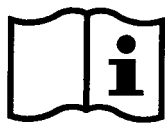


Product information

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User's 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



DE50100



100 tubes

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 or 50 assay tubes, permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

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.

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 125I 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 CA19-9, the unknown concentration of CA19-9 in patient samples can be determined.

Contents of the kit

1. **Anti-CA19-9 I-125** One bottle of TRACER (21 mL), ready to use, containing < 980 kBq 125I- anti-CA19-9 (1116NS19-9* - *see legal note*) antibody in buffer with red dye and 0.1 % KathonCG as preservative.
2. **ANTISERUM** One bottle (11 mL) of ANTISERUM, biotin labelled anti-CA19-9 (1116NS19-9*) in buffer with blue dye and 0.1 % KathonCG as preservative. Ready to use.
3. **CAL 0** Two bottles (2.5 mL) of DILUTION SERUM (S0), containing mouse serum and PBS-BSA buffer with 0.1 % NaN₃ as preservative. Ready to use.
4. **CAL 1 – 5** Five vials of STANDARDS S1-S5 (5 x 1 mL), containing 15-30-60-120-240 U/mL CA19-9 in human serum with 0.1 % KathonCG and NaN₃ as preservative. Ready to use.
5. **CONTROL 1 and 2** Two vials of CONTROL SERUM CI, CII (1 mL) containing CA19-9 in human serum with 0.1% KathonCG and NaN₃ as preservative. Ready to use.
The concentrations of controls are specified in the quality certificate enclosed.
6. **SORB CT** COATED TUBES, ready to use.
Reactive test tubes, 12x75 mm, packed in plastic boxes. 2 boxes, 2x50 pcs
7. **WASH SOLN 61x** One bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.1% KathonCG as preservative.

See *Preparation of reagents*.

Quality certificate

Pack leaflet

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 and 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. 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).

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.

Assay procedure

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

1. Label coated tubes in duplicate for each standard (S0, S1-S5), control serum (C1, CII) and sample (Mx). 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.

Table1. Assay Protocol, Pipetting Guide (all volumes in microliters)

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				
Repeat once washing step				
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				
Repeat twice washing step				
Count radioactivity (60 sec/tube)				
Calculate the results				

Optional protocol

Incubation times of **30 minutes** can be used in both reaction steps. Typical cpm outputs when using fresh and old tracer are shown below:

tracer	old		fresh	
shaking	60' + 60'	30' + 30'	60' + 60'	30' + 30'
T	205625	203818	383994	392467
S0	202	148	181	136
S1	929	758	1975	1570
S2	2424	1949	5374	4418
S3	5007	4139	11343	9542
S4	10711	8409	23700	19947
S5	19766	15966	46117	37960

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:

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

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.

Table2. Typical assay data

Tubes	Mean cpm	B/T%	CA19-9 U/mL
T	251 744		
S0	475	0.2	
S1	1 781	0.7	
S2	3 685	1.5	
S3	7 721	3.1	
S4	15 077	6.0	
S5	29 361	11.7	
CI	4 921	2.0	39.3
CII	10 062	4.0	78.3

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 0.3 U/mL, measured using fresh tracer.

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:

Limit of Blank (LoB): 0.94 U/mL

Limit of Detection (LoD): 1.85 U/mL

For results under LoB, should report as “analyte not detected”. For results between LoB and LoD, should report as “analyte detected”, concentration < 1.85 U/mL.

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

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 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.

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 antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti- HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). 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 infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain Kathon CG and NaN₃ as an antimicrobial agent. The total Kathon CG present in each pack is 64 mg. The total azide present in each pack is 12 mg.











Storage and shelf life

Store this product at a temperature of 2-8°C Shelf-life: 60 days from availability.

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.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta y advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore