103%, indicating no effect of matrix on the ability of the ELISA to accurately measure HER-2/neu protein in serum.

4.3.6 Hook Effect (Antigen Excess)

Extremely high concentrations of HER-2/neu seen in some malignant conditions may cause a "hook effect" in certain assay formats. 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. A 526 ng/ml stock solution of p105 HER-2/neu was serially diluted six times at 1:2 and assayed. A reportable concentration was obtained only when the expected concentration of the diluted sample approaches the range of the standard curve (0-35 ng/ml). Separation of sample and reagent addition by washes prevents high-dose hook effect in this device.

4.3.7 Parallelism (Dilution Studies)

Five separate clinical samples containing elevated levels of HER-2/neu, and two Bovine Serum-based controls containing spiked HER-2/neu were serially diluted two-fold for four dilutions. Recoveries were evaluated at each of the dilution points for mean and standard deviation and were comparable for all three ELISA kit lots. Serum samples diluted in kit Sample Diluent result in accurate recovery of analyte at a range of concentrations in human samples.

4.3.8 End-to-End Variation

The effect on HER-2/neu recovery when incubation times of critical reagent steps were varied from sample to sample within a single assay run was evaluated. When times extended well beyond normal usage, some increase or decrease in recovery was observed (15%). However, the expected variance in timing during normal use by skilled individuals should fall well short of these extended times tested and recoveries should be unaffected.

4.3.9 Plate Coating Variations

In order to evaluate antibody coating variations, a human serum sample was assayed over entire plates. Serum sample recovery, and therefore antibody coating, is consistent throughout plates and kit lots with 4.7-7.6 % CV.

4.3.10 Reproducibility

Intra- and inter-assay reproducibility were evaluated at OSDI and at two clinical trial sites. Three controls and a human serum pool were assayed for reproducibility and results are shown in Tables 4.3-1 and 4.3-2. Imprecision data pooled across HER-2/neu ELISA kit lots and across assay sites showed within run CV's of 6-7% and total CV's of 10-11% for clinically relevant controls. A low control (3 ng/ml), below the lowest normal patient sample, gave higher within run and total % CV's of 10.2 and 17.7, respectively. This is well within acceptable limits for an assay of this type and for its intended use.

Table 4.3-1 Imprecision for OSDI Site, All Microtiter ELISA Lots Combined

	Number of Runs	Number of Replicates	Mean (ng/mL)	Within Run		Total	
				STD DEV	%CV	STD DEV	%CV
Control 1	45	135	24.2	0.91	3.8	1.90	7.8
Control 2	45	135	9.5	0.45	4.7	0.86	9.1
Control 3	45	134	2.9	0.22	7.8	0.30	10.6
Control 4	45	135	9.2	0.42	4.5	0.77	8.3

Table 4.3-2 Imprecision for Three Sites and Three Kit Lots Combined

	Number of Runs	Number of Replicates	Mean (ng/mL)	Within Run		Total	
				STD DEV	%CV	STD DEV	%CV
Control 1	130	385	24.5	1.46	6.0	2.62	10.7
Control 2	131	390	9.8	0.69	7.0	1.03	10.4
Control 3	131	386	3.3	0.34	10.2	0.59	17.7
Control 4	130	385	9.5	0.65	6.9	1.02	10.8

4.3.11 Sensitivity (Detection Limit)

Sensitivity of the HER-2/neu Microtiter ELISA was evaluated at OSDI and at two clinical trial sites by determining the Minimum Detectable Dose (MDD). An MDD of 1.5 ng/mL was observed when assaying 232 replicates of Sample Diluent alone in three HER-2/neu ELISA kit lots. This MDD is acceptable for the intended use of this assay, where the lowest normal patient was 4.23 ng/ml and the cutoff determined in this study for elevated HER-2/neu values was 15 ng/ml.

4.4 CLINICAL STUDIES

4.4.1 Introduction

To assess the safety and effectiveness of the **OSDI HER-2/neu Microtiter ELISA**, clinical studies were performed at two investigational sites on patient samples from four different locations. All patients were studied retrospectively. Assay values were determined for surplus serum samples which had been collected and stored (-70° C) in specimen banks prior to the study. Studies performed at OSDI have shown no evidence of changes in HER-2/neu concentration in patient samples stored at -80° C for 5 ¾ years.

4.4.2 Metastatic Patient Serial Monitoring - HER-2/neu Management Value

The clinical utility of the OSDI HER-2/neu Microtiter ELISA in monitoring metastatic breast cancer patients with initial elevated HER-2/neu was evaluated using retrospective serum. Serial serum HER-2/neu values were measured over a 6 to 12 month period in clinically evaluable patients with Stage IV breast cancer undergoing therapy. 33.7 % had initial elevated serum samples (HER-2/neu concentrations equal to or greater than 15 ng/mL). These fifty-six patients were then evaluated for correspondence of changes in their serum HER-2/*neu* values with changes in their clinical course of disease.

A visit-to-visit analysis of the study results are presented in Table 4.4-1. The changes in serum HER-2/*neu* values from one visit to the next were calculated for each patient. These changes were separated into 2 groups: Group I had changes in serum HER-2/*neu* which paralleled the clinical course of disease and Group II had changes in serum HER-2/*neu* values that did not parallel the clinical course of disease. Clinical course or status was determined by the physician. Correspondence of HER-2/*neu* changes

to clinical status were determined as follows: An increase of 20% or greater from the previous visit was reflective of disease progression. If the change is less than a 20% increase from the previous visit, this was reflective of a lack of disease progression during therapy (including responding or stable disease). Stable and responding were consolidated since both reflect effectiveness of a current therapy. The 20% criterion was derived from the longitudinal variability in normal patients (determined from the HER-2/*neu* values in six serial samples from each of 38 healthy women).

Table 4.4-1: Correspondence of Metastatic Breast Cancer Patient Disease Status with Changes in Serum HER-2/neu: Visit-by-Visit

		Change in Clinical Status		
		Pro-gression	No Pro-gression	TOTAL
Change In HER-2/*neu*	≥20% ↑	52	33	85
	<20% ↑	55	96	151
	TOTAL	107	129	236

The following figures show typical examples of changes in HER-2/neu, over time, for 2 of the 56 metastatic breast cancer patients from the clinical study. Figure 4.4-1 shows a patient whose disease is responding and Figure 4.4-2 shows a patient with progressive disease.

Figure 4.4-1: Monitoring of a 38-year-old Stage IV Breast Cancer Patient with the OSDI HER-2/*neu* ELISA. Longitudinal changes in OSDI HER-2/*neu* values correlate with changes in disease status.

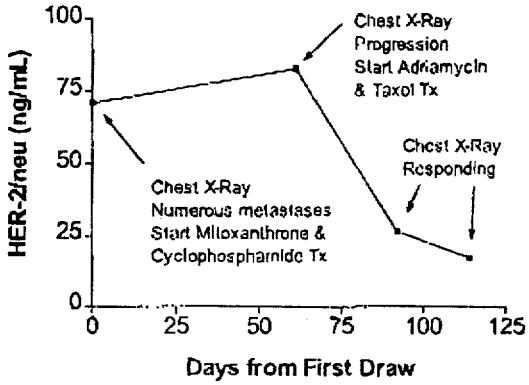
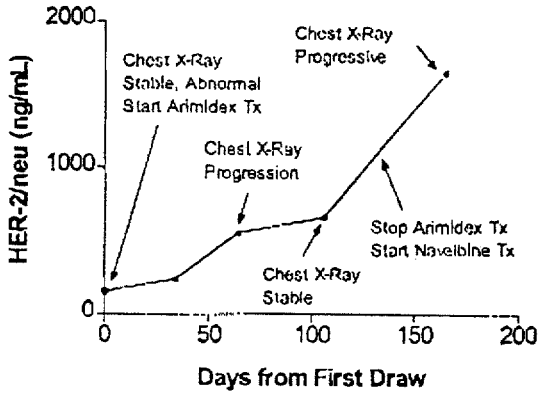


Figure 4.4-2: Monitoring of a 74-year-old Stage IV Breast Cancer Patient with the OSDI HER-2/*neu* ELISA. Longitudinal changes in OSDI HER-2/*neu* values correlate with changes in disease status.



These data support the clinical utility of the OSDI HER-2/neu Microtiter ELISA for use in management (monitoring) during the course of disease and therapy of metastatic breast cancer patients whose initial HER-2/neu levels are elevated above 15 ng/ml.

4.4.3 Distribution of HER-2/neu Concentrations; Sensitivity and Specificity

The HER-2/neu ELISA was used to estimate the clinical (cross-sectional) sensitivity in patients with breast cancer and characterize the frequency distribution of HER-2/neu ELISA values in a population of breast cancer patients by stage of disease. Specificity of the HER-2/neu ELISA was determined in patients with benign breast diseases, other non-malignant diseases, and in normal healthy individuals.

The Upper Limit of Normals in this study with the ELISA was 15 ng/ml, which was confirmed as an appropriate cutoff by ROC analysis to give 95% specificity. These two results validate the use of 15 ng/ml as the baseline for elevated HER-2/neu concentrations used in the metastatic patient serial monitoring portion of this study.

The longitudinal measurements for healthy women seen in this study showed a standard deviation of 10%. Ninety-five percent of the time, serial ELISA values should be within twice this SD. Therefore, the normal variability expected in longitudinal sample analysis would be 20%, and anything greater would be potentially indicative of changes due to cancer.

4.4.4 Conclusions from the Clinical Studies

The results of this retrospective clinical trial demonstrate that the OSDI Manual HER-2/neu Microtiter ELISA is reproducible, and is safe and effective for the management and follow-up of patients with metastatic breast cancer whose initial serum levels of HER-2/neu are 15 ng/ml or greater.

This study carefully examined the potential role of longitudinal serum HER-2/neu concentrations in the management of patients with metastatic breast cancer. The results are consistent with published reports showing HER-2/neu to be useful in the metastatic clinical setting. Data collected

from this study show that changes in serum HER-2/neu concentrations over time in initially elevated metastatic breast cancer patients reflect changes in clinical status such as response to therapy or progression of disease.

The reproducibility CV's obtained by the two clinical laboratories in the OSDI HER-2/neu ELISA fall within typical ranges for manual microtiter assays of <10% intra-assay and <15% interassay. The detection limit is acceptable for the intended use of this assay. This demonstrates that this assay should provide reliable and reproducible results when tested by different laboratories using different manufactured lots of reagents at different times.

5. CONCLUSIONS DRAWN FROM ALL THE STUDIES

Valid Scientific Evidence

The conclusions drawn from these studies are based upon valid scientific evidence. Data were gathered following a well-designed protocol, in a clinical research laboratory operating under the principles of Good Clinical Practices. Clinical data were gathered during well controlled investigations conducted by qualified experts. Patient case histories were well documented. The results of this study are comparable to literature reports of experiences with HER-2/neu assays.

Method Performance

OSDI HER-2/neu ELISA results are highly reproducible with a maximum inter-assay %CV pooled over reagent lots and clinical sites of 11% over the clinically useful range of the assay. The assay shows no interference by common serum components or by vitamins, over-the-counter drugs, chemotherapeutic agents or

HERCEPTIN®. Other performance characteristics including analytical sensitivity and specificity, cross-reactivity, linearity, and antigen excess hook effect were excellent.

Safety and Effectiveness

These clinical studies confirm the safety and effectiveness of the OSDI HER-2/neu Microtiter ELISA as an aid in the follow-up and management of metastatic breast cancer patients whose initial HER-2/neu concentration is 15 ng/ml or greater. The correspondence between HER-2/neu concentrations and the patients' clinical course of disease demonstrate that the OSDI HER-2/neu Microtiter ELISA may be used in conjunction with other clinical indicators to confirm disease progression and response to therapy in metastatic breast cancer patients with initial elevated levels of HER-2/neu.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

SEP 2 9 2000

Walter P. Carney, Ph.D.
Director
Oncogene Science
Bayer Corporation
80 Rogers Street
Cambridge, Massachusetts 02142-1168

Re: K994112
Trade Name: Oncogene Science Diagnostics, Manual HER-2/neu Microtiter ELISA
Regulatory Class: II
Product Code: NCW
Dated: June 30, 2000
Received: July 3, 2000

Dear Dr. Carney:

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 legally marketed predicate 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.

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" (21CFR 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 (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsma/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): K994112

Device Name: OSDI HER-2/neu Assay

Indications For Use:

The OSDI HER-2/neu Assay is an *in vitro*, diagnostic device intended for use in the quantitative determination of serum HER-2/neu in women with metastatic breast cancer who have an initial value of 15 ng/ml or greater. HER-2/neu values obtained may be used in the follow-up and monitoring of patients with metastatic breast cancer. HER-2/neu values should be used in conjunction with information available from clinical and other diagnostic procedures in the management of breast cancer. The clinical utility of the serum measurement of HER-2/neu as a prognostic indicator for early recurrence and in the management of patients on immunotherapy regiments has not been fully established.

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

Peter E Maffin

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K994112
Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ OR Over-the-counter Use _____
(per 21 CFR 801.109)

(Optional Format 1-2-96)