



MIS
P950011

Memorandum

Date . MAR 28

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of BIOMIRA Diagnostics, Incorporated's
TRUQUANT® BR™ RIA - Action

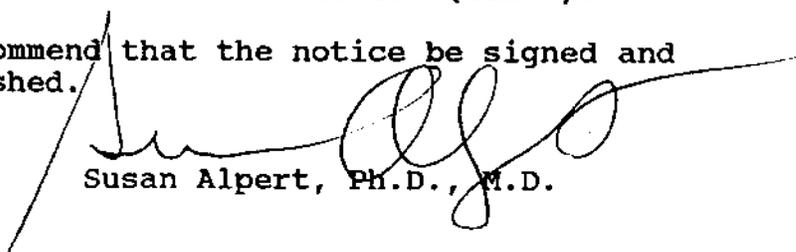
To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

- (1) a premarket approval order for the above referenced medical device (Tab B); and
- (2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.



Susan Alpert, Ph.D., M.D.

Attachments
 Tab A - Notice
 Tab B - Order
 Tab C - S & E Summary

DECISION.

Approved _____ Disapproved _____ Date _____

Prepared by Doreen Melling, CDRH, HFZ-440, 3/4/96, 594-1293

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

Biomira Diagnostics, Incorporated; PREMARKET APPROVAL OF
TRUQUANT® BR™ RIA

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application submitted by Mr. Thomas Tsakeris, Devices and Diagnostics Consulting Group, Rockville, Maryland, U.S. Representative for Biomira Diagnostics, Incorporated, 30 Meridian Road, Rexdale, Ontario, Canada, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of TRUQUANT® BR™ RIA. After reviewing the recommendation of the Immunology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on MAR 29 1996, of the approval of the application.

DATE: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file

with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated: _____.



Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

BIOMIRA Diagnostics, Inc.
c/o Thomas M. Tsakeris
Devices and Diagnostic Consulting Group
16809 Briardale Road
Rockville, Maryland 20855

MAR 29 1996

Re: P950011
TRUQUANT® BR™ RIA
Filed: February 24, 1995
Amended: April 5, April 12, May 31, August 23, August 31,
September 27, October 30, October 31, November 13, December 6,
1995; March 1, and March 7, 1996

Dear Mr. Tsakeris:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the BIOMIRA TRUQUANT® BR™ RIA. This device is an in vitro diagnostic device indicated for the quantitative determination of CA 27.29 antigen in serum or EDTA plasma of patients previously treated for stage II or stage III breast cancer. Serial testing for CA 27.29 antigen with TRUQUANT® BR™ RIA in patients who are clinically free of disease should be used in conjunction with other clinical methods used for the early detection of recurrence. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

In addition to the postapproval requirements in the enclosure, the postapproval reports must include the following information:

Results from your postapproval study designed to collect additional data on patients at the time of recurrence. Your approved protocol for this study is on file at CDRH.

Expiration dating for this device has been established and approved at 12 weeks at 2-8°C. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as

provided by 21 CFR 814.39(a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

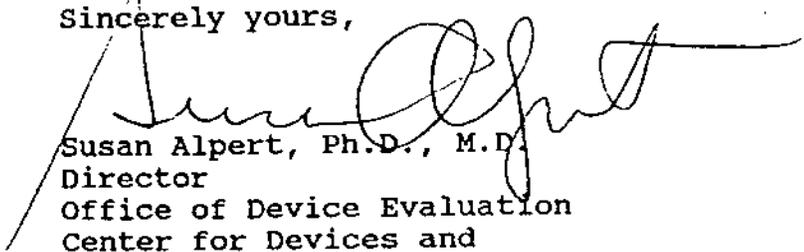
You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HF2-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter E. Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,



Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

I. GENERAL INFORMATION

Device Generic Name:

Radioimmunoassay for the measurement of CA 27.29 antigen in serum or plasma.

Device Trade Name:

TRUQUANT® BR™ RIA

Applicant's Name and Address:

BIOMIRA Diagnostics, Inc.
30 Meridian Road
Rexdale, Ontario, Canada
M9W 4Z7

U.S. Representative:

Mr. Thomas M. Tsakeris
Devices and Diagnostics
Consulting Group
16809 Briardale Road
Rockville, Maryland 20855

Premarket Approval (PMA) Number: P950011

An expedited review dated 4 April, 1995 was granted because it is believed that accurate and reliable information about the patient's disease status be available to assist the clinician. TRUQUANT® BR™ RIA device may offer significant advances in safety and effectiveness over other existing test modalities in the monitoring of treated breast cancer patients for recurrent disease.

Date of Panel Recommendation:

September 21, 1995

Date of Notice of Approval to the Applicant:

March 29, 1996

II. INDICATIONS FOR USE

TRUQUANT® BR™ Radioimmunoassay (RIA) is an *in vitro* diagnostic device indicated for the quantitative determination of CA 27.29 antigen in serum or EDTA plasma of patients previously treated for stage II or stage III breast cancer. Serial testing for CA 27.29 antigen with TRUQUANT® BR™ RIA in patients who are clinically free of disease should be used in conjunction with other clinical methods used for the early detection of recurrence.

Precautions and Warnings

Precautions:

For *in vitro* diagnostic use.

The usefulness of this device in Stage I patients has not been established.

Elevated levels of CA 27.29 are found in patients with other diseases, benign conditions and first trimester pregnancies. As is the case with all tumor markers, results from the TRUQUANT® BR™ RIA should be considered along with other clinical parameters. TRUQUANT® BR™ RIA incorporates BIOMIRA's proprietary antibody B27.29. Comparable assay results will not be obtained using other antibodies against CA 15-3 antigen. Read all safety precautions before proceeding with this test.

Warnings:

This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals and for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to regulation and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority. This kit contains radioactive material and should be stored and handled in specially designated areas. All radioactive waste must be stored in appropriately-labeled waste containers and disposed of according to local (state) and federal regulations.

Antigen for standards, controls and coated tubes are derived from human ascites or pleural effusions which have been individually tested in accordance with current serum-based FDA-cleared procedures and found to be negative for HbsAg, HCV and HIV. However, no test method can offer complete assurance that products derived from human fluids will not transmit infectious agents. Proper handling and disposal methods should be used.

Sodium azide is present in some kit components as a preservative at the final concentration of not more than 0.2 percent sodium azide can form explosive compounds when in contact with lead or copper plumbing and should always be flushed with large amounts of water. To prevent formation of toxic vapor, mixing with acidic solutions should be avoided.

TRUQUANT® BR™ RIA has been designed to minimize human anti-mouse antibody interference. However, caution should be exercised in interpreting any serum any result from *in vitro* diagnostic kits which employ murine antibodies because of the potential human anti-mouse responses resulting from usage of murine antibodies and/or their fragments *in vivo*.

Do not use kit components beyond the expiration date and do not mix reagents from kits with different lot numbers.

Avoid exposure of the reagents to excessive heat or light.

Background

Mucins are high-molecular weight glycoproteins found on the cell surface of epithelial tissues. The extent and nature of mucin glycosylation vary with tissue type and between normal and malignant cells. Some tumor-associated mucins are shed into the bloodstream and these can be quantitated by immunoassay with monoclonal antibodies.

The CA 27.29 antigen is a tumor-associated mucin found in carcinomas of the breast, ovary, pancreas, stomach, and liver and also in some nonmalignant inflammatory conditions. Serum levels of CA 27.29 antigen have been reported to correlate with clinical status, disease progression, and response to therapy in breast cancer patients.

III. DEVICE DESCRIPTION AND PRINCIPLE OF THE ASSAY

TRUQUANT® BR™ RIA is a solid-phase immunoassay which quantitates CA 27.29 antigen by competitive inhibition RIA. An ¹²⁵Iodine-labeled mouse monoclonal antibody, specific for CA 27.29 antigen, is added to antigen-coated polystyrene tubes with the specimen in the form of plasma or serum. The CA 27.29 antigen in the specimen inhibits the antibody from binding to the CA 27.29 antigen on the tube and after a subsequent wash step, bound radioactivity is determined. A calibration curve, generated from standards containing known quantities of CA 27.29 antigen, is used to determine the amount of antigen in the specimen.

IV. ALTERNATIVE PRACTICES AND PROCEDURES

Recurrence of disease in previously diagnosed breast cancer patients is currently monitored by radiographic procedures. Chest X-rays, bone and liver scintigraphy, computerized tomography, and magnetic resonance imaging are also commonly used procedures to monitor recurrence.

V. MARKETING HISTORY

TRUQUANT® BR™ RIA is marketed in Canada, Italy, Germany, UK, Argentina, South Korea, China, Austria, Turkey, Chile and Benelux. TRUQUANT® BR™ RIA has not been withdrawn from the market in any country.

VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Any adverse effects of the device on health would be indirect, since the device does not come in contact with the patient. A false-negative result could delay recognition of recurrence. In a case such as this, the patient could be adversely affected by a delay in early beneficial treatment or resumption of treatment. A false positive result could contribute to a medical decision which might cause a patient to undergo needless treatment or an unnecessary change in treatment. Risk of these events is low if the conventional "standard of care" procedures are also performed for determining recurrence of stage II and stage III breast cancer.

VII. SUMMARY OF STUDIES

1. Nonclinical Laboratory Studies

Characterization of the Antigen

CA 27.29 antigen was obtained from pleural effusions (PE's), ascites of patients with adenocarcinoma of breast or ovary, a source rich in high-molecular weight glycoproteins (mucins). This antigen is similar, if not identical to, the breast tumor-associated MUC-1 gene product, CA 27.29 antigen, as indicated by epitope mapping, inhibition of antibody binding, tracer exchange and clinical correlation studies.

Antigen was analyzed for purity (sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SDS-PAGE), and immunologic identity (Western blot).

Characterization of the Antibody

Immunogen for generation of monoclonal antibody (MAB) B27.29 was derived from ascites of patients with ovarian carcinoma and chocolate ovarian cysts. Clones were screened for reactivity of antibody with both immunogens and for inhibition of antibody binding by sera from patients with breast or ovarian carcinoma. Hybridomas were recloned twice, assessed for microbial contamination and characterized with respect to growth properties (doubling times, saturation density, plating efficiency), stability of antibody secretion and propensity for *in vivo* antibody production.

MAB B27.29 was purified by Protein A chromatography and subjected to biochemical and immunological characterization. MAB B27.29 has been isotyped as IgG₁, kappa. Epitope mapping indicated B27.29 recognizes a core peptide similar to that defined by other anti-MUC-1 antibodies.

Assay Performance Studies

1. Reproducibility Studies

A reproducibility panel comprised of a normal sample and a dilution series of pooled sera from breast cancer patients, adjusted to contain different levels of CA 27.29 antigen spanning the linear range of the standard curve was used. This panel was tested with TRUQUANT® BR™ RIA at four sites: M.D. Anderson Cancer Center, The University of Texas, Houston, TX; Bowman Gray School of Medicine, Comprehensive Cancer Center of Wake Forest University, Winston-Salem, NC; The Johns Hopkins Hospital, Baltimore, MD; and BIOMIRA Diagnostics Inc., Rexdale, Ont. Each member was tested in replicates of 20 on three separate days with one lot of TRUQUANT® BR™ RIA to assess intra-/inter-assay and inter-laboratory precision.

Intra-Assay Reproducibility

Data from replicate results on the first test day were arbitrarily chosen for calculation of intra-assay variation of samples within the assay calibration range (see **Table 1**).

Table 1: Intra-Assay Reproducibility

Panel Member	Mean (U/mL)	CV (%)			
		Site 1	Site 2	Site 3	Site 4
PM1	22	10.7	9.9	14.1	11.5
PM2	66	4.2	3.3	8.3	4.3
PM3	99	3.7	2.1	3.9	3.6
PM4	158	4.1	4.8	4.4	6.1

The intra-assay coefficient of variation (CV) was largest for the normal sample (PM1) and ranged from 10-14 percent among the four sites. The CVs for panel members containing low to high levels of CA 27.29 antigen, exhibited a lower range 2-8 percent and were relatively constant at each site.

The mean intra-assay variation for TRUQUANT® BR™ RIA was 10-14 percent for a sample negative for CA 27.29 antigen and 2-8 percent for samples from patients with breast cancer. This is acceptable for an assay of this type.

Inter-Assay Reproducibility

Mean data from replicate results over 3 test days were used to calculate inter-assay variation (See **Table 2**).

Table 2: Inter-Assay Reproducibility

Panel Member	Mean (U/mL) n=3	CV (%)			
		Site 1	Site 2	Site 3	Site 4
PM1	21	17.9	23.9	7.7	10.8
PM2	63	1.6	2.5	12.9	9.7
PM3	96	1.5	4.0	12.0	5.9
PM4	152	2.4	4.4	10.0	7.3

The inter-assay CV for PM1 was greater than for PM1-PM4, ranging from 8-24 percent among the clinical sites and BIOMIRA Diagnostics. The CVs for the other three members containing antigen spanned a lower range 2-13 percent and were relatively constant at each site.

TRUQUANT® BR™ RIA has a mean inter-assay CV of 15 percent for a serum that is negative for CA 27.29 antigen and 6 percent for samples from patients with breast cancer. This is acceptable for an assay of this type.

Inter-Laboratory Reproducibility

The mean inter-assay result for each panel member was used to compute variation among the laboratories (See **Table 3**).

Table 3: Inter-Laboratory Reproducibility

Panel Member	Mean (U/mL); n=4	CV (%)
PM1	21	5.1
PM2	63	2.9
PM3	96	4.9
PM4	152	6.1

The inter-laboratory variation for TRUQUANT® BR™ RIA with all panel members was 3-6 percent. The mean CV of 5 percent is indicative of an acceptable level of precision among various test sites.

Lot-to-Lot Reproducibility

Three combinations of components comprising distinct lots of antigen-coated tubes, standards and tracer were tested against six samples (PM1-PM4; Kit Positive Control 1, 51-99 U/mL; Kit Positive Control 2, 91-145 U/mL) falling within the linear range of the standard curve (0-200 U/mL CA 27.29 antigen). Replicates of 20 were performed and the mean kit lot results used to compute lot-to-lot variation (See Table 4). Reproducibility of test calibrators was also monitored.

Table 4: Lot-to-Lot Reproducibility

Sample	Mean (U/mL)			Mean (U/mL)	CV (%)
	Lot 1	Lot 2	Lot 3		
PM1	15	20	23	19	20.5
PM2	50	48	62	53	14.8
PM3	93	94	95	94	1.2
PM4	142	145	141	143	1.1
PC1	62	65	68	65	4.8
PC2	104	111	112	109	3.9

The serum that was negative for CA 27.29 antigen (PM1) exhibited the greatest variation among lots (CV, 21 percent), whereas sera containing low to high levels of antigen yielded lower CVs (1-15 percent). The inter-lot variation observed with standards (not tabulated) was essentially constant at 8-11 percent for the five calibrators.

TRUQUANT® BR™ RIA showed acceptable lot-to-lot reproducibility with the device controls (mean CV, 8.5 percent) and samples containing CA 27.29 antigen (mean CV, 5 percent).

2. Spike and Recovery

Normal human serum, spiked with varying amounts of CA 27.29 antigen within the linear range of the assay (0-200 U/mL), was tested for antigen recovery in three assays (See Table 5). The correlation between expected and mean observed levels was 0.99 for 5 samples and statistically significant ($P < 0.05$).

Table 5: Spike and Recovery

Spike Ratio (Ag:NHS)	Expected (U/mL)	Observed (U/mL)				% Recovery (Range)
		1	2	3	Mean	
10:90	32	30	29	35	31	94-108
20:80	48	52	44	46	47	92-107
40:60	81	80	80	81	81	99-101
60:40	113	110	123	112	115	97-109
80:20	146	143	152	151	148	98-104

TRUQUANT® BR™ RIA quantitatively determined CA 27.29 antigen within the calibration range of the assay.

3. Linearity (Specimen Dilution)

Clinical samples containing CA 27.29 antigen levels within (n = 5) and outside (n = 5) the assay calibration range were diluted 10-80 percent (after adjustment to < 200 U/mL, if necessary) and tested for recovery. With one exception, regression analysis indicated slopes were statistically not significant (P > 0.05). The recovered values for this sample however, were within the ranges observed for other specimens.

TRUQUANT® BR™ RIA exhibited assay linearity for CA 27.29 antigen determination upon sample dilution.

4. Stability

Adverse Shipping Study

Stability of TRUQUANT® BR™ RIA was determined under conditions of simulated adverse shipping. Lots packed in shipping containers were subjected to temperature stress (-20° and 37°C) and tested for performance with QC controls following return to 2-8°C. Lots stressed for 3 days were virtually unaffected and showed activity comparable to a reference kit maintained at 2-8°C.

TRUQUANT® BR™ RIA was stable for brief exposures to extremes of temperature which may be encountered during shipping.

Real-Time Study

Three lots of TRUQUANT® BR™ RIA consisting of unique components, i.e., standards, antigen-coated tubes and tracer, were monitored for stability at the recommended storage temperature (2-8°C) with QC and kit controls. Determinations within the 3 standard deviation specifications for the controls were considered indicative of acceptable stability. Data indicates antigen-coated tubes are stable for 18 months, standards for 15 months and tracer for 12 weeks.

TRUQUANT® BR™ RIA is stable at 2-8°C for duration of labeled expiration date.

5. Analytical Specificity

Studies were performed to determine the effects of physiological interfering substances (bilirubin, hemoglobin, lipids), therapeutic agents (chemical agents: Fluorouracil, Cytosan, Methotrexate, Adriamycin and Taxol/hormonal agents: Tamoxifen, Cytadren, Megace, Thadrozal, and CGS) and common medications or over-the-counter (OTC) drugs (anticoagulants: coumadin and heparin; vitamins: Vitamin E and C; Folic acid, Multivitamins, Vitamin B13, and B complex; analgesics: Tylenol, Tylenol 3, Baby aspirin and codeine) on the assay of CA 27.29 antigen by TRUQUANT® BR™ RIA. Varying concentrations of physiological substances were added to serum containing CA 27.29 antigen in mid-range of the assay calibration curve and the significance of regression coefficients determined. The recovery of antigen spiked at several concentrations into sera from patients who received chemotherapy, hormonal therapy, common and OTC drugs was compared with antigen spiked into samples from normal individuals by analysis-of-variance (ANOVA). There was no evidence of physiological, therapeutic or OTC substance interference with assay performance.

TRUQUANT® BR™ RIA can be used to quantitate CA 27.29 antigen in patients receiving common medications such as OTC drugs.

6. Analytical Sensitivity

The minimal detectable concentration of CA 27.29 antigen is defined as the concentration of antigen 2 standard deviations from the 0 U/mL standard. The mean of six assays incorporating replicate determinations (25) of the 0 U/mL standard was used to establish the analytical sensitivity. The analytical sensitivity of TRUQUANT® BR™ RIA was 7 U/mL.

2. Clinical Performance Studies

Description of Clinical Studies

TRUQUANT® BR™ RIA was evaluated in a controlled prospective clinical trial at five different clinical centers in the U.S. Studies at all sites were conducted under essentially the same protocol, under investigational review board (IRB) review and approval of the clinical protocol. Approved consent forms were used for all breast cancer patients enrolled in the study. Patient enrollment occurred after IRB approval. The study was conducted following the clinical site protocol and the testing was performed according to the TRUQUANT® BR™ RIA product insert. Test results were double masked, i.e., neither the clinician nor the patient were aware of any testing results throughout the study period.

The following study sites and investigators participated in the clinical trial:

The Johns Hopkins Hospital - Daniel W. Chan, Ph.D., DABCC
Department of Laboratory Medicine, Baltimore, Maryland

University of Minnesota Medical School - David T. Kiang, M.D., Ph.D.
Minneapolis, Minnesota

Bowman Gray School of Medicine - Hyman B. Muss, M.D.
Comprehensive Cancer Center of Wake Forest University
Winston-Salem, North Carolina

University of California at Los Angeles Medical School - Jan H. Wong,
M.D., Los Angeles, California

The University of Texas, M.D. Anderson Cancer Center - Herbert
Fritsche, Ph.D. Department of Laboratory Medicine, Houston, Texas
77030

The primary objective of the clinical studies was to demonstrate that TRUQUANT® BR™ RIA, through serial testing of Stage II and Stage III breast cancer patients exhibiting no clinical evidence of disease at study entry, can be used as an aid in the detection of recurrence.

Prospectively collected serial specimens from breast cancer patients in Stages II and III were evaluated at five different geographic clinical sites. Sample size calculations were based on an estimate of the proportion of patients in the study who might be positive for CA 27.29 antigen and have a recurrent breast cancer.

In addition, specimens from patients with other conditions, both malignant and non-malignant, were also tested to assess the distribution of CA 27.29 antigen levels in these populations. In order to establish an appropriate upper limit of normal, a normal range was established at each site by testing at each site approximately 200 apparently healthy female volunteers over the age of 30 years. An upper limit of normal range (99th percentile) was established as the test cutoff for each site.

Patient history information was collected on each breast cancer patient entered into the study. An initial history form which included demographics and other clinical information was used at patient entry. A follow-up history form was completed at each return visit. A blood specimen was drawn at the time of patient entry and at each return visit. The case history forms used for each study population are part of the clinical protocols.

Analysis of Clinical Studies

Clinical Study I: Determination of the Normal Range of Values for TRUQUANT® BR™ RIA in an Apparently Healthy Blood Donor Population.

Approximately two hundred retrospectively gathered specimens from healthy blood donors were assayed at each site to determine the distribution of CA 27.29 antigen level in an assumed healthy population. The distribution of the values from the blood donors at each of the five sites were tested for consistency with the normal distribution using the SPSS (Statistical Package for the Social Sciences) univariate analysis method. The data indicated that there was significant deviation from normality in all 5 sites. The observed distributions were asymmetric and were skewed to the right. A logarithmic transformation resulted in an overcorrection of the data, producing a skew to the left of the distribution. The transformed data was consistent with the normal distribution. The usual practice with devices of this type is to establish an upper limit to the normal range. For this analysis the upper limit of the normal range was established as the 99th percentile of the transformed distribution which was consistent with the normal distribution at each site. The upper limit of normal is calculated by taking the mean of the square root transformed variables and adding the standard deviation multiplied by 2.33. This value is then squared to provide a value in the units of the original measurements. Any value of CA 27.29 antigen above this value is considered above the normal range.

Since the upper limit to normal is constructed using normal probability theory, an assessment was made of the five site distributions and to all sites to determine if they deviated from normality. This procedure was done with SPSS univariate analysis program which employs the Kolmogorov-Smirnov test for normality.⁽⁶⁾ For the square root estimate it is necessary to square

the value. The square root transformed means, standard deviation, transformed upper limit to normal, and untransformed upper limit to normal by study site and over all is given in **Table 6**. One analysis utilized the respective upper limit from each institution to determine an indication of recurrence. If a study patient had at least one value above the limit for the study site at which they were enrolled, the patient was considered positive for the CA 27.29 antigen variable; otherwise the patient was considered negative.

Table 6 Normal Range of Values for TRUQUANT® BR™ RIA in a Healthy Population

Site	Number of Samples	Transformed Mean	Transformed Standard Deviation	Transformed Upper Limit of Normal	Untransformed Upper Limit of Normal
Johns-Hopkins	207	4.04	0.84	6.00	36.1
Minnesota	200	4.60	0.90	6.68	44.7
Bowman Gray	200	3.73	0.87	5.76	33.2
UCLA	198	3.73	0.89	5.81	33.8
MD Anderson	199	3.65	0.87	5.67	32.2
All	1004	3.95	0.88	6.14	37.7

Clinical Study II: Determination of the Distribution of CA 27.29 Antigen Levels in a Population with Confirmed Malignancies Other than Breast Cancer.

Four of the five study sites provided data on samples of cancers of other types.

Since the number of patients at each study site was small, especially when subclassifying by several cancer types, no attempt was made to obtain distributions of CA 27.29 antigen levels by study site. The distribution by cancer type across all study sites is given in **Table 7**.

Table 7 Distribution of TRUQUANT® BR™ RIA Assay Results for Other Malignancies

Cancer Type	Below Upper Limit (UL)	UL to 100 U/mL	100 U/mL to 200 U/mL	Greater than 200 U/mL
Colon	94	15	2	0
Liver	41	4	1	1
Lung	64	16	7	1
Ovary	71	28	22	8
Pancreas	19	4	2	0
Prostate	52	10	0	0
Stomach	19	1	0	2
Uterus	56	7	4	1
Other	76	10	1	1
TOTAL	492	95	39	14

With the exception of ovarian cancer, the other cancers have 73-87 percent of their assays in the normal range for the study sites in which they were measured.

Clinical Study III: Determination of CA 27.29 antigen Levels in Patients with Other Non-malignant Conditions.

Three of the five study sites provided data on samples of other non-malignant conditions.

Since the number of patients at each study site was small, especially when subclassifying by several non-malignant conditions, no attempt was made to obtain distributions of CA 27.29 antigen by study site. The distribution by non-malignant condition across all study sites is given in **Table 8**.

Table 8 Distribution of TRUQUANT® BR™ RIA Assay Results for Non-Malignant Conditions

Condition	Below Upper Limit (UL)	UL to 100 U/mL	100 U/mL to 200 U/mL
Benign Breast Disease	97	3	0
Cirrhosis, Hepatic Necrosis	40	7	1
Endometriosis	40	0	0
Hepatitis	30	6	0
Ovarian Cysts	55	1	0
Pericarditis	1	0	0
Pregnancy	69	1	0
Renal Impairment	48	2	1
Other	17	2	0
TOTAL	397	22	2

The data indicated that for the other non-malignant conditions assayed, 94 percent of the assays were in the normal range. This indicated that none of the tested conditions are likely to give abnormally high readings which might interfere with the monitoring function for Stage II and Stage III breast cancer.

Clinical Study IV: Relationship of levels of CA 27.29 antigen in serum to the recurrence of disease in Stage II and Stage III breast cancer patients exhibiting no evidence of disease.

Specimens were gathered from female patients with diagnosed breast cancer who had surgery to treat their primary tumor and whose tumor status at time of entry to this study was stage II or stage III, were 1- 4 years post-surgery, and had no evidence of disease (NED). These patients were monitored over the length of the study (18-24 months) with serial blood samples.

Data Admissibility Criteria for TRUQUANT® BR™RIA

1. Samples were repeated if samples measuring > 36 U/mL CA 27.29 antigen had a CV>15 percent based on the U/mL value, samples measuring 20-36 U/mL had a CV>20 percent, and samples measuring 10-19 U/mL had a CV>30 percent.
2. Samples above 200 U/mL were diluted (1:10) and reassayed.
3. The assay was repeated if either positive control was out of the range specified on the vial label.

Sample Size

The sample size was selected to provide an estimate of the proportion of patients in the study who might be positive for CA 27.29 antigen and have a recurrent breast cancer. The marginal distribution of a given cell in a fourfold table such as the one generated by a cross tabulation of the test result and the cancer result is known to be binomial.⁽¹⁾ From preliminary data, approximately 15-20 percent of the total patients in a breast cancer population with no evidence of disease might be positive for the test and approximately 15-20 percent might have a recurrence.⁽²⁾ If the TRUQUANT® BR™ RIA assay were ineffective, then the probability of having both a positive CA 27.29 antigen value and recurrent breast cancer would be 0.03 - 0.04 percent. To observe a difference in the tested population of ten percentage points in the positive test/positive recurrence cell of the cross tabulation, i.e., observing 0.13 in that cell, with a power of 80 percent at a 5 percent significance level, would require 172 patients to complete the study. This is computed by a method described in Fleiss⁽³⁾ which is based on a normal approximation of the binomial with correction for continuity. If the potential for missing data is about 25 percent, then approximately 230 patients would have to be recruited to have 172 patients completing the study.

Definitions of Patient Cancer Status

1. NED - no evidence of disease at entry into the study based on physical examination, patient complaints, and radiographic studies.
2. Evidence of recurrence or progression - by clinical symptoms and/or general or specific imaging.

Descriptive Statistics of the Study Sample

There were 244 patients enrolled in the prospective study. Of these, 51 patients had fewer than three samples collected and analyzed, an additional 27 did not meet the inclusion criteria, leaving 166 patients available for analysis with the following distribution by study site:

Table 9 Age Distribution of Study Site Populations

Site	Eligible	Mean Age (Yrs.)	Standard Deviation
Johns-Hopkins	63	48.6	8.3
Minnesota	23	56.3	11.8
Bowman Gray	39	56.1	12.6
UCLA	2	51.2	12.0
MD Anderson	39	51.9	9.6

Of these 166 patients, 26 patients showed evidence of recurrence as determined by the study criteria (a classification of recurrence in the patient record).

The average age at time of entry to the study of the patients eligible for analysis was 52 ± 11 years but there was a statistically significant difference between study sites. The mean ages given in the table above indicate a potential for three distinct groupings by age: 1) John Hopkins, 2) Minnesota and Bowman Gray, and 3) M.D. Anderson and UCLA. Additionally there was a statistically significant difference in the age at breast cancer detection among the study sites. Patients from Bowman Gray and Minnesota were older at the time of surgery than the patients at the other three sites. There were 155 women with stage II and 38 with stage III breast cancer and the proportion of these did not differ by study site. The distribution of nodal status for the population indicated that 74 women had 1-2 positive nodes, 81 women had 3-9 and 38 had 10 or more. A statistically significant difference for nodal status was observed with Johns Hopkins having a different distribution from the other study sites. Positive progesterone receptor (P-receptor) status of the primary tumor was found in 109 (23 patients had missing P-receptor status) and there was no difference among study sites for this variable. Estrogen receptor status indicated 125 were positive estrogen receptors (19 patients had missing E-receptor values), but M.D. Anderson had a statistically different distribution from the others.

The variables suspected of having a role in the recurrence of stage II or stage III breast cancer include estrogen or progesterone receptor status of the primary tumor, the number of positive nodes, stage, and serum CEA levels.

Statistical Analysis

The data were analyzed by a method to determine the relative risk as measured by the odds ratio. Because of a difference found in age at study entry, age at surgery and nodal status, the data were analyzed for three groups of patients: 1) Johns Hopkins; 2) Bowman Gray and Minnesota; and 3) M.D. Anderson and UCLA. A logistic regression analysis⁽⁴⁾, using SPSS, for the three groupings was performed on the study data with proportion of stage II and stage III study subjects with confirmed recurrent breast cancer as the dependent variable, and several independent variables including CA 27.29 antigen status (above or below the limit), age at surgery, estrogen or progesterone receptor status of the primary tumor, the number of positive nodes, stage, and serum CEA levels. The variables other than CA 27.29 antigen status are considered covariates and remain in the model only if the coefficients corresponding to those variable are statistically significant when adjusted for the other factors. The backward elimination method was used to identify relevant covariates.

The analysis for the third group, M.D. Anderson and UCLA did not result in the same pattern of agreement. Since no patients were found to have both a positive TRUQUANT® BR™ RIA test result and a recurrence of breast cancer, the logistic regression failed to find a statistically significant relationship for the coefficient of the CA 27.29 antigen assay.

The sensitivity and specificity were also calculated for the total population using the method given in Fleiss⁽³⁾ and the data in **Tables 10-12**.

Table 10 Breast Cancer Recurrence in Stage II/III Patients Compared to TRUQUANT® BR™ RIA Results

		Breast Cancer Recurrence		
		YES	NO	Total
ONE TRUQUANT® BR™ RIA Value above 37.7 U/mL	YES	15	8	23
	NO	11	132	143
	Total	26	140	166

Sensitivity = 58% (38-77)*

Specificity = 94% (90-98)

Predictive Value Positive = 65% (45-85)

Predictive Value Negative = 92% (88-97)

* The values in parenthesis are 95% percent confidence limits.

Table 11 Breast Cancer Recurrence in Stage II/III Patients Compared to TRUQUANT® BR™ RIA Results

		Breast Cancer Recurrence		
		YES	NO	Total
TWO TRUQUANT® BR™ RIA Values above 37.7 U/mL	YES	14	2	16
	NO	12	138	150
	Total	26	140	166

Sensitivity = 54% (34-73)* Predictive Value Positive = 88% (71-104)
 Specificity = 99% (97-101) Predictive Value Negative = 92% (88-96)
 * The values in parenthesis are 95% percent confidence limits.

Analysis of the data by the grouping suggested by the differences in entry indicators is summarized below:

Table 12 Comparison of Sensitivity, Specificity, Predictive Value Positive, Predictive Value Negative, and 95% Confidence Intervals by Group

Group	Sensitivity	Specificity	Predictive Value Positive	Predictive Value Negative
All	58%(37-77)*	88%(82-93)	47%(29-65)	92%(86-96)
Johns Hopkins	58%(28-85)	94%(83-99)	70%(35-93)	91%(80-97)
Bowman Gray/ Minnesota	64%(31-89)	88%(76-96)	54%(25-81)	92%(80-98)
UCLA/MD Anderson	33%(0.8-91)	79%(63-90)	11%(0.3-48)	94%(79-99)

* The values in parenthesis are 95% percent confidence limits.

The analysis indicates that for Johns Hopkins and the Bowman Gray and Minnesota groups, the sensitivity, specificity, and predictive values are in a range consistent with other markers of this type, especially those characteristics which indicate the absence of disease. The strong negative predictive value suggest that TRUQUANT® BR™ RIA may be particularly useful for following patients with no evidence of disease.

VIII. Conclusions Drawn from the Studies

The TRUQUANT® BR™ RIA performance specifications, including analytical sensitivity and specificity, reproducibility, linearity, stability, recovery and standardization procedures are within accepted standards for an assay of this type.

The distribution of the serum CA 27.29 antigen assay results found in apparently healthy patients as well as patients with various nonmalignant and malignant diseases when assayed using the TRUQUANT® BR™ RIA correlate well with the serum CA 27.29 concentrations reported in literature.

The results of the serial monitoring for patients with previously diagnosed Stage II or Stage III breast cancer provide reasonable assurance that the TRUQUANT® BR™ RIA is safe and effective as an aid in the management of such patients.

The correlation demonstrated between the serum CA 27.29 antigen concentrations and the recorded patient clinical status, which included X-ray, CAT scans and histologic examination to confirm the diagnosis and current condition, was within acceptable limits. The Center for Devices and Radiological Health (CDRH) has concluded that the device is safe and effective for the stated indication.

IX. Panel Recommendations

The Immunology Devices Panel recommended at a panel meeting on September 21, 1995 that the PMA for TRUQUANT® BR™ RIA was approvable with the following conditions:

1. An additional analysis and description of the seventeen patients listed as "false positives" in the original study.
2. A protocol or plan for an additional postapproval study designed to collect additional data on patients at the time of recurrence with a particular emphasis on establishing a correlation with extent of disease at time of recurrence. This study would be conducted over a period of approximately two years and include one hundred recurrences. Summaries are to be provided in an annual report.
3. A satisfactory copy of revised draft labeling for this product.

X. FDA Decision

CDRH concurred with the recommendation of the Panel and issued a letter to the applicant on November 7, 1995 advising that the PMA was subject to the conditions described above as recommended by the Panel and required by FDA. In amendments received by FDA on 6 December 1995 and 1 March 1996, the applicant submitted the required data. FDA issued an approval order on March 29, 1996. The applicant's manufacturing facility was inspected on January 15, 1996 and was found to be in compliance with the device Good Manufacturing Practice regulations.

XI. Approval Specifications

Directions for use: See attached labeling (Attachment A)

Conditions of Approval: CDRH's approval of this PMA is subject to full compliance with the conditions described in the approval order (Attachment B).

References

1. **Krutchkoff, R.** 1970. *Probability and Statistical Inference*. Gordon and Breach Science Publishers, New York.
2. **Haagensen, C.D.** 1986. Chapter 20: Local recurrence following mastectomy. In: *Diseases of the breast*, 3rd ed. Philadelphia, WB Saunders Co.
3. **Fleiss, J.** 1981. *Statistical Methods for Rates and Proportions*. John Wiley and Sons, New York.
4. **Breslow, N.** 1976. Regression analysis of the log odds ratio: A method for retrospective studies. *Biometrics* 32: 409.
5. **Lilliefors, H.W.** (1967). On the Komogorov-Smirnov test for normality with mean and variance unknown. *J. Amer. Statist. Assoc.* 62: 399-402.

TRUQUANT® BR™ RIA

INTENDED USE

TRUQUANT® BR™ Radioimmunoassay (RIA) is an *in vitro* diagnostic device indicated for the quantitative determination of CA 27.29 antigen in serum or EDTA plasma of patients previously treated for stage II or stage III breast cancer. Serial testing for CA 27.29 antigen with TRUQUANT® BR™ RIA in patients who are clinically free of disease should be used in conjunction with other clinical methods used for the early detection of recurrence.

Catalog numbers: 3 1064 (100 tubes), 3 1095 (500 tubes)



The 100-tube kit contains not more than 10 microcuries (370 kilobecquerels) of radioactive ¹²⁵I monoclonal anti-CA 27.29; the 500-tube kit contains not more than 50 microcuries (1850 kilobecquerels).

For *in vitro* diagnostic use.

The usefulness of this device in Stage I patients has not been established.

Elevated levels of CA 27.29 are found in patients with other diseases, benign conditions and first trimester pregnancies.

As is the case with all tumor markers, results from the TRUQUANT® BR™ RIA should be considered along with other clinical parameters.

TRUQUANT® BR™ RIA incorporates BIOMIRA's proprietary antibody B27.29. Comparable assay results will not be obtained using other antibodies against CA 15-3 antigen.

Read all safety precautions before proceeding with this test.

SUMMARY AND EXPLANATION OF THE TEST

Breast malignancy is the single most prevalent form of cancer in women in the United States. Early detection of the recurrence of breast cancer is key to the management of the disease (1). Tumor-associated mucinous antigens such as CA 15-3 antigen, detected in serum and plasma often reflect changing clinical status (2). Mucins (large molecular weight glycoproteins) associated with breast carcinoma encoded by the human MUC 1 gene are identified by several names including MAM 6, milk mucin, CA 27.29 and CA 15-3. Numerous monoclonal antibodies directed against mucinous antigens have been developed (3-11) that bind distinct and overlapping epitopes (12). Some have found utility in immunoassays for quantitation of this antigen in serum/plasma of patients with breast cancer. TRUQUANT® BR™ RIA employs a monoclonal antibody (B27.29) which is reactive with the core peptide of the malignant breast carcinoma-associated mucin antigen encoded by the MUC 1 gene. (12).

PRINCIPLE OF THE PROCEDURE

TRUQUANT® BR™ RIA is a solid-phase, competitive inhibition radioimmunoassay. Polystyrene tubes, coated with CA 27.29 antigen, are incubated with standards, controls and serum/EDTA plasma samples together with the ¹²⁵Iodine-labelled B27.29 monoclonal antibody. During the incubation, CA 27.29 antigen in the sample competes with the solid-phase antigen for binding to the ¹²⁵Iodine-labelled B27.29 tracer antibody. Unbound material is removed by washing the tubes and radioactivity of bound antibody is measured in a gamma counter. Bound radioactivity is inversely proportional to the concentration of CA 27.29 antigen in the sample. Standards are used to construct a curve from which the concentrations of positive controls and CA 27.29 antigen in a test sample are determined.

WARNINGS

This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals and for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to regulation and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority. This kit contains radioactive material and should be stored and handled in specially designated areas. All radioactive waste must be stored in appropriately-labelled waste containers and disposed of according to local (state) and federal regulations.

Antigen for standards, controls and coated tubes is derived from human ascites or pleural effusions which have been individually tested in accordance with current serum-based FDA-cleared procedures and found to be negative for HBsAg, HCV and HIV. However, no test method can offer complete assurance that products derived from human fluids will not transmit infectious agents. Proper handling and disposal methods should be used.

Sodium Azide (NaN₃) Warning

Sodium azide is present in some kit components as a preservative at a final concentration of not more than 0.2%. Sodium azide can form explosive compounds when in contact with lead or copper plumbing and should always be flushed with large amounts of water. To prevent formation of toxic vapor, mixing with acidic solutions should be avoided.

TRUQUANT® BR™ RIA has been designed to minimize human anti-mouse antibody interference. However, caution should be exercised in interpreting any serum assay result from *in vitro* diagnostic kits which employ murine antibodies because of the potential human anti-mouse responses resulting from usage of murine antibodies and/or their fragments *in vivo*.

Do not use kit components beyond expiry date and do not mix reagents from kits with different lot numbers.

Avoid exposure of the reagents to excessive heat or light.

Safety Measures

Safe handling of radioactive and biohazardous materials should include the following:

1. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used.
2. Do not pipette radioactive solutions by mouth.
3. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves.
4. Radioactive materials must be stored in their original containers or in containers providing equivalent radiation protection, in designated areas.
5. A record of disposal of all radioactive materials must be kept.
6. Radioactive materials must be used only in designated areas.
7. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.
8. Radioactive spills must be handled in accordance with established procedures.
9. Radioactive materials must be disposed of according to the regulations and guidelines of the agencies with jurisdiction over the laboratory.
10. Avoid contamination of the reagents by using a fresh pipette tip for each reagent, by closing the vial immediately after use and by returning the reagents to the refrigerator as soon as possible.

MATERIALS SUPPLIED

Antigen-Coated Tubes (T)

One hundred (4 x 25) or 500* (20 x 25) polystyrene tubes (12 x 75 mm) coated with human CA 27.29 antigen supplied in heat-sealed foil pouches containing desiccant. Store refrigerated and protected from moisture. Reseal pouches after opening. Stable at 2-8°C for duration of labelled expiry date.

Radiolabelled Tracer Antibody (¹²⁵I)

One (five vials*) vial (20 mL) ¹²⁵I-labelled B27.29 murine monoclonal antibody in buffered solution with 1 mg/mL sodium azide as preservative. Total radioactivity: ≤ 0.5 μCi/mL (≤ 18.5 kBq/mL). Store refrigerated. Stable at 2-8°C for duration of labelled expiry date.

Positive Controls (+),(++)

One (two vials*) vial (0.5 mL) positive control containing 75 ± 24 U/mL human CA 27.29 antigen (+) and one vial (0.5 mL) positive control containing 118 ± 27 U/mL human CA 27.29 antigen (++) in buffered protein solution with 1 mg/mL sodium azide as preservative. Store refrigerated. Stable at 2-8°C for duration of labelled expiry date.

Standards (1-5)

One set of standards containing human CA 27.29 antigen in buffered protein solution with 1 mg/mL sodium azide as preservative. Standards consist of one (five vials*) vial (30 mL) of 0 units per millilitre (U/mL) CA 27.29 antigen (1) and one (two vials*) vial (0.5 mL) each of 25, 50, 100 and 200 U/mL CA 27.29 antigen, (2) to (5), respectively. Store refrigerated. Stable at 2-8°C for duration of labelled expiry date.

Sample Diluent

The 0 U/mL standard (1) serves also as patient sample diluent.

* 500-Tube kit

MATERIALS REQUIRED BUT NOT PROVIDED

Well-type gamma counter compatible with standard 12x75mm tubes suitable for measuring ¹²⁵I. The counting efficiency of the instrument should be greater than 40%. If less, a counting time longer than 1 minute should be used. Refer to the instrument manual for complete operating instructions.

Rack shaker - set at approximately 275 ± 25 rpm to agitate tubes

Precision pipettes with disposable tips capable of delivering 25 μL, 50 μL, 200 μL and 500 μL

Aspiration device

Device for delivering 2 mL of wash solution (Re-pipetting pump is suggested)

De-ionized or distilled water

Disposable plastic wrapping film.

Test tube rack for 12 x 75mm tubes.

Test tubes for dilution of sera.

STORAGE INFORMATION

Store the reagents between 2 - 8°C.

SPECIMEN COLLECTION AND HANDLING

The patient need not be fasting, and no special preparations are necessary. Serum or EDTA plasma may be tested with this assay. It is recommended that the same type of specimen (serum or EDTA plasma) be used throughout the course of study of an individual patient. Specimens containing particulate matter should be clarified by centrifugation before testing. Samples should be collected by non-traumatic venipuncture and the serum (if applicable) promptly separated from the clot. Samples which are to be tested within 24 hours may be stored at 2 - 8°C. Samples which are not to be tested within 24 hours should be stored frozen (-20°C or lower) and tested within 12 months. It is recommended that samples be tested as soon as possible.

It is important that samples tested in the assay be free of microbial contamination which may result in erroneous results. Normal sample collection and handling should be sufficient to avoid contamination.

TEST PROCEDURE

Preparation for the Assay

Bring all samples to room temperature (18 - 25°C). Swirl gently (avoid foaming) to mix.

Prepare a 1 in 21 dilution of each patient sample. Pipette 500 μ L of 0 U/mL standard (1) into a test tube then add 25 μ L of patient sample and mix well. Assay performance is validated for assays in which standards, controls and samples are all pipetted within 60 minutes.

NOTE: Do not dilute standards and positive controls. Standards and controls are pre-diluted 1 in 21 and ready to use.

All standards (1-5), controls (+, ++) and patient samples should be tested simultaneously and in duplicate.

Label the coated tubes (T) provided in the kit and place the tubes in the test tube rack.

Place test tube rack, patient serum samples and the reagents from the TRUQUANT® BR™ RIA kit in the designated work area.

Finish the assay without interruption.

Assay Procedure

- A. Pipette in duplicate 50 μL of each standard (1-5), Positive Control (+, ++) and diluted patient sample directly to the bottom of appropriately-labelled CA 27.29 antigen-coated tubes (T).

NOTE: It is important, due to the small sample size utilized, that the sample be pipetted directly to the bottom of the tube. Failure to do so may adversely affect assay precision. Use a fresh pipette for each sample.

- B. Pipette 200 μL of the ^{125}I -labelled mouse monoclonal antibody B27.29 (^{125}I) into each tube. For a large number of samples, a repeater pipette is very convenient for this step.
- C. Cover all tubes with disposable plastic wrapping film.
- D. Incubate with agitation (275 ± 25 rpm) for 3 hours \pm 5 minutes at room temperature (18 - 25°C).
- E. Aspirate the tubes and wash with 2 mL of de-ionized or distilled water. Aspirate and repeat the wash. A manual re-pipetting pump is suggested for this step. Ensure careful removal of liquid after each wash.
- F. Measure the bound radioactivity in the tubes in a gamma counter. Radioactivity must be measured within 48 hours.

CALCULATION OF RESULTS

- A. Construct the standard curve by plotting the mean counts per minute (cpm) for each CA 27.29 standard on the vertical (Y) axis against the concentration of the corresponding CA 27.29 standard on the horizontal (X) axis. Draw the curve by a "best-fit" regression method. Data reduction programs may be used; logistic or four parameter logistic data reduction programs are recommended for best results.
- B. Determine the concentration of CA 27.29 antigen in the Positive Controls and patient samples from the standard curve.

NOTE: Do not multiply the result by 21. The standard curve already corrects for the 1 in 21 dilution of patient samples.

- C. Patient samples reading over 200 U/mL should be further diluted with the 0 U/mL standard (1) and re-analyzed for more accurate results. A dilution of 1 in 10 of such samples is recommended.

D. Example of TRUQUANT® BR™ RIA standards and controls.

Standard U/mL	CPM	Mean CPM	Calculated U/mL
0	80976		
	82011	81494	
25	66183		
	66083	66133	
50	53006		
	52445	52726	
100	36849		
	37493	37171	
200	23681		
	23469	23575	
Positive Control (+)	44101		
	43979	44040	75
Positive Control (++)	33616		
	34102	33859	118

Example of Standard Curve*

* For illustration purposes only. Do not use in place of assay results.

QUALITY CONTROL

Before CA 27.29 antigen levels in patient samples are calculated, the validity of each assay must first be established. An invalid test may indicate a procedural or technical fault or component problem and implies values derived for patient samples may not be reliable.

Verify assay validity by (i) computing mean dose for duplicate determinations of each positive control and (ii) computing variation of duplicate dose determinations from the mean of each positive control.

The assay is considered to be valid when the following criteria are met:

1. The mean dose of each positive control is within the specified limits, i.e.

Positive Control (+)	51-99 U/mL
Positive Control (++)	91-145 U/mL

2. Each replicate of duplicate determinations for each positive control is $\pm 10\%$ of the observed dose for the (+) and (++) Positive Controls.

Repeat test if assay is invalid. Consult Technical Service if assay fails repeat test.

PERFORMANCE CHARACTERISTICS

Precision

Precision was assessed with a 4-member panel consisting of a normal sample and a dilution series of pooled sera from patients with breast cancer, adjusted to contain CA 27.29 antigen within the linear range of the standard curve. The reported ranges represent data obtained at 4 test sites.

Intra-assay (Within-Run)

Statistics were based on replicates of 20 determinations per assay. Data represent mean results of a single assay at 4 test sites.

Sample	Mean (U/mL)	SD (U/mL)	CV (%)
Normal	22.4	2.6	11.6
Malignants:			
Low	65.8	3.3	5.0
Medium	98.6	3.3	3.3
High	157.8	7.7	4.9

Inter-assay (Run-to-Run)

Statistics were based on replicates of 20 determinations over 3 assays. Data represent mean results of 3 assays at 4 test sites.

Sample	Mean (U/mL)	SD (U/mL)	CV (%)
Normal	20.7	3.1	15.0
Malignants:			
Low	63.4	4.2	6.6
Medium	96.5	5.7	5.9
High	152.4	9.2	6.0

Analytical Sensitivity

The minimum detectable concentration of CA 27.29 antigen was determined to be 7 U/mL. The minimum detectable concentration is defined as that concentration of CA 27.29 antigen two standard deviations from the 0 U/mL standard.

Spike and Recovery Studies

A normal serum sample containing a low endogenous level of antigen was spiked with varying amounts of CA 27.29 antigen. The mean recoveries from duplicate determinations for 3 assays are reported.

ENDOGENOUS U/mL	ADDED U/mL	EXPECTED U/mL	ASSAY 1		ASSAY 2		ASSAY 3	
			OBS.* U/mL	REC. %	OBS. U/mL	REC. %	OBS. U/mL	REC. %
14	18	32	30	94	29	91	35	108
13	36	49	52	105	44	90	46	94
10	71	81	80	99	80	99	81	101
6	107	113	110	97	123	109	112	99
3	142	145	143	99	152	105	151	104

* OBS.: Observed; REC.: Recovered

Parallelism

The dilution characteristics of sera were investigated using the 0 U/mL standard to dilute sera containing CA 27.29 antigen. Antigen levels in proportional dilutions were determined in duplicate and the average result, corrected for the appropriate dilution factor, reported for three assays.

TRUQUANT® BR™ RIA Dilution Linearity

Specificity

The specificity of TRUQUANT® BR™ RIA was analyzed by testing sera containing the compounds tabulated below. These compounds and sample conditions were determined to have no effect on assay performance.

The effect of icteric, lipemic and hemolysed samples on quantitation of CA 27.29 antigen was simulated by addition of bilirubin, lipids and hemoglobin to serum. The physiological substances showed no interference with assay performance at the concentrations tested.

Physiological Interfering Substances:	Range
Bilirubin	9-600 mg/L
Lipids	0.7-10 g/L
Hemoglobin	1.5-100 mg/L

To assess the effect of chemotherapeutic and hormonal agents, prescription drugs and over-the-counter (OTC) preparations on assay performance, serum samples from cancer patients receiving therapy, prescription medications or admitting to use of OTCs and from apparently normal healthy individuals were spiked with CA 27.29 antigen and quantitated. None of the agents listed below interfered with antigen recovery.

Chemotherapeutic Agents:	Hormonal Agents:
Adriamycin	Cytadren
Cytoxan	Megace
Fluorouracil	Tamoxifen
Methotrexate	
Taxol	

Prescription and Over-the-Counter Drugs:		
Anticoagulants	Analgesics	Vitamins
Coumadin	Acetylsalicylic Acid	Vitamin B
Heparin	Tylenol	Vitamin C
		Vitamin E
		Multivitamins

EXPECTED VALUES

Based on a study of CA 27.29 antigen levels in 1004 sera from apparently healthy female blood donors greater than 30 years of age 37.7 U/mL was determined as the upper limit of normal (cut-off) for the TRUQUANT® BR™ RIA. In this study a mean value of 15.6 U/mL was obtained. The upper limit of normal was determined from the 99th percentile of the distribution based on normal theory. Each laboratory should define their own upper limit of normal.

A change from normal range of CA 27.29 antigen levels detected by TRUQUANT® BR™ RIA to values greater than the upper limit of normal in stage II or stage III breast cancer patients considered free of disease should be used in conjunction with other clinical methods used for the early detection of recurrence provided alternative causes of elevated CA 27.29 antigen can be excluded. It is recommended that after a suitable interval a follow-up sample be tested with TRUQUANT® BR™ RIA to confirm diagnosis in asymptomatic patients.

TRUQUANT® BR™ RIA Clinical Results for Other Malignancies

With the exception of ovarian cancer, 73-87% of patients with other cancers have TRUQUANT® BR™ RIA values within the normal range.

Cancer Type	Total Number Tested	Number Below Upper Limit	Upper Limit to 100 U/mL	100 U/mL to 200 U/mL	Above 200 U/mL
Colon	111	94	15	2	0
Liver	47	41	4	1	1
Lung	88	64	16	7	1
Ovary	129	71	28	22	8
Pancreas	25	19	4	2	0
Prostate	62	52	10	0	0
Stomach	22	19	1	0	2
Uterus	68	56	7	4	1
Other	88	76	10	1	1
Total	640	492	95	39	14

TRUQUANT® BR™ RIA Clinical Results for Other Non-malignant Conditions

Normal range values for TRUQUANT® BR™ RIA were observed in 94% of samples from patients with non-malignant conditions. Based on the clinical results, these conditions are not likely to cause abnormally high TRUQUANT® BR™ RIA values which would interfere with the monitoring of patients with stage II or stage III breast cancer.

Non-Malignant Condition	Total Number Tested	Number Below Upper Limit	Upper Limit to 100 U/mL	100 U/mL to 200 U/mL
Benign Breast Disease	100	97	3	0
Cirrhosis, Hepatic Necrosis	48	40	7	1
Endometriosis	40	40	0	0
Hepatitis	36	30	6	0
Ovarian Cysts	56	55	1	0
Pericarditis	1	1	0	0
Pregnancy	70	69	1	0
Renal Impairment	51	48	2	1
Other	19	17	2	0
Total	421	397	22	2

TRUQUANT® BR™ RIA Clinical study

TRUQUANT® BR™ RIA was evaluated in a prospective clinical trial at five centers in the United States. Serial TRUQUANT® BR™ RIA values were determined for Stage II and Stage III breast cancer patients who were lymph node positive at time of surgery but clinically free of disease. Logistic regression analysis of the clinical data showed that TRUQUANT® BR™ RIA was a significant and independent predictor of recurrent breast cancer in the study population.

Clinical Data

TRUQUANT® BR™ RIA test results can be interpreted based on a single value above the upper limit of normal or with two consecutive values above the upper limit of normal as shown with the following clinical study tables.

Breast Cancer Recurrence¹ in Stage II or Stage III Patients Compared to TRUQUANT® BR™ RIA Results

		Breast Cancer Recurrence		TOTAL
		YES	NO	
ONE TRUQUANT® BR™ RIA value above 37.7 U/mL	YES	15	8	23
	NO	11	132	143
TOTAL		26	140	166

Sensitivity: 58%
 Specificity: 94%
 Predictive value of a Positive: 65%
 Predictive value of a Negative: 92%

		Breast Cancer Recurrence		TOTAL
		YES	NO	
TWO TRUQUANT® BR™ RIA values above 37.7 U/mL	YES	14	2	16
	NO	12	138	150
TOTAL		26	140	166

Sensitivity: 54%
 Specificity: 99%
 Predictive value of a Positive: 88%
 Predictive value of a Negative: 92%

¹ Evidence of recurrence established using clinical symptoms and/or general or specific imaging techniques including chest-xray, mammogram, magnetic resonance imaging, computerized tomography, bone scan, liver scan and ultrasound.

SUMMARY OF ASSAY PROTOCOL

1. Bring all reagents to room temperature and swirl to mix.
2. Dilute patient sample by adding 25 μL of sample to 500 μL of 0 U/mL standard (1 in 21 dilution) and mix well.
3. Pipette in duplicate 50 μL of standards, controls and diluted patient samples directly to the bottom of appropriately marked CA 27.29 antigen coated tubes.
4. Add 200 μL of ^{125}I -labelled B27.29 tracer antibody to each tube.
5. Cover and incubate with agitation (275 ± 25 rpm) for 3 hours \pm 5 minutes at room temperature (18 - 25°C).
6. Aspirate contents of tubes, wash twice with 2 mL of de-ionized or distilled water and count in a gamma counter.
7. Construct a standard curve using values obtained for each standard.
8. Determine the concentration of CA 27.29 antigen in the positive controls and samples from the standard curve.

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