OVARIAN CANCER ANTIGEN (CA-125)

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10103

Enzyme Immunoassay for the Quantitative Measurement of Ovarian Cancer Antigen (CA-125) in Human Serum

Intended use

The CA-125 ELISA test is primarily intended for use as a monitoring and screening test. An abnormal result (i.e., elevated serum CA-125 level) can indicate ovarian cancer and suggests the need for further clinical management. The serum CA-125 test appears to be a useful tumor marker for patients in clinical remission, following treatment. Post-operative serum CA-125 values that fail to return to normal, strongly suggest the presence of residual tumor. Tumor recurrence is often accompanied by a rise of serum CA-125 before progressive disease is clinically evident.

Introduction

One in every 70 American women will develop ovarian cancer in her life. There are approximately 20,000 new cases of ovarian cancer diagnosed every year and more than 12,000 women die each year because of it. Ovarian cancer is the most malignant type of gynecological cancers, with an overall 5-year survival rate of only 30%. This is because diagnosis is often not made until the advanced stage. Cancer Antigen 125 (CA-125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA-125 is associated with a high molecular weight glycoprotein. Serum concentrations of this tumor marker can be detected and measured by a murine monoclonal antibody. Published studies have indicated that elevated serum CA-125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma. Serum CA-125 levels higher than normal can also be found in individuals with adenocarcinoma of the fallopian tube endometrium, certain non-gynecologic malignancies and some non-malignant conditions.

The serum CA-125 concentration is greater than 35 units per mL in about 60% of women with ovarian cancer. More than 80% of patients with disseminated ovarian cancer have serum CA-125 concentrations greater than 35 units per mL. The serum CA-125 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). Serum levels of CA-125 greater than 35 units per mL. combined with pelvic examination increases the test specificity. Serial determinations of serum CA-125 further enhance the positive predictive value of the test for ovarian cancer. Serum CA-125 concentration may be useful in monitoring patients with diagnosed ovarian cancer. A persistently high serum CA-125 may be associated with progressive malignant disease and poor therapeutic response.On the other hand, a declining CA-125 value appears to be indicative of a favorable prognosis and a good response to treatment. Residual disease is confirmed in 95% of patients with serum CA-125 concentrations greater than 35 units per mL.However, negative results do not necessarily exclude the disease. To date, CA-125 is the most sensitive marker for residual epithelial ovarian cancer. CA-125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

Test principle

The CA-125 Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay.The assay system utilizes one monoclonal anti-CA-125 antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-CA 125 antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CA-125 antibody coated microtiter wells. Then CA-125 antibody labeled with horseradish peroxidase (conjugate) is added. If human CA-125 is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the CA-125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 3 hour incubation at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of CA-125 is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- Monoclonal anti-CA-125 -antibody coated microtiter plate 96 wells
- Enzyme conjugate reagent 12 mL
- TMB Substrate 12 mL
- Stop Solution 12 mL
- CA-125 reference standards, containing 0 , 15, 50, 100, 200, and 400 Unit / mL of CA-125, in liquid form (ready to use) or lyophilized form
- Wash Buffer Concentrate(50X) ,15 mL

Materials required but not provided:

- Precision pipettes: 40µL-200µL, 200-1000µL
- Disposable pipette tips
- Distilled water
- Vortex mixer
- Absorbent paper or paper towel
- Microtiter plate reader
- Graph paper

Specimen collection and preparation

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical . Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA,heparin, or oxalate may interfere with test procedures and should be Avoided.
3. Specimens should be capped and may be stored up to 48 Hours at 2 - 8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

- All reagents should be brought to room temperature(18-22°C) and mixed by gently inverting or swirling prior to use.Do notinduce foaming.
- If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstitutedstandards should be sealed and stored at 2-8°C.
- Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into 735 mL of distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

Assay procedure

1. Secure the desired number of coated wells in the holder. Dispense 50µL of CA-125 standards,specimens,and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispense 100µL of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have a complete mixing in this setup. Incubate at 37°C for 3 hours.
3. Prepare TMB substrate 15 minutes before use.
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer(1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Dispense 100µL of TMB substrate into each well.Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.

6.Stop the reaction by adding 100μL of Stop Solution to each well.Gently mix for 10 seconds until the blue color completely changes to yellow.
7.Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note

1.The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2.It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

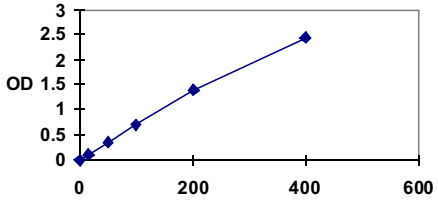
Calculation of results

Calculate the mean absorbance value for each set of CA-125 reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per mL on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CA-125 in units per mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of standard curve

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against CA-125 concentrations shown in the X axis.

CA-125 Values (U/mL)	Absorbance (450nm)
0	0.010
15	0.105
50	0.347
100	0.703
200	1.411
400	2.437



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 standard curve and data.

Performance characteristics

1. Accuracy:
Comparison between Our Assay and commercial available Kits provide the following data
N = 62
Correlation Coefficient = 0.981
Slope = 0.933
Intercept = 2.06
Mean (Our Kits) = 38.79
Mean (Abbott) = 35.88

2. Precision:

1). Intra-Assay:

Concentrations	N	Mean	S.D.	% CV
Level	20	16.27	0.970	5.96
Level I	20	86.26	4.290	4.97

2). Inter-Assay

Concentrations	N	Mean	S.D.	% CV
Leve I	10	17.19	1.698	9.87
Level I	10	88.62	6.160	6.95

3. Linearity

Two patient sera were serially diluted with 0 U/mL standard in a linearity study. The average recovery was 99.0 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	218.02	218.02	
2x	109.01	110.62	101.5
4x	54.51	53.02	97.3
8x	27.25	26.11	95.8
16x	13.63	14.80	108.6
Average Recovery: 100.8 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	260.33	260.33	
2x	130.17	128.23	98.5
4x	65.08	64.98	99.8
8x	32.54	31.76	97.6
16x	16.27	15.11	92.9
Average Recovery: 97.2 %			

4.Sensitivity
The sensitivity is defined as the concentration of CA-125 that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable concentration of this assay is estimated to be 5.0 U/mL.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.
1)As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2)Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.
3)The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

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