The CA-125 EIA test is primarily intended for use as a monitoring and Intended use

gynecologic malignancies and some non-malignant conditions. CA-125 levels higher than normal can also be found in individuals with endometroid, clear-cell and undifferentiated ovarian carcinoma. Serum elevated serum CA-125 levels can be found in individuals with serious murine monoclonal antibody. Published studies have indicated that CA-125 is associated with a high molecular weight glycoprotein. Serum is a surface antigen associated with epithelial ovarian cancer. In serum, often not made until the advanced stage. Cancer Antigen 125 (CA-125) for patients in clinical remission, following treatment. Post-operative management. The serum CA-125 test appears to be a useful tumor marker can indicate ovarian cancer and suggests the need for further clinical screening test. An abnormal result (i.e., elevated serum CA-125 level) 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 with 96 wells.
- Enzyme conjugate reagent, 12 ml.
- CA-125 reference standards containing; 0,15,50, 100, 200, and 400 Unit/ml of CA-125,Liquid,ready to use 1 set.
- TMB Substrate ,12 ml
- Stop Solution, 12ml.
- Wash Buffer Concentrate(50X),15ml.

Materials required but not provided:
- Precision pipettes and tips, 0.04–0.2ml
- 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 (One year from the date of
   manufacture). 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
1. All reagents should be brought to room temperature (18-22°C)
   and mixed by gently inverting or swirling prior to use. Do
   NOT induce foaming.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of
   distilled water. For example, Dilute 15 ml of Wash Buffer (50x)
   into 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
   100µl 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 complete mixing
   in this setup. Incubate at 37°C for 3 hours.
3. 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.
4. Dispense 100µl of TMB substrate reagent into each well. Gently
   mix for 10 seconds. Incubate at room temperature, in the dark, for
   20 minutes.
5. 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.
6. 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
resulting 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.

<table>
<thead>
<tr>
<th>CA-125 Values (U/ml)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.010</td>
</tr>
<tr>
<td>15</td>
<td>0.105</td>
</tr>
<tr>
<td>50</td>
<td>0.347</td>
</tr>
<tr>
<td>100</td>
<td>0.703</td>
</tr>
<tr>
<td>200</td>
<td>1.411</td>
</tr>
<tr>
<td>400</td>
<td>2.437</td>
</tr>
</tbody>
</table>

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.

Expected values and sensitivity
Healthy women are expected to have CA-125 assay values below 35
U/ml. The minimum detectable concentration of CA-125 in this assay is
estimated to be 5 U/ml.

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 wash procedure is critical. Insufficient washing will result in poor precision
   and falsely elevated absorbance readings.
3. Heterophilic antibodies such as human anti-mouse antibodies (HAMA) are
   frequently found in the serum of human subjects. Those antibodies can cause
   severe interference in many immunodiagnostic procedures. This assay has been
   designed to minimize that kinds of interference. Nevertheless, complete
   elimination of this interference from all patient specimens cannot be guaranteed.
   A test result that is inconsistent with the clinical picture and patient history
   should be interpreted with caution.

DAI Code # 16
References


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