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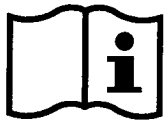

Useŕs Manual

CA125 IRMA

The CA125 IRMA system provides a direct in vitro quantitative determination of the cancer associated antigen CA125 in human serum

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1. DESCRIPTION

The CA125 IRMA system provides a direct *in vitro* quantitative determination of the cancer associated antigen CA125 in human serum in the range of 0-500 U/mL. Each kit contains material sufficient for 100 assay tubes, permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

2. INTRODUCTION

CA125 is a coelomic epithelium-related antigen carried by a high molecular weight glycoprotein complex (200 - 1000 kDa). This antigen was originally defined by the OC125 monoclonal antibody, which was obtained by immunization of mice with OVCA 433 cell line, derived from a papillary serous cystadenocarcinoma of the ovary (Bast et al., J Clin Invest 68:1331-1337, 1981).
CA125 is expressed in most epithelial ovarian carcinomas, especially in those of non-mucinous type. High serum levels are mainly found in ovarian cancer, but can also be observed in a lesser extent in other neoplasms (breast, colon, lung, pancreas, uterus and liver) and in non-malignant conditions as endometriosis, pericarditis, cirrhosis, menses and first trimester pregnancy.
The CA125 assay is not useful as a screening test. However, it has a significant clinical interest in the differential diagnosis of ovarian cancer, the evaluation of treatment efficiency, the early detection of relapse after a first-line therapy and in the long-term follow up of patients in remission.

3. PRINCIPLE OF METHOD

The technology uses two monoclonal antibodies of high affinity in an immunoradiometric assay (IRMA) system. The ^{125}I labelled signal-antibody (OC125*) binds to an epitope of the CA125 molecule spatially different from that recognised by the biotin-capture-antibody (M11*). The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a **capture antibody - antigen - signal antibody** complex, also referred to as a “sandwich”.
During an incubation period of 2 hours with shaking, the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter.
The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of CA125, the unknown concentration of CA125 in patient samples can be determined.
*Fujirebio Diagnostics Inc. antibodies

4. CONTENTS OF THE KIT

1. One bottle of TRACER (11 mL), ready to use, containing < 980 kBq 125I-anti- CA125 antibody and biotin-capture antibody in buffer with red dye and 0.1 % NaN3.
2. One bottle (5 mL) of STANDARD ZERO (S0) in equine serum with 0.1% NaN3.
3. Five vials of STANDARDS S1-S5 (5 x 1 mL), containing 15-30-80-200-500 U/mL CA125 in human serum with 0.1% NaN3. Assay calibration was performed using Fujirebio Diagnostics Inc. CA125II RIA.
4. Two vials of CONTROL SERUM (1 mL) containing app. 50 U/mL and 100 U/mL CA125 in human serum with 0.1% NaN3. The concentrations of controls are specified in the quality certificate enclosed.
5. Two boxes of COATED TUBES, ready to use: 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
6. One bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.1% NaN3. See Preparation of reagents.
7. Quality certificate
8. Pack leaflet

5. MATERIALS, TOOLS AND EQUIPMENT REQUIRED

- common laboratory equipment
- 100 µl precision micropipette
- 100 µl repeating pipette
- 2000 µl repeating pipette or dispenser
- horizontal shaker (at least 600 rpm)
- plastic foil to cover tubes
- absorbent tissue
- gamma-counter with software

6. SPECIMEN COLLECTION AND STORAGE

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Hemolyzed and lipemic specimens may give false values and should be avoided.
Samples with a CA125 concentration higher than 500 U/mL should be diluted with S0 zero standard and reassayed. Recommended dilution: 10-fold (450 µL S0 + 50 µL sample).

7. PREPARATION OF REAGENTS, STORAGE

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiration date is given on the package label and in the quality certificate. Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution, store at 2-8°C until the expiration date of the kit.

CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

8. ASSAY PROCEDURE

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S0-S5), control serum and sample. Optionally, label two test tubes for total counts (T).
2. Pipette 100 µL of standards, controls and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
3. Pipette 100 µL of tracer into each tube.
4. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm recommended).
5. Incubate tubes for 2 hours, shaking at room temperature.
6. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Return the tube-rack to an upright position and repeat step-6 two more times.
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the CA125 concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Tracer	**100**	**100**	**100**	**100**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

9. CALCULATION OF RESULTS

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}5} / C_{\text{I-II}} / M_x\ (\text{cpm}) - S_0\ (\text{cpm})}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of CA125. Determine the CA125 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Out of fitting programs applied for computerized data processing, spline fittings are recommended.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	CA125 U/mL
T	291557		
S0	278	0.1	
S1	2177	0.7	
S2	3610	1.2	
S3	8361	2.9	
S4	20729	7.1	
S5	46876	16.1	
CI	5642	1.9	51.7
CII	10973	3.8	105.9

10. PERFORMANCE CHARACTERISTICS

Specificity
The antibodies used in this assay guarantee a measurement completely specific for CA125.

Sensitivity
Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:
Limit of Blank (LoB): 0.56 U/mL
Limit of Detection (LoD): 1.08 U/mL
For results under LoB, should report as “analyte not detected”. For results between LoB and LoD, should report as “analyte detected”, concentration < 1.08 U/mL.

Precision and reproducibility
Four serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 20 independent assays. Values obtained are shown below.

Intra-assay		**Inter-assay**	
Mean (U/mL)	**CV%**	**Mean (U/mL)**	**CV%**
24.39	1.99	24.31	5.71
44.26	1.42	44.67	3.11
154.59	2.31	157.0	3.12
305.87	1.98	316.42	2.80

Linearity – dilution test
Six individual serum samples were serially diluted with zero-standard and measured according to kit protocol. Mean recovery after dilution was 92.6%. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:
$Y = 0.9899X - 4.2676 \quad R^2 = 0.9967 \quad n = 20$

Recovery
Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of CA125. The average per cent recovery for 3 serum samples spiked with CA125 at 3 levels each was 103.37%, with a range of 99% to 107%.

Hook effect
No hook effect is observed for concentrations lower than 25000 U/mL.

Expected Values
It is recommended that each laboratory determine a reference range for its own patient population.
Serum samples from 408 presumably healthy, non-pregnant female blood donors were evaluated:

Samples	408
Mean (U/mL)	16.53
Median (U/mL)	14.03
Samples < 35 U/mL	386 (94.6%)
Samples < 55 U/mL	404 (99.0%)

Method comparison
The CA125 IRMA (Y) was compared to the Fujirebio Diagnostics Inc. CA125II RIA (X) on 135 specimens ranging from 5 to 500 U/mL. Linear regression analysis yielded the following results:
$Y = 1.0168X + 1.0221 \quad R^2 = 0.9442$

11. PROCEDURAL NOTES

- The non-respect of the instructions in this insert may affect results significantly.
- Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
- **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.
- **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

12. LIMITATIONS

- The CA125 assay should not be used as a cancer screening test.
- CA125 assay values greater than or equal to 35 U/mL can be found in some healthy individuals and in patients with non-malignant conditions.
- A CA125 value below 35 U/mL does not indicate the absence of residual ovarian cancer.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

13. PRECAUTIONS

Radioactivity
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1), Hepatitis B surface Antigen (HBsAg) and Treponema antibody.
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Chemical hazard
Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 38.5 mg.

14. LEGAL NOTE

CA125™ is a trade mark of Fujirebio Diagnostics Inc. (FDI). The present CA125 IRMA is based on the use of the OC125 and M11 antibodies, which are available exclusively through FDI, and its licensed distributors.

	Used by	LOT	Batch code
	Temperature limitation	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device	AS	Antiserum
	Manufacturer		
REF	Catalogue number		
	Radioactive material		