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Userś Manual

CEA15-3 IRMA

The CA15-3 IRMA system provides a direct *in vitro* quantitative determination of the cancer associated antigen CA15-3 in human serum.

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REF DE53100

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Vers. ACE130501
/AR
Updated 130607

Intended Use

The CA15-3 IRMA system provides a direct *in vitro* quantitative determination of the cancer associated antigen CA15-3 in human serum in the range of 0-300 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.

Introduction

CA15-3 is a high molecular weight mucin glycoprotein encoded by the MUC-1 gene. This antigen can be detected by the use of two monoclonal antibodies: DF3 and 115D8. The DF3 antibody was prepared against a membrane-enriched fraction of a human breast carcinoma (Kufe et al., Hybridoma 1984;3(3):223-232) and the 115D8 antibody was raised against antigens of human milkfat globule membranes (Hilkens et al., Prot Biol fluids 1981;29:813-816).
The concentration of CA 15-3 antigen is frequently elevated in the serum of patients with breast cancer as well as with other malignancies, such as ovary and lung cancer. Some non-malignant disorders (ex. endometriosis, pelvic inflammatory disease and hepatitis) and physiological conditions like pregnancy and lactation can also raise CA15-3 levels.
The CA15-3 assay is not useful as a screening test. However, it has a significant clinical interest in the differential diagnosis and prognosis of breast 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.

Principle of method

This immunoradiometric assay is based on a two-step procedure. In the first step the serum sample is incubated in streptavidin coated tubes with a biotin labeled, capture monoclonal antibody (115D8*). During this incubation period the immuno-complex is immobilized on the reactive surface of the test tubes. After incubation the tubes are washed. In the second stage ^{125}I-labelled, signal monoclonal antibody (DF3*) is added and it binds to an epitope of the CA15-3 molecule different from that recognised by the capture antibody, resulting in the formation of a capture antibody - antigen - signal antibody complex, also referred to as a “sandwich”.
The reaction mixture is then discarded, the test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.
The concentration of antigen is directly proportional to the radioactivity measured in the test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of CA15-3, the unknown concentration of CA15-3 in patient samples can be determined.
**Fujirebio Diagnostics Inc. antibodies*

Contents of the kit

1. One bottle of TRACER (21 mL), ready to use, containing < 980 kBq of ^{125}I-anti- CA15-3 antibody in buffer with red dye and 0.1 % NaN_3.
2. One bottle of ANTISERUM (21 mL), ready to use, containing biotinilated capture antibody in buffer with blue dye and 0.1 % NaN_3.
3. One bottle (10 mL) of DILUENT (DIL) containing equine serum and PBS buffer with 0.1% NaN_3.
4. Six vials of STANDARDS S0-S5 (6 x 0.5 mL), containing CA15-3 in human serum with 0.1% NaN_3. The concentrations of standards are specified in the quality certificate enclosed.
5. Two vials of CONTROL SERA (2 x 0.5 mL) containing low and high concentrations of CA15-3 in human serum with 0.1% NaN_3. The concentrations of controls are specified in the quality certificate enclosed.
6. Two boxes of COATED TUBES, ready to use: 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
7. One bottle of WASH BUFFER CONCENTRATE (40 mL), containing 0.1% NaN_3. *See Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

- common laboratory equipment
- 10 µl precision micropipette
- 200 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

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 CA15-3 concentration higher than 300 U/mL should be diluted with diluent and reassayed. Recommended dilution: 10-fold (450 µL DIL + 50 µL sample).

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 (40 mL) to 1400 mL distilled water to obtain 1440 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.

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. Label two test tubes for total counts (T).
2. Pipette 10 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 200 µL of antiserum into each tube (except T).
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).
5. Incubate tubes for 1 hour, shaking at room temperature.
6. Add 2.0 mL of diluted wash buffer to each tube. Decant the fluid of all tubes by the inversion of the rack.
7. Return the tube-rack to an upright position, and repeat the washing step two more times. In the upside down position place the rack on an absorbent paper for 2 minutes at least.
8. Pipette 200 µL of tracer into each tube.
9. 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).
10. Incubate tubes for 1 hour, shaking at room temperature.
11. Wash according to steps 6 and 7.
12. Count each tube for at least 60 seconds in a gamma counter.
13. Calculate the CA15-3 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		10		
Control			10	
Sample				10
Antiserum		200	200	200
Shake for 1 hour at room temperature				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Repeat the washing procedure 2 more times				
Tracer	200	200	200	200
Shake for 1 hour at room temperature				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Repeat the washing procedure 2 more times				
Count radioactivity (60 sec/tube)				
Calculate the results				

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:

$$B/T(\%) = \frac{S1\text{-}5 \,/\, C\ I\text{-}II \,/\, Mx\ (cpm) - S0\ (cpm)}{T(cpm)} \times 100$$

Plot the B/T(%) for each standard versus the corresponding concentration of CA15-3. Determine the CA15-3 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%	CA15-3 U/mL
T	342248		
S0	161	0.05	
S1	6136	1.8	15
S2	12336	3.6	30
S3	24193	7.1	65
S4	42404	12.4	130
S5	76043	22.2	300
CI	10039	2.9	24.1
CII	19442	5.7	49.6

Performance characteristics

Specificity

The antibodies used in this assay guarantee a measurement completely specific for CA15-3.

Sensitivity

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:
Limit of Blank (LoB): 0.24 U/mL
Limit of Detection (LoD): 1.35 U/mL
For results under LoB, should report as “analyte not detected”. For results between LoB and LoD, should report as “analyte detected”, concentration < 1.35 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 15 independent assays. Values obtained are shown below.

Intra-assay		**Inter-assay**	
Mean (U/mL)	**CV%**	**Mean (U/mL)**	**CV%**
14.32	1.62	15.59	3.44
31.14	1.33	33.33	3.95
87.50	1.84	88.44	4.66
217.15	1.36	221.16	3.12

Linearity – dilution test

Five individual serum samples with concentrations between 200 and 300 U/mL were diluted ten-fold gravimetrically with the diluent and measured according to the kit protocol. The results of recovery after dilution were 83.9%, 85.6%, 84.1%, 92.1% and 90.6%, respectively.

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of CA15-3. The average per cent recovery for 5 serum samples spiked with CA15-3 at 3 levels each was 105.9%, with a range of 104% to 108%.

Hook effect

No hook effect is observed for concentrations lower than 7500 U/mL.

Expected Values

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

Samples	295
Mean (U/mL)	14.67
Median (U/mL)	14.24
Samples < 30 U/mL	294 (99.7%)
Samples < 25 U/mL	287 (97.3%)

Method comparison

The DE53100 IRMA (Y) was compared to IBA-CIS CA15-3 IRMA (X) as a reference method on 172 specimens ranging from 5 to 300 U/mL. Linear regression analysis yielded the following results:

$Y = 1.0454X – 0.675 \quad R^2 = 0.9698$

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!** 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.

Limitations

- The CA15-3 assay should not be used as a breast cancer screening test.
- CA15-3 assay values greater than or equal to 30 U/mL can be found in some healthy individuals and in patients with non-malignant conditions.
- A CA15-3 value below 30 U/mL does not indicate the absence of residual breast 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.

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 96 mg.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	AS	Antiserum
	Manufacturer	DIL	Diluent
REF	Catalogue number		Store between 2-8°C
	Radioactive material		CE

Legal note

CA15-3® is a registered mark of Fujirebio Diagnostics Inc. (FDI). The present CA15-3 IRMA is based on the use of the DF3 and 115D8 antibodies, which are available exclusively through FDI, and its licensed distributors.