



FOR THE DETERMINATION OF CA 125 ASSAY VALUES IN SERUM

FUJIREBIO DIAGNOSTICS, INC. (FDI)

Caution: Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory, and use is restricted to by or on the order of a physician.

The **FDI CA 125 II™ RIA** is based on the use of the OC 125 antibody as the tracer antibody, which is available exclusively through FDI, and its licensed distributors. Performance characteristics of kits which employ the OC 125 system are not transferable to those diagnostic kits using different antibodies. The assay value for 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 CA 125 II assay used. Assay values obtained with different assay methods cannot be used interchangeably (1).

FDI CA 125 II RIA

For the determination of CA 125 assay values in serum

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 exercises of regulatory authority.

INTENDED USE

The FDI CA 125 II RIA is an *in vitro* device for the quantitative measurement of OC 125 reactive determinants associated with a high-molecular-weight glycoprotein in serum of women with primary epithelial invasive ovarian cancer, excluding those with cancer of low malignant potential. The FDI CA 125 II RIA is indicated for use as an aid in the detection of residual ovarian carcinoma in patients who have undergone first-line therapy and would be considered for diagnostic second-look procedures. An assay value of greater than or equal to 35 U/mL is predictive of residual disease, provided that alternative causes of an elevated CA 125 assay value can be excluded (see under **LIMITATIONS OF THE PROCEDURE**). It is recommended that the assessment and treatment of patients with ovarian cancer and the use of the FDI CA 125 II RIA be under the order of a physician trained and experienced in the management of the gynecologic cancers.

SUMMARY AND EXPLANATION OF THE TEST

CA 125 assay values are defined by using the OC 125 monoclonal antibody which was produced using lymphocytes from a mouse immunized with OVCA 433, a cell line derived from a papillary serous cystadenocarcinoma of the ovary (2). The CA 125 II RIA is a second-generation assay for the detection of OC 125 reactive determinants on a high-molecular-weight glycoprotein in serum. The assay utilizes M11 monoclonal antibody, as the capture antibody coated onto polystyrene beads that bind molecules containing OC 125 reactive determinants (3). These reactive determinants are quantified using radioiodinated OC 125 antibody as tracer. Since the original CA 125 assay has been defined based on its reactivity with OC 125 antibody, this reagent continues to be used in the CA 125 II RIA to insure the integrity of the assay.

OC 125 reactive determinants can be found in a high percentage of non-mucinous epithelial ovarian tumors (4) and are found in the serum of women bearing such tumors (5,6). OC 125 reactive determinants are not present in the surface epithelium of either normal fetal or adult ovaries, with the exception of inclusion cysts, areas of metaplasia, and papillary excrescences. In fetal tissue, OC 125 reactive determinants have been observed in the amnion and in the derivatives of the coelomic epithelium. Among adult tissues, OC 125 reactive determinants have been identified in the epithelium of fallopian tubes, endometrium, and endocervix (7).

The OC 125 reactive determinants, isolated from either cell culture or serum, are found on a heterogeneous, high-molecular-weight (200 - 1,000 kilodalton) glycoprotein. The determinant is proteinaceous in nature, but has nondeterminant associated carbohydrate moieties (8).

In women with primary epithelial ovarian carcinoma who had undergone first-line therapy and were candidates for diagnostic second-look procedures, a CA 125 assay value greater than or equal to 35 U/mL was found to be indicative of the presence of residual tumor (9-11). Assuming the physician cannot identify alternative causes for an elevated CA 125 assay value, a CA 125 assay value determined to be greater than or equal to 35 U/mL provides substantial evidence that residual tumor is present.

A CA 125 assay value below 35 U/mL does not indicate the absence of residual ovarian cancer because patients with histopathologic evidence of ovarian carcinoma may have CA 125 assay values within the range of healthy individuals (5,17-21, 23,24). Clinical decisions should not be based on an FDI CA 125 II RIA value below 35 U/mL.

CA 125 assay values are elevated in most patients with active epithelial ovarian cancer, including those with stage I disease. In addition, CA 125 assay values are elevated in 1% - 2% of healthy individuals and may be elevated in diseases other than ovarian carcinoma, including both benign and malignant disorders (see Table 4).

CA 125 assay values greater than or equal to 35 U/mL may be found in patients with nonmalignant conditions, such as pericarditis, cirrhosis, severe hepatic necrosis, endometriosis (Stages II-IV), first trimester pregnancy, and ovarian cysts or in patients with non-ovarian malignancies, such as uterine carcinoma, hepatoma, pancreatic adenocarcinoma, and lung cancer (5,6,12-16). CA 125 assay value elevations have been found in women following abdominal or pelvic irradiation. The FDI CA 125 II RIA is a one-time test for the quantitative measurement of CA 125 assay values in the serum of women who have undergone first-line therapy and would be considered for diagnostic second-look procedures. It should be used in conjunction with all clinical information derived from diagnostic tests, physical examination, and full medical history in accordance with appropriate patient management procedures. It is recommended that the FDI CA 125 II RIA be used by or under the order of a physician trained and experienced in the management of gynecologic cancers (29).

PRINCIPLES OF THE PROCEDURE

The FDI CA 125 II RIA is a one-step "sandwich" radiomunoassay. Polystyrene beads coated with the M11 capture antibody reacting with molecules containing OC 125 reactive determinants are incubated with the patient sample, standards or control, and tracer. The tracer, composed of ¹²⁵I-labeled (mouse monoclonal) OC 125, quantifies the number of OC 125 reactive determinants and is distinct from the solid phase antibody. During this incubation, molecules which contain OC 125 reactive determinants form "sandwich" complexes with the monoclonal antibodies. Unbound materials present in the patient sample are removed by aspiration of the fluid and washing of the beads. The bound radioactivity is proportional to the concentration of the OC 125 reactive determinant (antigen) in the patient sample within the working range of the assay. A standard curve is obtained by plotting the U/mL of CA 125 II Standards vs. bound radioactivity (counts per minute [CPMs]). The CA 125 assay values for patient samples and controls, run concurrently with the standards, can be determined from the standard curve.

REAGENTS

- 1 **COATED BEADS** CA 125 II Capture Antibody (M11, mouse, monoclonal), 1 vial containing 100 polystyrene beads.
 - 2 **STANDARD/DILUENT** CA 125 II Standard/Diluent (with preservative), 0 U/mL* 1 vial, 5.0 mL.
 - 3 **STANDARDS** CA 125 II Standards, 15, 30, 80, 200, and 500 U/mL* (in recalcified defibrinated human plasma** with preservative) 5 vials, 0.8 mL each.
 - 4 **CONTROLS** CA 125 II Control L: Low control in recalcified defibrinated human plasma** (with preservative, assay value and range are printed on vial label), 1 vial, 0.8 mL.
CA 125 II Control H: High control in recalcified defibrinated human plasma** (with preservative, assay value and range are printed on vial label), 1 vial, 0.8 mL.
 - 5 **TRACER** ¹²⁵I-OC 125 (mouse) monoclonal (in sodium citrate buffer with protein stabilizers and preservative), 2 vials, 5.0 mL each. Radioactivity maximum: less than 1.30 µCi/mL (less than 48.2 kBq/mL).
- * CA 125 assay values are expressed as units per mL (U/mL). A unit is an arbitrary value related to an FDI-maintained reference standard. There is no generally available reference standard at this time.
** Tested in accordance with current FDA-required assays for blood-borne pathogens.

Materials supplied perform 100 tests. DISCARD any component 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 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 is to be disposed of into appropriately labeled waste containers, according to State or Federal requirements. Radioactive material should be stored in a properly designated area.
- 6 Sodium azide has been used in FDI CA 125 II RIA reagents as a preservative. Sodium azide has been reported to form lead or copper azide 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 MATERIAL 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 Store all reagents at 2° - 8°C when received. Avoid freezing of reagents.
- 2 All reagents must be brought to room temperature (20° - 30°C) before use and immediately 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. Reference should be made to the instruction manual supplied with the instrument. It is recommended that counters used have efficiencies of greater than 60%. If counter efficiency is less than 60%, longer counting periods should be used.

PATIENT SAMPLE COLLECTION AND HANDLING

Serum is required for testing with the FDI CA 125 II RIA. Separation of serum from clot should be done within 24 hours after collection. Whenever possible, patient samples should be clear and nonhemolyzed. Patient samples containing particulate matter should be clarified by centrifugation at 1,000g for 15 minutes before testing. Serum should be fresh, not frozen, and thawed repeatedly. If serum is to be stored for future repeat tests, aliquot samples to avoid repeated freezing and thawing.

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

The assay should not be performed until at least 3 weeks after the completion of primary chemotherapy and at least 2 months following abdominal surgery.

MATERIALS PROVIDED

FDI CA 125 II RIA Kit, 100 Test Unit (refer to **REAGENTS** for a list of material provided). An appropriate number of the following accessory sets are provided for test performance.

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips to deliver 0.1 mL (± 1%).
- Distilled or deionized water for use in bead washing operation.
- 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. It is recommended that counters used have an efficiency greater than 60%. If counter is less than 60%, longer counting periods should be used.
- Bead dispenser device, e.g., commercial single- or multiple-bead dispenser, or nonmetallic forceps.
- Rectilinear graph paper or appropriate software for the construction of a point-to-point curve.

CA 125 II RIA PROCEDURE

NOTE: Each standard, control, and patient sample should be assayed in duplicate each time the assay 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 contamination.

- 1 Identify reaction tray wells for testing the standards, controls, and patient samples. Six standards and two controls should be run with each series of

unknown samples. Reaction trays containing standards and controls should be subject to the same manipulations and incubation times as the sera being tested.

- 2 Pipette 0.1 mL (100 µL) of standards, controls, and patient samples into their assigned wells.
- 3 Dispense one bead into each reaction well. Beads may be transferred by use of clean forceps or by use of a single- or multiple-bead dispensing device.
- 4 Pipette 0.1 mL (100 µL) of ¹²⁵I-OC 125 (tracer, mouse monoclonal) into each of the wells. The tracer contains a red dye to offer visual assurance that the reagent has been pipetted into all appropriate wells.
- 5 Make sure that each bead is completely covered. Apply a cover sealer to each tray. Gently tap the tray to assure mixing of the solutions and to eliminate any air bubbles trapped in the reaction wells. Be careful not to splash liquid onto cover.
- 6 Incubate the trays at room temperature (20° - 30° C) for 20 ± 2 hours.
- 7 At the end of the 20-hour incubation, carefully remove and discard the cover sealer. Wash each bead according to the appropriate procedure below.

Wash Procedure

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 additional 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 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 with a Cornwall Syringe or equivalent, 5 mL of distilled or deionized water. The bead must be totally immersed throughout the wash procedure. Care should be taken not to overflow the well. Repeat the wash procedure two additional times for a total wash volume of 15 mL.

- 8 Transfer the beads from the reaction trays to the transfer trays containing the plastic tubes by aligning the numbers and FDI logo on the bottom of the transfer system with the numbers and logo on the reaction tray. Invert both the tube rack and 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.
- 9 Properly identify all tubes either prior to or after the transfer of beads.
- 10 Place the counting tubes in a suitable well-type gamma counter. Count the radioactivity in each tube for 1 minute. All standards, controls, and patient samples must be counted together.

PROCEDURE FOR ASSAY OF PATIENT SAMPLES WITH GREATER THAN 500 U/ML

If in an initial assay, a patient sample is found to contain a CA 125 assay value of greater than 500 U/mL, a 10-fold dilution of the patient sample with an appropriate amount of CA 125 II Diluent is recommended, e.g., a 10-fold dilution is prepared by adding 0.05 mL (50 µL) of patient sample to 0.45 mL (450 µL) of CA 125 II diluent. Mix thoroughly before assaying. Perform the assay according to **CA 125 II RIA PROCEDURE**. Multiply the value in U/mL by a factor of 10 (see Table 2). Continue with 10-fold dilutions until the U/mL assay value for the sample falls within the standard curve. Multiply the U/mL assay value by the appropriate dilution factor.

FDI CA 125 II RIA PROCEDURE SUMMARY

- 1 Allow all reagents and patient samples to reach room temperature before use.
- 2 Pipette 0.1 mL of standards, control, or patient sample into appropriate well of reaction tray.
- 3 Dispense one antibody-coated bead into each well.
- 4 Pipette 0.1 mL of ¹²⁵I OC 125 (mouse, monoclonal) into each well. Ensure that beads are completely covered.
- 5 Apply adhesive cover sealer and gently tap tray to mix reagents and to ensure beads are covered and that air bubbles are released.
- 6 Incubate for 20 ± 2 hours at room temperature.
- 7 Remove cover sealer, aspirate the liquid, and wash each bead three times with a total of 15 mL distilled or deionized water.
- 8 Remove all excess liquid from tray by aspiration.
- 9 Immediately transfer beads to the counting tubes.
- 10 Count radioactivity for 1 minute per tube.
- 11 Construct standard curve by plotting average CPM (Y axis) vs. concentration (x axis) for CA 125 II standards. Connect points with straight line segments.
- 12 Determine unknown concentration from standard curve.

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 samples with less than 300 CPM, duplicates may differ more than 15% from the mean CPM. Since such samples fall well below the 35 U/mL cutoff assay value, retesting of these samples may not be necessary.
- 2 Ensure that values for the controls fall within the limits indicated on the vial labels.
- 3 Ensure that the slope of the line segment between the 30 U/mL standard and 80 U/mL standard is at least 30 CPM/U. The slope can be calculated using the following equation:
$$\text{Slope} = \frac{\text{CPM}_{80 \text{ std}} - \text{CPM}_{30 \text{ std}}}{50}$$
- 4 Ensure that Y intercept (CPM) as extrapolated from the line segment between the 30 U/mL standard and 80 U/mL standard is in a range between -1,100 CPM to + 450 CPM.

This can be calculated using the following equation:

$$\text{Y intercept} = \text{CPM}_{80 \text{ std}} - (80 \times \text{slope})$$

If conditions 2, 3, and 4 are not met, the assay should be repeated.

RESULTS

- 1 Construct a standard curve by plotting the average CPM obtained for each CA 125 II Standard on the vertical (Y) axis vs. the corresponding CA 125 II standard U/mL 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. A representative standard curve is shown in Figure 1.

Example Equation:

A)
$$m = \frac{\text{CPM}_H - \text{CPM}_L}{U_H - U_L}$$

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

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

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

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

B)
$$U_{\text{unk}} = \frac{\text{CPM}_H - \text{CPM}_L}{m}$$

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

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

Example Calculation: (Numbers taken from Table 1)

A)
$$m = X$$

$$\text{CPM}_H = \mathbf{11,714}$$

$$\text{CPM}_L = \mathbf{4,193}$$

$$U_H = 80$$

$$U_L = 30$$

$$m = \frac{\text{CPM}_H - \text{CPM}_L}{U_H - U_L}$$

$$X = \frac{11,714 - 4,193}{80 - 30} = \frac{7,521}{50} = 150.4$$

B)
$$U_{\text{unk}} = X$$

$$\text{CPM}_{\text{unk}} = 5,552$$

$$U_{\text{unk}} = \frac{\text{CPM}_H - \text{CPM}_L}{m}$$

$$X = \frac{80 + [(5,552 - 11,714)/150.4]}{X} = \frac{80 + (-41.0)}{X} = 39.0 \text{ U/mL}$$

- 2 Using the mean CPM value for each patient sample, determine the corresponding CA 125 II assay value in U/mL from the standard curve.
- 3 Reassay samples in which the CA 125 assay values fall within the range of 26 - 52 U/mL (grey zone). If initial and retest values are in agreement relative to the cutoff point (>35 U/mL), values can be averaged. If initial and retest values are in discord, report the value which is < 35 U/mL.

TABLE 1. EXAMPLE OF CPM FOR FDI CA 125 II RIA STANDARDS						
U/mL on Vial	0	15	30	80	200	500
CPM	141	2,305	4,336	11,104	28,116	71,297
	157	2,087	4,050	12,324	30,454	75,127
Average CPM	149	2,196	4,193	11,714	29,285	73,212

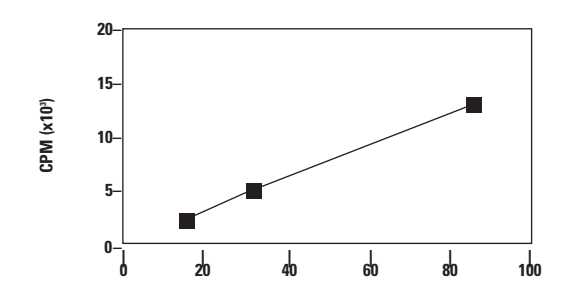
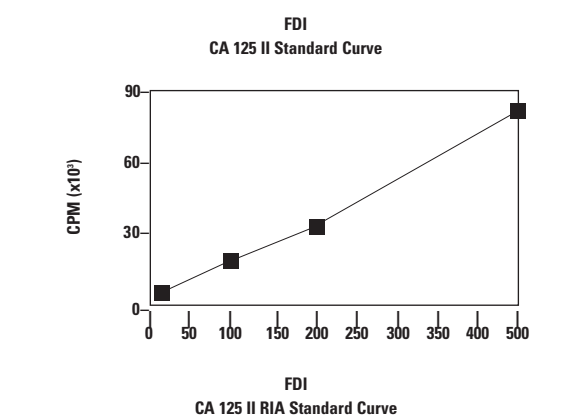


Figure 1. Example of standard curve for demonstration purposes only. Do not use in place of standard curve for assay.

TABLE 2. EXAMPLE OF ASSAY VALUES OF PATIENT SAMPLES					
Patient Supply	CPM	Average CPM	CA 125 U/mL From Curve	Multiply Dilution Factor	U/mL
A	5,235 5,868	5,552	39.0	1	39.0
B	21,981 23,113	22,547	154.0	1	154.0
C	44,950 46,750	45,850	313.1	1	313.1
D	104,276 107,932	106,104	>500		
Sample D Diluted (1:10)	23,376 25,390	24,383	166.5	10	1,665.0

LIMITATIONS OF PROCEDURE

- 1 CA 125 assay values greater than or equal to 35 U/mL may be found in 1% -2% of healthy individuals and in patients with nonmalignant conditions, such as pericarditis, cirrhosis, severe hepatic necrosis, endometriosis (Stages II-IV), first trimester pregnancy, and ovarian cysts or in patients with non-ovarian malignancies, such as uterine carcinoma, hepatoma, pancreatic adenocarcinoma, and lung cancers (5-7,12-16).
A CA 125 II assay value below 35 U/mL does not indicate the absence of residual ovarian cancer because patients with histopathologic evidence of ovarian carcinoma may have CA 125 II assay values within the range for healthy individuals (5,7,12,17-21). Clinical decisions should not be based on a CA 125 assay value below 35 U/mL.

The FDI CA 125 II RIA is a one-time test for the quantitative measurement of CA 125 assay values in the serum of women who have undergone first-line therapy and would be considered for diagnostic, second-look procedures. It should be used in conjunction with all clinical information derived from diagnostic tests, physical examination, and full medical history in accordance with appropriate patient management procedures. It is recommended that the FDI CA 125 II RIA be used by or under the order of a physician trained and experienced in the management of gynecologic cancers.

2 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) since possible interference has not been investigated above these concentrations. A patient sample containing particulate matter should be clarified by centrifugation at 1,000g for 15 minutes before testing.

3 In clinical testing very high concentrations of CA 125 levels may result in a prozone effect in a simultaneous sandwich format. This prozone or "hook" effect is not pronounced, in that, addition of greater than 100,000 U/mL of antigen is required before an erroneous assay value below the 35 U/mL cutoff is found.

4 Individuals receiving mouse immunoglobulin by parenteral routes may produce antimouse antibodies. Serum from such individuals may produce erroneous results (25-28).

5 The assay should not be performed until at least 3 weeks after the completion of primary chemotherapy and at least 2 months after abdominal surgery. This is recommended because it is not clear what effect, if any, these procedures may have on the CA 125 assay value.

6 Considering the coefficient of variation for the FDI CA 125 II RIA, any CA 125 assay value which falls in the range of 26 - 52 U/mL is considered suspect and should be repeated.

7 It is not possible to extrapolate the standard curve above 500 U/mL. Therefore, values obtained for samples with CPM greater than that obtained for the 500 U/mL standard should not be considered valid. Samples above 500 U/mL must be diluted and retested as described in **PROCEDURE FOR ASSAY OF PATIENT SAMPLES WITH GREATER THAN 500 U/ML**.

EXPECTED VALUES

Women With Ovarian Carcinoma at Second Look

A multicenter clinical study was conducted in women with primary epithelial ovarian carcinoma who had completed first-line therapy, were clinically free of disease, and were scheduled for diagnostic second-look procedures. The objective of this clinical study was to determine the specificity, sensitivity, and positive and negative predictive values of CA 125 assay value results. Subsequent studies demonstrate concordance between the CA 125 RIA and the CA 125 II RIA.

The following inclusion criteria were used: 1) Histopathologic confirmation of primary epithelial ovarian cancer; 2) Second-look surgery performed by laparotomy; 3) No clinical evidence of disease after first-line therapy; and 4) CA 125 assay values obtained in blinded fashion within 7 days prior to diagnostic second-look surgery. Subsequent to defining patients who fulfilled these criteria, those patients who had either a history of or the presence of a second non-ovarian primary malignancy were excluded. The final study population was comprised of 73 women with a mean age of 56 ± 11 years. There were 11 women with stage I, 10 with stage II, 46 with stage III, and 6 with stage IV ovarian carcinoma. (Standard clinical staging as described by FIGO, International Federation of Gynecology and Obstetrics: classification and staging of malignant tumors in the female pelvis. Acta Obstet. Gynecol. Scand. 50:1, 1971.) Histopathologic types included 39 women with serous, 2 with mucinous, 3 with endometrioid, 2 with clear cell, 9 of mixed type, and 18 with unclassified adenocarcinomas.

Fifty-eight of 73 patients had serum CA 125 II assay values that were negative (less than 35 U/mL) and 15 had positive values (greater than or equal to 35 U/mL). Although no patient who entered into this study had clinically apparent disease at the time of diagnostic second-look surgery, 36 of 73 patients had evidence of residual disease based upon the histopathologic assessment of samples obtained during diagnostic second-look surgery. Fifteen of these 36 patients with residual disease had a CA 125 assay value greater than or equal to 35 U/mL. Moreover, no patient (0 of 37) with a negative diagnostic second look surgery had a serum CA 125 assay value greater than 35 U/mL, i.e., there were no false-positive assay results in this study population. However, there were a substantial number of false-negative CA 125 assay values, occurring in 21 of 36 patients.

Of the 37 patients without evidence of recurrent disease at diagnostic second-look surgery, all 37 had true-negative assay results, indicating a specificity of 100% with a lower confidence limit of 92%. In addition, of 15 patients with a positive serum CA 125 II assay value, all 15 were true positives, resulting in positive predictive values of 100% with a lower confidence limit of 82%. The sensitivity (42%) of the FDI CA 125 II RIA is not high when compared to histopathologic assessment at diagnostic second-look surgery; however, all patients included in this study had no clinical evidence of disease and therefore represent a population group with minimal disease that already have been treated.

Extended Study in Women With Ovarian Cancer

The 73 patients discussed above met all of the criteria for inclusion in the clinical study. Data were also available from an additional 51 patients included in the multicenter study and from 17 patients reported in the literature (11). Although these additional 68 patients do not meet all of the strict requirements for inclusion in the study, they are representative of patients encountered in routine gynecologic practice. Findings for the original study, for the extended study, and for the two data sets combined are as follows in Table 3.

TABLE 3. SUMMARY OF CLINICAL STUDIES			
	Original Study	Extended Study	Combined Study (N=141)
Sensitivity	15/36 (42%)	18/36 (50%)	33/72 (46%; 34-58%*)
Specificity	37/37 (100%)	31/32 (97%)	68/69 (99%; 91-99%)
Positive Predictive Value	15/15 (100%)	18/19 (95%)	33/34 (97%; 84-99%)
Negative Predictive Value	37/58 (64%)	31/49 (63%)	31/49 (63%; 54-73%)
* 95% Confidence Interval.			

Results from these studies indicate that an elevated CA 125 II assay value (greater than or equal to 35 U/mL) following the first-line therapy for primary epithelial ovarian carcinoma is specific and predictive of residual disease prior to diagnostic second-look surgery. A negative CA 125 assay value (less than 35 U/mL) obtained following first-line therapy in patients with primary epithelial invasive ovarian cancer does not exclude residual disease.

Apparently Healthy Subjects, Patients With Nonmalignant Conditions, and Patients With Gynecologic and Other Malignancies

The objective of this study was to define the distribution of CA 125 II assay values in apparently healthy subjects, patients with nonmalignant conditions, and patients with gynecologic and other types of malignancies. Sera from six investigational sites were evaluated as part of a retrospective study. The sera from 3,294 subjects were analyzed in this study; 573 males, 2,701 females. Six hundred and seventy-four of the 1,659 apparently healthy and non-hospitalized females were 50 years of age or greater.

For the group of 809 apparently healthy females, the median CA 125 II assay value was 10.5 U/mL. Employing a reference assay value of 35 U/mL, 1.6% of the apparently healthy females had CA 125 assay values greater than or equal to 35 U/mL. Four (0.5%) of these healthy female population had CA 125 assay values > 65 U/mL. Also analyzed were a group of non-hospitalized females (n=855) which included a large subset greater than 50 years and included females with a variety of benign conditions (22). In this non-hospitalized population the median CA 125 assay value was 10.4 U/mL. Employing a reference value of 35 U/mL, 2.2% had CA 125 assay values greater than or equal to 35 U/mL. One (0.1%) had CA 125 assay values > 65 U/mL.

The distribution of CA 125 assay values in apparently healthy individuals, non-hospitalized females, and subjects with benign and malignant diseases are summarized in Table 4.

TABLE 4. SUMMARY OF DISTRIBUTION OF CA 125 II RIA ASSAY VALUES IN WOMEN AND MEN

A. HEALTHY SUBJECTS					
Healthy Subjects	Number	<35 U/mL	>35 <65 U/mL	>65 <100 U/mL	>100 U/mL
Males	344	340	4	0	0
Females	809	794	11	3	1
< 50 years	685	673	9	3	0
> 50 years	124	121	2	0	1
Smokers	46	44	0	0	2
Total	1,199	1,178	15	3	3

B. NON-HOSPITALIZED WOMEN					
Healthy Subjects	Number	<35 U/mL	>35 <65 U/mL	>65 <100 U/mL	>100 U/mL
< 50 years	300	290	9	1	0
> 50 years	550	541	8	0	1
Total	855*	836	17	1	1
*Total includes 5 individuals whose age was unreported.					

C. NON-MALIGNANT CONDITIONS					
Population	Number	<35 U/mL	>35 <65 U/mL	>65 <100 U/mL	>100 U/mL
Males	77	68	6	3	0
Females	491	421	47	11	12
Gynecologic Diseases	339	291	37	4	7
Pregnancy	39	28	4	3	4
Gastrointestinal Diseases	156	144	8	3	1
Lung Disease	27	19	4	4	0
Breast Diseases	7	7	0	0	0
Total	568	489	53	14	12

D. MALIGNANT DISEASES					
Population	Number	<35 U/mL	>35 <65 U/mL	>65 <100 U/mL	>100 U/mL
All Patients					
Males	149	121	12	6	10
Females	523	346	41	28	108
Gynecologic Cancers					
Total	336	192	26	21	97
Ovarian	118	16	11	9	82
Other	218	176	15	12	15
Gastrointestinal Cancers	227	198	13	6	10
Lung Cancer	48	27	9	5	7
Breast Cancer	61	50	5	2	4
Total	672	467	53	34	118

SPECIFIC PERFORMANCE CHARACTERISTICS

Reproducibility

The reproducibility of the FDI CA 125 II RIA was evaluated as a balanced three-way crossed classification. It was carried out over 3 consecutive days, at six sites, using aliquots from 14 samples (12 serum and 2 positive controls), and kits from three CA 125 II RIA lots. Each assay value was the average of duplicate determinations. The variances were examined for apparent sample to sample differences to determine whether results could be pooled for further analysis. The reproducibility was determined separately for each of the 14 serum samples because there was an increase in variance with increase in concentration of antigen in serum samples. Mean CA 125 assay values for site, day, and lot for each sample are presented in Table 5. There were no consistent patterns in the order of the site, day, or lot means over the 14 samples.

TABLE 5. ESTIMATES OF REPEATABILITY (So), AMONG SITES' STANDARD DEVIATION (Ss), REPRODUCIBILITY (Sx), AND COEFFICIENT OF VARIATION (CVx)						
Sample	S2o	S2s	S2x	Sx	Mean	CVx*
High	42.07	7.73	49.80	7.06	11.87	6.31
Low	10.75	4.75	15.50	3.94	56.19	7.01
1	1.90	0.60	2.50	1.58	14.61	10.81
2	5.03	3.32	8.35	2.89	33.31	6.68
3	12.40	2.04	14.44	3.80	50.36	7.55
4	700.72	311.90	1012.62	31.82	400.48	7.95
5	531.70	233.67	775.37	27.48	321.30	8.55
6	423.74	32.87	506.61	22.51	279.60	8.05
7	191.32	164.66	355.98	18.87	198.29	9.52
8	32.95	11.13	44.08	6.64	84.29	7.04
9	38.28	20.19	58.47	7.65	91.46	8.36
10	6.93	3.32	10.25	3.20	43.83	7.30
11	4.12	0.59	4.71	2.17	21.63	10.04
12	4.37	2.20	6.57	2.56	25.56	10.02
* CVx = (Sx/mean) x 100.						

Estimates of Sx² were calculated using the formula:

Sx² = So² + Ss²

Where So² is the sum of the variance components due to error, lot, day, and lot x day, while Ss² is the variance components associated with site and the interactions site X lot and site X day. Estimates used in the calculations of reproducibility standard deviation (Sx) and the coefficient of variation (CVx). Negative variance estimates were converted to zeroes for the calculations. The estimates of CVx fall within a narrow range: 6.31 - 10.81, showing good reproducibility.

Precision

A study was carried out to determine the precision of the FDI CA 125 II RIA at 12 concentrations of antigen ranging from 14 - 400 U/mL. Four replicates of each sample were assayed on 3 days at 6 sites, using kits from three master lots. Results are shown in Table 6.

TABLE 6. PRECISION AT VARYING CONCENTRATIONS OF OC 125 REACTIVE DETERMINANTS (ANTIGEN)				
Sample	N	Mean (U/mL)	Root MSE* (U/mL)	CV(%)**
1	212	14.4	1.24	8.6
2	208	33.3	2.49	7.5
3	212	50.4	3.87	7.7
4	203	398.8	39.25	9.8
5	210	321.4	25.16	7.8
6	212	279.6	21.57	7.7
7	198	198.3	15.08	7.6
8	200	84.4	6.56	7.8
9	212	91.5	7.07	7.7
10	210	43.9	3.12	7.1
11	212	21.5	2.07	9.6
12	212	25.5	2.06	8.1
* Square root of the mean square error (MSE) from analysis variance.				
** Calculated by dividing the Root MSE by the corresponding sample mean.				

Recovery Studies

This recovery study was performed to determine the effect of serum matrix on quantitation of CA 125 assay values detected with the FDI CA 125 II RIA. Serum from a patient of CA 125 assay value was added to 10 individual sera from 10 healthy individuals and compared with the value obtained by adding the same sample to the CA 125 II Zero Standard/Diluent. Three CA 125 levels were prepared in each individual sample: high (H), 285.2 U/mL; medium (M), 135.2 U/mL; and low (L), 69.8 U/mL. Recoveries of CA 125 from these samples are shown in Table 7.

TABLE 7. RECOVERY				
CA 125 II U/mL				
Sample	N	Expected	Observed	95% Confidence Interval
H	30	285.2	281.4	266.1, 296.7
M	30	135.2	128.8	121.6, 136.0
L	30	69.8	66.0	61.71, 70.6

Linearity Studies

The linearity of the FDI CA 125 II RIA was tested with serial dilutions of 12 individuals with elevated CA 125 assay values. Dilutions were prepared in CA 125 II Zero Standard/Diluent. Regression analysis comparing observed and expected CA 125 assay values for the samples yielded an average intercept of -6.5, and an average slope of 1.009. A slope of 1 and intercept of 0 demonstrate linearity. The mean percent recovery for the 12 samples was 93.8% with a range of 83.9% - 101.4% for the individual samples.

A small number of clinical patient samples have been observed which do not dilute linearly. These patient samples had clearly elevated levels and were always well above the reference level of 35 U/mL.

Interfering Substances

Therapeutic and toxic levels of acetaminophen, digoxin, gentamicin, lithium, phenobarbital, phenytoin, quinidine, salicylate, theophylline, amikacin, amitriptyline, caffeine, carbamazepine, chloramphenicol, clonazepam, cortisol, cyclosporine, desipramine, disopyramide, estriol, ethosuximide, imipramine, kanamycin, lidocain, methotrexate, NAPA, netilmicin, primidone, propranolol, streptomycin, valproic acid, and tobramycin were tested with the FDI CA 125 II RIA. No significant differences were observed among therapeutic and toxic drug levels compared with control CA 125 assay values, indicating that these drugs did not interfere with the FDI CA 125 II RIA.

Hemoglobin (up to 0.5 g/dL) and bilirubin (up to 30 mg/dL) do not interfere with the FDI CA 125 II RIA.

Lipemia, as indicated by triglyceride concentrations up to 1,000 mg/dL, does not interfere with the FDI CA 125 II RIA.

Individuals receiving mouse immunogloblin by parenteral routes may produce antimouse antibodies. Serum from such individuals may produce erroneous results in the CA 125 II RIA (25-28).

Minimum Detectable Dose

An estimate of the minimal detectable dose for the FDI CA 125 II RIA was determined based on data obtained by using multiple kits. The sensitivity of the CA 125 II RIA is approximately 0.4 U/mL. This was calculated as the concentration which was distinguishable from zero, that is, two standard deviations above zero.

Parallelism

The relationship between OC 125 reactive determinants (antigen) derived from OVCA 433 cell culture supernatants and that found in the sera of a patient with ovarian cancer was evaluated using the CA 125 II RIA. The data indicate no significant difference in linearity or parallelism between the patient sample antigen and the tissue culture antigen used to produce standards.

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