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Immunoassay

REF **CMB0601 / CMB0602 / CMB0603 / CMB0604 / CMB0605**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

CA125 CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of CA125 (Cancer Antigen 125) in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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Introduction

CA125 (Cancer Antigen 125) 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. Serum CA125 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.

Serum CA125 assay values are useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer. In a review of nine published studies, the overall correlation reported between CA125 serum levels and the course of the disease was 87%.[1] Serum levels of CA 125 greater than 35 units per mL, combined with pelvic examination increases the test specificity. Serial determinations of serum CA125 further enhances the positive predictive value of the test for ovarian cancer. Serum CA125 concentration may be useful in monitoring patients with diagnosed ovarian cancer. Persistently rising CA125 assay values may be associated with malignant disease and poor response to therapy, whereas decreasing CA125 assay values may indicate a favorable response to therapy.[2-4]

In women with primary epithelial ovarian carcinoma who had undergone first-line therapy and were candidates for diagnostic second-look procedures, a CA125 assay value greater than or equal to 35 U/mL was found to be indicative of the presence of residual tumor. However, a CA125 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 CA125 assay values within the range of normal individuals.[5]

Elevations of CA125 assay values have been reported in approximately 1-2% of healthy individuals, and in individuals with nonmalignant conditions such as cirrhosis,[6] hepatitis, endometriosis,[7] first trimester pregnancy, ovarian cysts, and pelvic inflammatory disease. Elevations of CA125 assay values during the menstrual cycle have also been reported. Non-ovarian malignancies in which elevated CA125 assay values have been reported include cervical, liver, pancreatic, lung, colon, stomach, biliary tract, uterine, fallopian tube, breast, and endometrial carcinomas.[8]

To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer.

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-CA125 coated microparticles and enzyme labeled CA125 antibody are combined. CA125 present in the sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 being sandwiched between the microparticles and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the CA125 within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of CA125 in the samples.

Materials provided

1. Calibrators

6 vials lyophilized Calibrator A through F. The matrix is PBS (phosphate buffered saline) buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Invert the calibrator several times to mix it completely. Then allow the reconstituted material to stand for at least 30 minutes.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

CA125 antibody coated microparticles in Tris-HCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal Anti-CA125 in PBS buffer containing casein and BSA (bovine serum albumin). Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the CA125 calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hy-

pochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 ℃, under which conditions the stability will be retained for 2 months. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
8. Avoid grossly hemolytic, lipemic or turbid samples.
9. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
12. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
13. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample cups or tubes on the sample rack, 25 μL of samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of CA125 in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tubes or cups and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch

code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a CA125 exceeding 1000 U/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

- The concentration of the sample after dilution should not be less than 200 U/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of CA125 in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the acceptable ranges. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. CA125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated CA125 value alone is not of diagnostic value as a test for cancer and should not be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.
6. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
7. This test measures concentrations within the range of 2- 1000 U/mL. If CA125 concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:1 of this test, under this condition, allowing samples to be up to approximately 2000 U/mL.

Biological Reference Interval

A normal value of less than 35 U/mL (95^{th} percentile) was obtained by testing serum samples from 651 individuals defined as normal by clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (U /mL)	Within-run	Total
			%CV	%CV
1	80	25.08	2.64	3.67
2	80	38.66	2.33	4.01
3	80	226.87	2.67	4.21

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 0.5 U/mL
Limit of Detection: 1.0 U/mL
Limit of Quantitation: 2.5 U/mL with a coefficient of variation of ≤ 20 %.

3. Analytical Specificity

Cross reaction: the following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration
AFP	500 ng/mL
CEA	160 ng/mL
CA19-9	400 U/mL

Interference: No interference with 20 mg/dL of bilirubin, 500 mg/dL of hemoglobin, 3 g/dL of triglycerides.

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a microparticle based CA125 reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	357	4.7649	0.9449	0.9892

5. High Dose Hook Effect

A sample spiked with CA125 up to 50,000 U/mL was determined, the concentration result obtained was ≥1000 U/mL.

Literature References

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