



FUJIREBIO DIAGNOSTICS, INC. (FDI) CA 15-3[®] RIA

Radioimmunoassay for the quantitative determination of DF3 antibody-defined antigen in serum or plasma of patients previously treated for stage II or stage III breast cancer. FDI 1-800-342-9225 PRODUCT INFORMATION (IN USA ONLY)

The **FDI CA 15-3** RIA is based on the use of FDI's proprietary DF3 antibody as the tracer antibody, which is available exclusively through FDI and its licensed distributors. Performance characteristics of kits which employ the DF3 system are not transferable to those diagnostic kits using different antibodies.

The concentration of DF3 antibody-defined antigen in a given patient sample determined with different assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Assay values obtained with different assay methods cannot be used interchangeably.

This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories, or hospitals and only 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. In the United States, its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

INTENDED USE

The FDI CA 15-3 RIA is an *in vitro* test for the quantitative determination of DF3 antibody-defined antigen, encoded by the MUC 1 gene, in serum and plasma of patients previously treated for stage II or stage III breast cancer. Serial test results obtained with the FDI CA 15-3 RIA, in patients who are clinically free of disease, should be used in conjunction with all relevant information derived from diagnostic tests, physical examination, and full medical history in accordance with appropriate patient management procedures used for early detection of recurrence.

SUMMARY AND EXPLANATION OF THE TEST

Breast cancer is the most common malignancy among women in the United States.(1) It is estimated that approximately 184,000 new cases of breast cancer will be diagnosed each year and that approximately 44,000 women will die of the disease.(2) One of every nine women in the U.S. will develop breast cancer and approximately 30% of women who have this malignancy will die of the disease. Metastatic disease may be present at the time of initial diagnosis and can occur at any time following primary therapy. Up to 70% of patients with metastases will respond to systemic treatment with cytotoxic drugs or endocrine therapy; therefore, early detection of recurrence is important to patient management.(3) The median survival following diagnosis of recurrent disease is approximately 2 years, but may range from a few months to decades.(4,5)

In patients previously treated for stage II or stage III breast cancer, early detection of recurrence cannot be readily accomplished by routine clinical or diagnostic studies alone. The use of a circulating serum tumor marker assay, such as FDI CA 15-3 RIA, can be useful in the identification of these patients.

PRINCIPLES OF THE PROCEDURE

The FDI CA 15-3 RIA utilizes two monoclonal antibodies (115D8 and DF3) which react with DF3-reactive determinants, expressed by human breast carcinoma cells. The 115D8 antibody was raised against antigens of human milkfat globule membranes. (6-9) The DF3 antibody was prepared against a membrane-enriched fraction of a human breast carcinoma.(10,11) The results of research studies indicate that the concentration of the DF3-reactive determinants is frequently elevated in the serum of patients with breast cancer as well as with other malignancies, such as lung cancer, and in some non-malignant disorders.(12-15) CA 15-3 assay values were not elevated in the sera of the majority of normal individuals or those with nonmalignant conditions.(12-15)

DF3 antibody-defined antigen in a given patient sample determined with different assays and from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Assay values obtained with different assay methods cannot be used interchangeably.

FDI CA 15-3 RIA is a solid phase radioimmunoassay based on the "forward sandwich" principle. Polystyrene beads coated with murine monoclonal 115D8 antibody are incubated with a specimen along with appropriate standards and controls. During this incubation, 115D8 reactive determinants present in the specimen are bound by the antibody on the solid phase. Unbound material present in the specimen are removed by aspiration and washing. In the second step, the beads are incubated with murine monoclonal DF3 labeled with ¹²⁵I which detects DF3-reactive determinants bound to the bead during the first incubation step. The unbound, labeled antibody is removed by aspiration and washing the beads. The bound radioactivity, determined by counting the beads in a gamma counter, is proportional to the CA 15-3 assay value in the specimen within the dynamic range of the assay. A standard curve is obtained by plotting the CA 15-3 assay values of the standards versus the bound radioactivity. The CA 15-3 assay values of the specimens and the controls are obtained by converting counts per minute (CPM) into CA 15-3 assay values using the standard curve from the same assay.

REAGENTS

- 1 **COATED BEADS** CA 15-3 115D8 (murine monoclonal antibody) Coated Beads, 1 vial, containing 100 beads.
- 2 **STANDARD/DILUENT** CA 15-3 0 U/mL Standard/Diluent (buffered protein solution with preservative), 0 U/mL*, 1 vial, containing 100 mL.
- 3 **STANDARDS** CA 15-3 Standards (antigen diluted in a buffered protein solution containing preservative), prediluted to 25, 50, 100, 200 U/mL*, 4 vials containing 1.2 mL each. Concentration of each standard is indicated on the vial.
- 4 **CONTROL** CA 15-3 Positive Control (with preservative), diluted re-calcified human plasma.** Concentration and range of the control are indicated on the vial.
- 5 **TRACER** CA 15-3 ¹²⁵I-DF3 (murine monoclonal antibody), in TRIS buffer with protein stabilizers and preservative, 2 vials (10 mL each). Maximum radioactivity: 1.0 µCi/mL (37.1 kBq/mL).

- * CA 15-3 assay values are expressed as units per mL (U/mL). A unit is an arbitrary value related to an FDI-maintained reference antigen preparation. There is no generally available reference standard at this time.
- ** Tested in accordance with current FDA-required assays for blood-borne pathogens.

Materials supplied are sufficient to perform 100 tests. DISCARD any components remaining after the performance of 100 tests (100 beads). REAGENTS MUST NOT BE MIXED FROM DIFFERENT KIT LOTS.

PRECAUTIONS FOR USERS

- 1 Do not eat, drink, smoke, or apply cosmetics in any laboratory in which radioactive materials are handled.
- 2 Do not pipette reagents and samples by mouth.
- 3 A lab coat or other suitable protective clothing and disposable gloves should be worn throughout the testing procedure.
- 4 All spillage should be immediately and thoroughly wiped up and contaminated material added to appropriate waste.
- 5 The user should store the radioactive material until used in the original shipping package or in a container providing equivalent radiation protection. The refrigerator should be properly marked with a radiation hazard sign. Pursuant to a Certificate of Registration received after filing form NRC-483, laboratories may receive products containing ¹²⁵I in units not exceeding 10 µCi each and may not possess, at any one time, at any one location of storage or use, a total amount of ¹²⁵I in excess of 200 µCi. Licensees in Agreement States are to refer to the appropriate regulations of their own state. Radioactive waste should be disposed of in appropriately labeled waste containers, according to State or Federal requirements. Radioactive material should be stored in a properly designated area.
- 6 Sodium azide is used in the FDI CA 15-3 RIA reagents as a preservative. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. Lead or copper azide may be explosive on percussion or hammering. Flush drains thoroughly with water after disposing of solutions containing sodium azide.
- 7 **CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIALS FROM WHICH THESE PRODUCTS HAVE BEEN DERIVED WERE FOUND NEGATIVE WHEN TESTED FOR HEPATITIS B SURFACE ANTIGEN, HEPATITIS C, HIV I AND HIV II ANTIBODIES, AND HIV ANTIGEN. HOWEVER, NO KNOWN TEST METHOD CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.**
- 8 For *in vitro* diagnostic use only. Not for internal or external use in humans or animals.
- 9 Do not use kit components beyond the expiration date.
- 10 Do not mix reagents from different kit lots.
- 11 Avoid microbial contamination of reagents in vials.

STORAGE INFORMATION

- 1 All reagents should be stored at 2° - 8° C upon receipt and should not be used beyond expiration date appearing on labels. Avoid freezing reagents.
- 2 All reagents must be brought to room temperature (20° - 30° C) before use and returned to 2° - 8° C for storage. (Bead containers should be brought to room temperature before opening and tightly closed thereafter.)

INSTRUMENT

Commercially available well-type gamma counters are routinely employed to determine radioactivity. Refer to the instruction manual supplied with the instrument. Counters used must have greater than 60% efficiency. If counter efficiency is less than 60%, longer counting periods should be used.

PATIENT SAMPLE COLLECTION AND HANDLING

Serum or plasma are both suitable for use with the FDI CA 15-3 RIA. When preparing serum samples, separate serum from the clot within 24 hours following collection. Whenever possible, patient samples should be clear and non-hemolyzed. Patient samples containing particulate matter should be clarified by centrifugation at 1,000g for 15 minutes before testing. Preferably, samples should be fresh; avoid repeated freeze-thaw cycles. If serum is to be stored for future or repeat tests, aliquot samples to avoid repeated freeze-thaw cycles.

If the test is to be run within 24 hours after collection, store the patient sample at 2° - 8° C. If testing will be delayed, the patient sample should be aliquoted and frozen at -20° C.

MATERIALS PROVIDED

FDI CA 15-3 RIA Kit, 100 Tests (refer to **REAGENTS** for a list of materials provided). Sufficient quantity of the following accessories are provided to perform 100 tests:

- Reaction trays.
- Adhesive cover sealers.
- Transfer trays pre-filled with plastic test tubes (for transfer of beads from reaction trays).

MATERIALS REQUIRED BUT NOT PROVIDED

- Second level of CA 15-3 control in the range of 80 - 120 U/mL.
- Precision pipettes with disposable tips to deliver 1.0 mL, 0.2 mL, and 0.020 mL (± 1%).
- Plastic tubes that can hold 2 mL for specimen and control preparation.
- Vortex mixer to mix the specimen and control dilutions.
- Distilled or deionized water for use in bead washing.
- Device for delivery of wash solution, such as a Cornwall syringe, Gorman-Rupp pump, or equivalent.
- An aspiration device for washing coated beads, e.g., cannula, aspiration tip, or commercial multiwash device with vacuum source and a liquid trap for retaining aspirated fluids.
- A well-type gamma counter with an efficiency greater than 60%. If counter efficiency is less than 60%, longer-counting periods should be used.
- Bead dispensing device, e.g., commercial single- or multiple-bead dispenser or non-metallic forceps.
- Rectilinear graph paper or appropriate software for the construction of a point-to-point curve.

CA 15-3 RIA PROCEDURE

NOTE: Each standard, control, and patient sample should be assayed in duplicate each time the test is performed.

CAUTION: ALLOW PATIENT SAMPLES AND REAGENTS TO REACH ROOM TEMPERATURE (20° - 30°C) BEFORE USE. USE A CLEAN PIPETTE OR DISPOSABLE TIP FOR EACH TRANSFER TO AVOID CROSS-CONTAMINATION.

- 1 Five standards and two controls should be run with each series of patient samples. Standards and controls should be subjected to the same incubation times and reagents as the samples being tested. Identify reaction tray wells on data sheet and on reaction trays using a waterproof marker.
- 2 Prepare a 1:51 dilution of the controls and samples to be tested.
 - a) Mark the pre-dilution tubes to match the identification number of the samples or controls.
 - b) Add 1.0 mL (1,000 µL) of CA 15-3 0 U/mL Standard/Diluent to each tube, using a precision pipette.
 - c) Add 0.020 mL (20 µL) of each sample or control to the corresponding pre-dilution tube with a precision pipette. Use a clean, disposable tip for each sample and the control.
 - d) Mix the samples and controls carefully with a Vortex mixer immediately before testing.
- 3 Pipette 0.2 mL (200 µL) of the diluted specimens and controls to their assigned wells. Use a separate tip for each sample and control.

Note: Diluted specimens should be saved and stored at 2° - 8° C until the assay data has been evaluated. These dilutions may be used for further dilution and retesting if the initial test results are greater than 200 U/mL. (See PROCEDURE FOR ASSAY OF PATIENT SAMPLES WITH GREATER THAN 200 U/mL.)

- 4 Pipette 0.2 mL (200 µL) of each Standard into wells. Use a clean tip for each Standard. **(Standards are provided prediluted for user convenience. Do not dilute standards.)**
- 5 Dispense one bead into each reaction well. Beads may be transferred by use of clean, non-metallic forceps or by use of a single- or multiple-bead dispensing device. If more than one tray is used, delay dispensing beads into the next tray until the amount of time that has passed is equivalent to the time required to wash one tray.
- 6 Apply a cover sealer to each tray. Gently tap the tray to eliminate any air bubbles trapped in the reaction wells. Make sure that each bead is completely covered by the sample, control, or standard. Be careful not to splash liquid onto cover. Mark the time the beads were dispensed on the cover sealer.
- 7 Incubate the trays at room temperature (20° - 30° C) for 2 hours ± 5 minutes.
- 8 At the end of the incubation period, carefully remove and discard the adhesive cover sealers. Wash each bead three times according to the appropriate procedure below.

Semi-Automated

Commercial rinsing/aspiration systems which are semi-automated are recommended. Each well is aspirated, then beads are washed with 5 mL of distilled or deionized water. Repeat this wash procedure two more times for a total rinse volume of 15 mL. To ensure an adequate washing, *beads must be lifted off the bottom of the reaction well during the wash process* and then lowered to the bottom to ensure all liquid has been aspirated.

Manual

Aspirate each well using a disposable pipette or cannula attached to a vacuum source. Rinse each bead by placing the pipette or cannula, attached to the vacuum source, adjacent to the bead in the bottom of the well and slowly add 5 mL of distilled or deionized water. The bead must be fully immersed throughout the wash procedure. Take care not to overflow the well. Repeat the wash procedure two additional times for a total wash volume of 15 mL.

- 9 Pipette 0.2 mL (200 µL) of ¹²⁵I-DF3 (murine), monoclonal, to each well containing a bead. If several trays are used, it may be necessary to delay dispensing the radiolabeled antibody, to compensate for the delay that will be incurred during the washing procedure.
- 10 Apply a cover sealer to each tray. Make sure that beads are completely covered with liquid. Tap the trays gently to release any air bubbles that may be trapped in the solution. Be careful not to splash liquid onto cover. Mark the time the tracer was added on the cover sealer.
- 11 Incubate the trays at room temperature (20° - 30° C) for 3 hours ± 10 minutes.
- 12 At the end of the incubation period, remove and discard the adhesive cover sealers. Wash beads three times as described above.
- 13 Transfer the beads from the reaction trays to the tube rack transfer system by aligning the numbers and letters on the bottom of the transfer system with the numbers and letters on the reaction tray simultaneously. Tap the tray lightly to transfer the beads to the counting tubes. Tear off the protective flap of the transfer system only after the beads have been transferred.
- 14 Identify all tubes either prior to, or after, transferring the beads.
- 15 Place the counting tubes in a suitable well-type gamma counter. Count the radioactivity in each tube for 1 minute. All standards, controls, and samples must be counted together.

Procedural Notes

- 1 The incubation time should be consistent for each bead throughout an assay. Care should be taken to ensure that the washing procedure does not result in differences in incubation times from bead to bead.
- 2 The beads may be stored in the tube rack overnight at room temperature (20° - 30°C) before counting.

PROCEDURE FOR ASSAY OF PATIENT SAMPLES WITH GREATER THAN 200 U/ML

Accurate determination of CA 15-3 assay values for specimens which exceed 200 U/mL requires that the specimen be further diluted and reassayed. An additional 10-fold or greater dilution of the specimen with an appropriate amount of CA 15-3 0 U/mL Standard/Diluent is recommended. For example: Retrieve the initial 1:51 dilution of the specimen (Step 2 of **CA 15-3 RIA Procedure**). In a separate test tube make an additional 10-fold dilution (1:510) by adding 0.05 mL (50 µL) of the initial specimen dilution to 0.45 mL (450 µL) of CA 15-3 0 U/mL Standard/Diluent. Mix thoroughly before assaying. Perform the assay according to Assay Procedure, starting with Step 3 and using this dilution as the specimen. Obtain results by multiplying the value obtained from the standard curve by 10 (the dilution factor).

NOTE: Standards are supplied pre-diluted 1:51; therefore, the correction factor for the additional 10-fold dilution of the sample is 10 (NOT 510).

FDI CA 15-3 RIA PROCEDURE SUMMARY

Bring all reagents and specimens to room temperature (20° - 30° C). Assay each standard, control, and specimen in duplicate.

First Incubation

- 1 Mark reaction trays and data sheets.
- 2 Dilute samples and the controls, 1:51, by adding 20 µL of sample or controls to 1,000 µL CA 15-3 0 U/mL Standard/Diluent in a tube marked for proper identification of samples and controls.
- 3 Pipette 200 µL of diluted samples and controls into their assigned wells.
- 4 Pipette 200 µL of each standard into its assigned wells. **(Do not dilute standards as directed in Step 2 above.)**
- 5 Dispense one bead into each well.
- 6 Apply adhesive cover sealer and gently tap tray to ensure that beads are covered and that air bubbles are released.
- 7 Incubate for 2 hours ± 5 minutes at room temperature (20° - 30° C).
- 8 Remove adhesive cover sealer, aspirate the liquid, and wash each bead three times with 5 mL distilled or deionized water.

Second Incubation

- 9 Pipette 200 µL of ¹²⁵I-DF3 murine monoclonal into all wells.
- 10 Apply adhesive cover sealer and gently tap tray to cover beads and release air bubbles.
- 11 Incubate for 3 hours ± 10 minutes at room temperature (20° - 30° C).
- 12 Remove cover, aspirate the liquid from wells, and wash beads as in Step 8.
- 13 Transfer beads to the counting tubes.
- 14 Count tubes for 1 minute.

QC CRITERIA

- 1 Values for duplicates should be within 15% of the mean CPM; duplicate values that differ from the mean by greater than 15% should be considered suspect and the sample should be retested. **However, for the 0 U/mL Standard/Diluent, and samples with less than 300 CPM, duplicates may differ more than 15% from the mean CPM.**
- 2 Ensure that the values for the controls fall within the limits indicated on the vial labels. Ensure that other commercial or laboratory-supplied control(s) are within the limits established for these materials.

RESULTS

- 1 Construct a standard curve by plotting the average CPM obtained for each standard on the vertical (Y) axis vs. the corresponding U/mL standard value on the horizontal (X) axis using rectilinear graph paper. Connect the points with straight line segments. Alternatively, enter the data on a computer with the appropriate software for the construction of a point-to-point curve. Values for duplicates should not differ from each other by more than 15% of the mean CPM. Duplicate values, except the 0 U/mL standard, that differ from the mean by greater than 15% should be considered suspect and repeated. A representative standard curve is shown in Figure 1.
- The U/mL value for a sample may also be calculated from the mean CPM. Below is an example calculation for Sample A (Table 1 and Figure 1) using the mean CPM.
- 2 Using the mean CPM value obtained for each specimen or control, determine the corresponding CA 15-3 assay value in U/mL directly from the standard curve.

Note: Because the standards have been prediluted and values assigned accordingly, the unit values of the unknown specimens can be determined directly from the standard curve. A multiplication factor is necessary only when a dilution greater than 1:51 is used.

Example Equation:

A)

$$m = \frac{CPM_H - CPM_L}{U_H - U_L}$$

m = Slope of the line segment in which the sample is located

$$CPM_H = \text{Mean CPM value of the standard which defines the top of the line segment}$$

$$CPM_L = \text{Mean CPM value of the standard which defines the bottom of the line segment}$$

$$U_H = \text{U/mL value of the standard which defines the top of the line segment}$$

$$U_L = \text{U/mL value of the standard which defines the bottom of the line segment}$$

B)

$$U_{unk} = U_H + ((CPM_{unk} - CPM_H)/m)$$

$$U_{unk} = \text{U/mL value of unknown}$$

$$CPM_{unk} = \text{Mean CPM of the unknown}$$

Example Calculations: (Numbers taken from Table 1)

A)

$$m = \text{Slope}$$

$$CPM_H = 6,976 \text{ CPM}$$

$$CPM_L = 3,648 \text{ CPM}$$

$$U_H = 100 \text{ U/mL}$$

$$U_L = 50 \text{ U/mL}$$

$$m = \frac{CPM_H - CPM_L}{U_H - U_L}$$

$$6,976 - 3,648 = 3,328 \text{ CPM}$$

$$100 - 50 = 50 \text{ U/mL}$$

$$3,328/50 = 66.6 \text{ CPM/U/mL}$$

B)

$$U_{unk} = X$$

$$CPM_{unk} = 5,220$$

$$U_{unk} = U_H + ((CPM_{unk} - CPM_H)/m)$$

$$X = 100 + ((5,220 - 6,976)/66.6)$$

$$X = 100 + (-26.4)$$

$$X = 73.6 \text{ U/mL}$$

NOTE: Accurate determination of CA 15-3 assay values for specimens which exceed 200 U/mL requires that the specimen be further diluted and reassayed.

TABLE 1: EXAMPLE RESULTS FROM AN FDI CA 15-3 RIA STANDARD CURVE					
U/mL	0	25	50	100	200
CPM	546	2,125	3,789	6,659	12,509
	449	1,939	3,507	7,293	11,789
Average CPM	498	2,032	3,648	6,976	12,149

EXAMPLE OF ASSAY VALUES FROM UNKNOWN SPECIMENS					
Specimen	CPM	Average CPM	CA 15-3 U/mL from Curve	Multiply by Additional Dilution Factor	Final CA 15-3 U/mL
A	3,378 2,866	3,122	41.8	N/A	41.8
B	15,296 17,404	16,350	>200	—	—
B ¹	4,926 5,626	5,276	74.4	x10	744

¹ 1:10 dilution of sample B.

FDI CA 15-3 RIA STANDARD CURVE

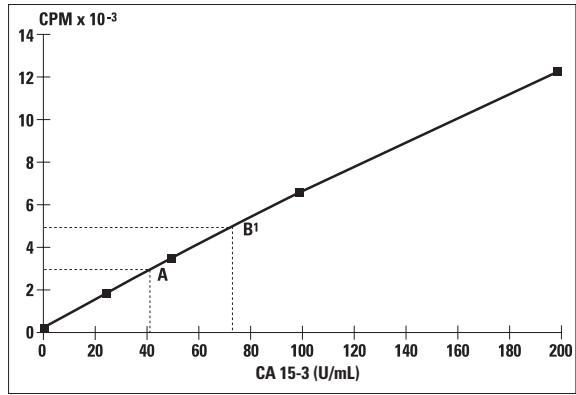


Figure 1: Example of standard curve. For demonstration purposes only.

LIMITATIONS OF PROCEDURE

- CA 15-3 assay values are frequently elevated in the serum of patients with breast cancer and in 34.4% of individuals with other malignancies. Conversely, the CA 15-3 assay is elevated in only 2.5% of normal individuals and 4.8% of individuals with nonmalignant conditions.
- The FDI CA 15-3 RIA is a test for the quantitative measurement of DF3 antibody-defined antigen in the serum and plasma of patients previously treated for stage II or stage III breast cancer. Serial test results obtained with the FDI CA 15-3 RIA, in patients who are clinically free of disease, should be used in conjunction with all relevant information derived from diagnostic tests, physical examination, and full medical history in accordance with appropriate patient management procedures used for early detection of recurrence.
- The assay should not be performed on hemolyzed (indicated by hemoglobin concentrations greater than 0.5 g/dL) or lipemic (indicated by triglyceride concentrations greater than 1,000 mg/dL) samples or those containing elevated bilirubin concentrations (greater than 30 mg/dL) because possible interference has not been investigated above these concentrations. Patient samples containing particulate matter should be clarified by centrifugation at 1,000*g* for 15 minutes before testing.
- With some immunoassays very high antigen levels can result in a prozone or “hook effect.” For the CA 15-3 RIA, antigen levels must be greater than 50,000 U/mL before the assay yields erroneous results of less than 200 U/mL.
- Individuals receiving mouse immunoglobulin by parenteral routes or who have otherwise been exposed to mice may produce anti-mouse antibodies. Serum from such individuals has been reported to produce falsely elevated values in assays which employ mouse monoclonal antibodies.
- It is not possible to extrapolate the standard curve above 200 U/mL. Therefore, values obtained for samples with CPM greater than that obtained for the 200 U/mL standard should not be considered valid. Samples above 200 U/mL must be diluted and retested as described in **PROCEDURE FOR ASSAY OF PATIENT SAMPLES WITH GREATER THAN 200 U/ML**.
- Assay values obtained with different assay methods or from different manufacturers cannot be used interchangeably.

EXPECTED VALUES

An increase to greater than 35 U/mL in serial CA 15-3 assay values, in the serum or plasma of patients previously treated for stage II or stage III breast cancer, who are clinically free of disease, should be used in conjunction with all information derived from other diagnostic tests, physical examination and full medical history in accordance with appropriate patient management procedures used for the early detection of disease recurrence, provided other causes of elevated CA 15-3 assay values can be excluded.

Apparently Healthy Subjects, Patients With Nonmalignant Conditions, and Patients With Malignancies Other Than Breast Cancer

Distribution of CA 15-3 assay values was investigated in a total of 5,682 subjects comprised of 3,799 normal female patients (normals), 884 patients with nonmalignant conditions, and 999 patients with malignant conditions other than breast cancer. CA 15-3 assay values were elevated in 2.5% of normals, 4.8% of patients with nonmalignant conditions, and in 34.4% of patients with malignant conditions other than breast cancer.

Reference Concentration (Cutoff)

A receiver-operator characteristics analysis was conducted to determine the optimum reference concentration. The total population analyzed consisted of 3,216 normal female patients (normals) and 321 female patients with active/metastatic breast cancer. Optimal sensitivity and specificity of the assay were obtained with a reference concentration (cutoff) of 35 U/mL. The same cutoff is appropriate when monitoring breast cancer patients without current evidence of disease who were initially stage II or stage III and who have undergone treatment; however, slight differences in sensitivity and specificity may be observed in this population.

Analytical and Clinical Performance in Breast Cancer Patients

One hundred seventy-three stage II and III breast cancer patients, who were clinically free of disease following surgery, were identified from previously conducted prospective trials of adjuvant therapies. The group included patients who subsequently experienced disease recurrence and patients who experienced no disease recurrence during follow-up. Serial plasma samples from 154 patients who met pre-established eligibility criteria were selected and analyzed in a blinded manner using the FDI CA 15-3 RIA and another commercial method. The analytical and clinical performance of the two assays were compared for all patients meeting requirements as described below.

Analytical Performance in Breast Cancer Patients

Analytical performance of the CA 15-3 assay was evaluated using specimens from 154 eligible breast cancer patients. All samples with values less than 200 U/mL (total 152) were analyzed using the FDI CA 15-3 RIA and another commercial method. The assays were compared for analytical correlation using Bablock-Passing and linear regression analysis. The other commercial method served as the comparative method. The results are summarized below:

Total Patients	Statistical Method	Slope	Intercept	Correlation
152	Bablock-Passing	0.604	7.76	0.669 ¹ (p=0.001)
152	Linear Regression	0.741	6.84	0.865 ² (p=0.001)

¹ Spearman's Rank.

² Pearson's.

The data were also analyzed to determine the concordance (agreement), relative sensitivity, and relative specificity between the two assays using their respective cutoff values. Ninety-two percent of the samples tested using both assays were in agreement.

Comparative Assay	CA 15-3		Total
	≥ Cutoff	< Cutoff	
≥ Cutoff	13	8	21
< Cutoff	4	129	133
Total	17	137	154

Relative sensitivity = 62% (13/21).

Relative specificity = 97% (129/133).

Concordance = 92% (142/154).

Clinical Performance in Breast Cancer Patients

Clinical performance of the FDI CA 15-3 RIA was evaluated in those patients who had samples taken within 6 months of the date of relapse. For patients without relapse, the last sample prior to discontinuation of follow-up was used. The results of the FDI CA 15-3 RIA were compared to another commercial assay and are summarized below.

Diagnosis	# of Evaluable Patients	CA 15-3		Other Assay	
		≥Cutoff	<Cutoff	≥Cutoff	<Cutoff
Recurrence	29	11	18	12	17
No Recurrence	105	4	101	8	97
Total	134	15	119	20	114

Clinical sensitivity was 38% for the CA 15-3 RIA and 41% for the other commercial method. Clinical specificity was 96% for the CA 15-3 RIA and 92% for the other commercial method.

Data were also evaluated using the Cox regression model which indicates that CA 15-3 assay value, age at surgery, and number of positive lymph nodes were statistically significant predictors of breast cancer recurrence in this study population.

NOTE: Factors such as the frequency at which samples are taken and other differences between study populations may affect the clinical performance observed.

SPECIFIC PERFORMANCE CHARACTERISTICS

Reproducibility

Twenty-four coded samples (2 sets of 12 samples) and one kit control were tested in duplicate by five laboratories using three CA 15-3 RIA lots on each of 3 days. The order of the coded samples was randomized for each day's testing. The estimates of intra-assay precision (CVe) ranged from 4.7% - 9.0%. The estimates of combined inter-assay-laboratory-kit lot precision (CVx) ranged from 7.0% - 11.5%. Samples with a mean CA 15-3 assay value around the reference value of 35 U/mL had CVx's in the range of 5.8% - 8.5%.

Recovery Studies and Dilution Linearity

Recovery of exogenous antigen and linearity upon dilution of endogenous antigen was evaluated to determine the effects of serum matrix and antigen dilution on quantitation of CA 15-3 assay values using the FDI CA 15-3 RIA. In the recovery study, a known amount of antigen was added to 10 different serum samples. The average recovery observed was 109% for both antigen diluted in the CA 15-3 0 U/mL Standard/Diluent and in patient samples.

In the Linearity study, 1:2 serial dilutions (up to 1:16) of 12 different patient samples were analyzed and percent recovery calculated. The average recovery observed across all samples was 99.6%.

Interfering Substances

The effect of a number of potentially interfering substances, including naturally-occurring compounds and chemotherapeutic agents, was evaluated by addition of these substances to patient samples. No interference from the following materials was observed at the concentrations indicated:

PHYSIOLOGICAL SUBSTANCES	
Substance	Dosed Value
Bilirubin ¹	30 mg/dL
Lipids	1,000 mg/dL
Hemoglobin	0.5 gm/dL

¹ Either conjugated or unconjugated bilirubin.

CHEMOTHERAPEUTIC DRUGS	
Drug	Therapeutic Range
Fluorouracil	500 ± 260 µmol/L
Cytosax	254.4 ± 45 µmol/L
Mutamycin	0.52 - 2.7 µg/mL
Leucovorin	96 ± 38 µmol/L
Thioplex	0.1 - 1.3 µg/mL
Novantrone	180 - 1474 ng/mL
Methotrexate	2.4 x 10 ⁴ - 1.9 x 10 ⁶ mol
Navelbine	260 - 1230 ng/mL
Velban	2 - 12 ng/mL
Taxol	1.3 - 13.0 µmol/L
Adriamycin	0.13 - 0.74 µg/mL

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Fujirebio Diagnostics, Inc. 201 Great Valley Parkway Malvern, PA 19355 610-240-3800 800-342-9225	Fujirebio Europe BV Takkebijsters 69c 4817 BL Breda The Netherlands
	

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