Enzyme Immunoassay for the quantitative determination of Ovarian Cancer Antigen CA125 in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of the Cancer Antigen CA125 concentration in human serum.

INTRODUCTION

Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma.

The serum CA125 concentration is greater than 35 units per ml in 60% of women with ovarian cancer and >80% of patients with disseminated ovarian cancer. The serum CA125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serial determinations of serum CA125 as well as pelvic examination increase the test specificity. Serum CA125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

PRINCIPLE OF THE TEST

The CA125 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[iii] The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA125 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at 37°C for 90 minutes, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20

minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Antibody-Coated Wells (1 plate, 96 wells)
  Microtiter Wells coated with CA125 MoAb
- Reference Standard Set (1.0 ml/vial)
  Contains 0, 15, 50, 100, 200, and 400 Unit/ml of CA125 in bovine serum with preservatives; liquid, ready to use
- CA125 Enzyme Conjugate Reagent (13 ml)
  Contains CA125 MoAb conjugated to horseradish peroxidase with preservatives
- TMB Reagent (11 ml)
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- Stop Solution -1N HCl (11 ml)
  Diluted hydrochloric acid

Materials required but not provided:

- Precision pipettes and tips: 100 µl
- Disposable pipette tips.
- Distilled water.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Specimens which cannot be assayed within 24 hours of collection should be frozen at –20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. <u>It is recommended that the wells be read within 15 minutes following addition of Stop Solution.</u>

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 100 µl of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100 µl Enzyme Conjugate Reagent into each well.
3. Mix gently for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at 37°C for 90 minutes.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well.
10. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Read the optical density at 450 nm with a microtiter plate reader **<u>within 15 minutes</u>**.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA125 in U/ml from the standard curve.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against CA125 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

CA125 Values (U/ml)	Absorbance (450 nm)
0	0.051
15	0.178
50	0.488
100	0.929
200	1.620
400	2.865

EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA125 assay values below 35 U/ml. The minimum detectable concentration of CA125 in this assay is estimated to be 5 U/ml.

Performance Characteristics

1. Accuracy

A statistical study using patient samples demonstrated good correlation of results with the commercially available kits as shown below:

Comparisons between CA 125 ELISA Test and Abbott AxSym CA 125 ELISA kits provide the following data:

N = 142
Correlation coefficient = 0.978
Slope = 0.951

Intercept = 29.98
CA 125 ELISA Test Mean = 393 U/mL
Abbott AxSym Mean = 403 U/mL

2. Sensitivity

The minimal detectable concentration of CA 125 by this assay is estimated to be 5 U/mL.

3. Hook Effect

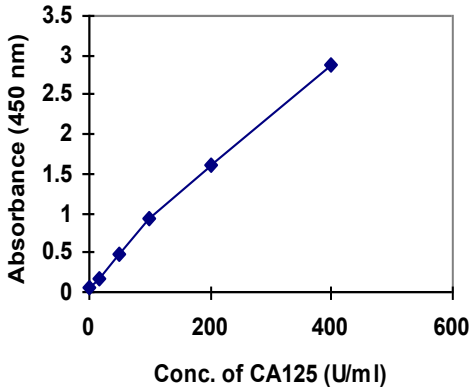
No hook effect was observed in this assay at CA 125 concentrations up to 100,000 U/ml.

4. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of six different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4	5	6
# Replicates	24	24	24	24	24	24
Mean CA 125 (U/ml)	10	29	52	123	253	380
S.D.	1.0	1.3	3.5	11	7.6	13
C.V. (%)	9.7	4.4	6.8	9.3	3.0	3.4



b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4	5	6
# Replicates	20	20	20	20	20	20
Mean CA 125 (U/ml)	11	28	55	126	249	361

S.D.	0.4	1.4	1.6	8.5	13	8.0
C.V. (%)	3.2	5.1	2.9	6.8	5.1	2.2

5. Specificity

The following were tested for cross-reactivity at concentrations up to the levels indicated below. The cross reactivities were probably due to the fact that cancer cells had been metastasized through the body to other organs, including the ovaries.

Material Tested	Test Concentration	Produced Intensity Equivalent to CA 125 (U/mL)
AFP	50,000 ng/mL	<5
	25,000 ng/mL	<5
	10,000 ng/mL	0
CEA	100,000 ng/mL	5
	50,000 ng/mL	<5
	25,000 ng/mL	<5
	10,000 ng/mL	0
Estradiol	1,000 U/mL	<5
	100 U/mL	<5
CA 15-3	3,190 ng/mL	14
CA 19-9	1,351 ng/mL	<5
PAP	6,000 ng/mL	0
	12,000 ng/mL	<5

6. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known human CA 125 levels were combined and assayed in duplicate. The mean recovery was 99.7%.

	Expected Conc. (U/ml)	Observed Conc. (U/ml)	% Recovery
1	359.00	355.00	98.9
.	373.50	353.00	94.5
2	147.00	159.00	108.2
.	96.00	88.00	91.7
3	65.50	66.00	100.8
.	66.50	66.00	99.2
4	11.45	13.00	113.5
.	12.15	11.00	90.5
5			
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6			
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	Average Recovery = 99.7%		

b. Linearity

Four patient samples were serially diluted to determine linearity. The mean recovery was 106.3%.

#	Dilution	Expected Conc. (U/ml)	Observed Conc. (U/ml)	% Expected
1.	Undiluted 1:2 1:4 1:8 1:16 1:32 1:64	----- 170.52 85.26 42.63 21.32 10.66 5.33	341.04 168.14 82.23 43.09 23.81 11.69 5.83	----- 98.6 96.4 101.1 111.7 109.7 109.5
				Mean = 104.5%
2.	Undiluted 1:2 1:4 1:8 1:16 1:32	----- 87.84 43.92 21.96 10.98 5.49	175.67 83.96 42.92 21.63 13.48 6.56	----- 95.6 97.7 98.5 122.8 119.6
				Mean = 106.8%
3.	Undiluted 1:2 1:4 1:8 1:16 1:32	----- 90.13 45.06 22.53 11.27 5.63	180.25 82.00 41.40 22.48 12.14 7.02	----- 91.0 91.9 99.8 107.8 124.7
				Mean = 103.0%
4.	Undiluted 1:2 1:4	----- 175.45 87.72 43.86	350.89 165.10 84.27 42.20	----- 94.1 96.1 96.2

	1:8 1:16 1:32 1:64	21.93 10.97 5.48	26.86 14.20 6.92	122.5 129.5 126.2
				Mean = 110.8%

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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