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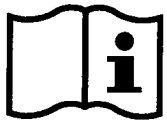

Useŕs Manual

CA19-9 IRMA

The CA19-9 IRMA system provides a direct in vitro quantitative determination of the cancer associated antigen CA19-9 in human serum

IVD

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Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

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1. DESCRIPTION

The CA19-9 IRMA system provides a direct *in vitro* quantitative determination of the cancer associated antigen CA19-9 in human serum in the range of 0-240 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

CA19-9 is a tumour associated mucin-type high molecular weight glycoprotein antigen circulating in the blood and can be found in tissues. This test is useful in monitoring patients with confirmed pancreatic cancer whose CA19-9 levels are above the cut off at the time of the diagnosis. Patients negative for the Lewis blood group antigen have no CA 19-9 in their blood even in the presence of malignant disease.

3. PRINCIPLE OF METHOD

CA19-9 concentrations are determined by using the 1116NS19-9* antibody recognising a specific epitope. The CA19-9 molecule presents the same epitope on its surface in different locations. The assay technology uses a monoclonal antibody of high affinity in an immunoradiometric assay (IRMA) system.
The biotin-capture-antibody (1116NS19-9*) binds to the specific epitope of the CA19-9 molecule in the first one-hour reaction step on a test tube shaker. After washing the reaction tubes a spatially different but otherwise the same epitope is recognised by the ^{125}I labelled signal-antibody (1116NS19-9*) in the second step during a one-hour incubation on shaker. The same but differently labelled antibodies react in two separate step with the antigens present in standards or samples, which leads to the formation of a **capture antibody-antigen-signal antibody** complex, that can be referred to as a “homo-sandwich”. The immun-complex is immobilized to the reactive surface of streptavidin coated test tube. The reaction mixture is then discarded and the 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 CA 19-9, the unknown concentration of CA19-9 in patient samples can be determined.

4. CONTENTS OF THE KIT

1. One bottle of TRACER (21 mL), ready to use, containing <980 kBq ^{125}I-anti-CA19-9 (1116NS19-9* - *see legal note*) antibody in buffer with red dye and 0.1 % KathonCG as preservative.
2. One bottle (11 mL) ANTISERUM, biotin labelled anti-CA19-9 (1116NS19-9*) in buffer with blue dye and 0.1 % KathonCG as preservative. Ready to use.
3. Two bottles (2.5 mL) of DILUTION SERUM (S0), in mouse serum matrix with 0.1 % KathonCG as preservative. Ready to use.
4. Five vials of STANDARDS S1-S5 (5 x 1 mL), containing CA19-9 in human serum with 0.1 % KathonCG as preservative. Ready to use. The concentrations of standards are specified in the quality certificate enclosed.
5. Two vials of CONTROL SERUM CI, CII (1 mL) containing CA19-9 in human serum with 0.1 % KathonCG as preservative. Ready to use. 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 (20 mL), containing 0.1 % KathonCG as preservative. *See Preparation of reagents.*
8. Quality certificate
9. Pack leaflet

5. MATERIALS, TOOLS AND EQUIPMENT REQUIRED

- Test tube rack
- precision pipette with disposable tips for 100 µl
- repeating pipettes for 200 and 2000 µl
- 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. Haemolysed and lipemic specimens may give false values and should be avoided.
Samples with a CA19-9 concentration higher than 240 U/mL should be diluted with Dilution serum and reassayed. Recommended dilution: 10-fold (450 µL S0 + 50 µL sample).

7. PREPARATION OF REAGENTS, STORAGE

Store the “ready to use” reagents between 2-8°C after opening. At this temperature these reagents are 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 1200 mL distilled water to obtain 1220 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, S_1-S_5), control serum (CI, CII) and sample (M_x). 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 antiserum 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 1 hour, 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 one more time.
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. 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.
12. Return the tube-rack to an upright position and repeat step-6 two more times.
13. Count each tube for at least 60 seconds in a gamma counter.
14. Calculate the CA19-9 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/S0		**100**		
Control			**100**	
Sample				**100**
Antiserum		**100**	**100**	**100**
Shake for 1 hour at room temperature				
Wash Buff.		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash Buff.		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Tracer	**200**	**200**	**200**	**200**
Shake for 1 hour at room temperature				
Wash Buff.		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash Buff.		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash Buff.		**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:

$$B/T(\%) = \frac{S_{1\text{-}5} / C_{\text{I-II}} / M_x \text{ (cpm)} - S0 \text{ (cpm)}}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of CA19-9. Determine the CA19-9 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%	CA19-9 U/mL
T	382 114		
S0	153	0.04	
S1	6 055	1.58	
S2	11 986	3.14	
S3	23 154	6.06	
S4	44 918	11.8	
S5	85 919	22.5	
CI	9 011	2.36	22.4
CII	27 325	7.15	73.1

10. PERFORMANCE CHARACTERISTICS

Specificity
The antibody used in this assay guarantees a completely specific detection of CA19-9.

Sensitivity
The analytical sensitivity or minimum detectable dose (MDD) is calculated by the interpolation of the mean counts of dilution serum plus 2 standard-deviations from the standard curve. Determination was carried out using 20 replicates of dilution serum response. The value of analytical sensitivity is 0.1 U/mL, measured using fresh tracer.

Precision and reproducibility
Three serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 65 independent assays. Values obtained are shown below.

Intra-assay		**Inter-assay**	
Mean (U/mL)	CV%	Mean (U/mL)	CV%
2.15	8.5	2.15	15.6
36.5	6.1	35.7	6.2
216.4	2.0	194.3	5.4

Linearity – dilution test
Five individual serum samples were serially diluted with zero-standard and measured according to kit protocol. Mean recovery after dilution was 108.0%. The following equation obtained for expected (Y) versus measured (X) concentration demonstrates the good linearity:
$Y = 0.9723X - 1.57 \quad R^2 = 0.9963 \quad n = 15$

Recovery
Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of CA19-9. The average per cent recovery for 6 serum samples spiked with CA19-9 at 3 levels each was 94.4%, with a range of 86.7 % to 101.1 %.

Hook effect
No high dose hook effect is observed for concentrations lower than 1 500 000 U/mL.

CUT OFF and distribution of values
It is recommended that each laboratory determine a cut off for its own patient population.
CUT OFF value: 37 U/mL
Serum samples from 494 presumably healthy blood donors were evaluated:

Samples	494
Samples with 0 U/mL	39
Mean (U/mL)	4.87
Median (U/mL)	3.22
Samples < 37 U/mL	493

Method comparison
The RK-199CT IRMA (Y) was compared to the Fujirebio Diagnostics Inc. CA19-9 RIA (X) on 60 specimens ranging from 0 to 240 U/mL. Linear regression analysis yielded the following results:
$Y = 1.106*X - 1.524 \quad R^2 = 0.893$

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 CA19-9 assay should not be used as a cancer screening test.
- CA19-9 assay values greater than or equal to 37 U/mL can be found in some healthy individuals and in patients with non-malignant conditions.
- 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 Kathon CG as an antimicrobial agent. The total Kathon CG present in each pack is 64 mg.

14. LEGAL NOTE

*CA19-9™ is a trade mark of Fujirebio Diagnostics Inc. (FDI). The present CA19-9 IRMA is based on the use of the 1116NS19-9 antibody.

	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		