



OncoEIA-CA 19-9

Enzyme-linked Immunosorbent Assay for Quantitative Determination of Cancer Antigen 19-9 in Human Serum

1. INTENDED USE

1.1. This kit is intended for quantitative determination of Cancer Antigen 19-9 in human serum.

1.2. CA 19-9 is a carbohydrate antigen and a marker of pancreatic, stomach, liver and colorectal cancer. Its concentration is increased in 45% of patients with pancreatic cancer. In patients with benign pancreatic tumors increased CA 19-9 concentration is detected only in 12% cases and does not exceed 45 U/ml. It should be taken into consideration that CA 19-9 excretion depends on biliary tract patency. That is why inadequate increase of CA 19-9 concentration may be caused by cholestasis.

Quantitative determination of CA 19-9 in serum, especially in combination with CEA, is useful for monitoring of pancreatic, stomach, liver and intestinal cancer and for control of treatment efficiency.

2. ASSAY DESCRIPTION AND PRINCIPLES

2.1. Kit Contents:

- 12 microtitration strips, 12x8 wells, coated with anti-CA 19-9 monoclonal antibodies, packed in the foil bag, labeled "Strips with monoclonal antibodies against CA 19-9", 1 bag;
- CA 19-9 calibrators containing known CA19-9 concentrations. Exact CA 19-9 concentrations are indicated on vial labels, 6 vials .
- anti-CA 19-9 antibodies conjugated with HRP, concentrated solution, labeled "Conjugate E", 1 vial.
- conjugate diluent, labeled "sample diluent", 1 vial.
- sample diluent, labeled "sample diluent", 1 vial.
- concentrated wash buffer, labeled "Buffer P", 1 vial.
- tetramethylbenzidine diluent, labeled "TMB diluent", 1 vial;
- "Stop reagent", 1 vial.
- control containing known CA 19-9 concentration, labeled "Control", 1 vial.
- two adhesive plate sealers

2.2. "Onco EIA-CA 19-9" reagents are sufficient for determination of 41 unknowns, 6 calibrators, 1 control in duplicates, provided that all the strips are used simultaneously.

NOTE :if used partially, kit should be utilized within a month after opening.

2.3. Assay principle. "Onco EIA-CA 19-9" is a "sandwich" type of solid-phase enzyme linked immunoassay, based on two monoclonal antibodies that is specific to different determinants of CA 19-9 molecule. One of these antibodies is conjugated with horseradish peroxidase; the other is immobilized on inner surface of microwells. During incubation CA 19-9 molecules from the serum sample binds to immobilized antibodies. Then the strips are washed to remove any material not bound to the inner surface of the wells. Then the wells are incubated with anti CA 19-9 antibodies conjugated with horseradish peroxidase. The quantity of the bound conjugate is directly proportional to CA 19-9 concentration in the sample. The unbound material is removed by washing and TMB substrate solution is added to the wells. During the incubation with TMB substrate solution the coloring appears. The color intensity is in direct proportion to the CA 19-9 concentration in the sample. Optical density of the solution in the wells is measured and the CA 19-9 concentration in the samples is calculated using the calibration curve..

3. PERFORMANCE CHARACTERISTICS

3.1. Specificity. No cross-interaction between CA 19-9 monoclonal antibodies and the following oncomarkers: AFP, PSA, CEA, CA 125 and CA 15-3 antigens.

3.2. Coefficient of variation (intra-assay precision) between the results of CA 19-9 determination in the same sample is less than 8%.

3.3. Linearity (Dilution test). Dilution of serum sample containing predetermined CA 19-9 concentration with sample diluent leads to linear recovery of CA 19-9 in diluted samples in concentration range between Calibrator №2 and Calibrator №6.

3.4. Recovery. To determine this parameter, equal volumes of control and Calibrator №3 were mixed. Then the correspondence between the calculated CA 19-9 concentration in the obtained sample and the measured concentration was determined. Recovery range is 90–110%.

3.5. Detectability. Minimal detectable concentration for " OncoEIA-CA 19-9" assay is 2U/ml.

3.6. clinical tests. Serum samples taken from 78 apparently healthy people (both males and females) at the age of 20 to 55 years were assayed with "OncoEIA CA 19-9" kit. Mean CA 19-9 concentration was 6.7 U/ml. this limit should be considered as guideline only.

3.7. It is highly recommended for each laboratory to determine its own reference range of CA 19-9 concentrations.

4. WARNINGS AND PRECAUTIONS

4.1. All the components are non-toxic.

4.2. Stop reagent is an irritant . Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water.

4.3. It is highly recommended to handle kit components in accordance with established good laboratory practice. The operator should wear disposable latex or plastic gloves and handle patients samples as if capable of transmitting infectious agents.

5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

- digital variable pipettes that cover volume range from 0.050 to 0.100 ml with appropriate disposable tips;
- 8-channel digital variable pipette that covers volume range from 0.050- 0.250 ml with appropriate disposable tips;
- orbital microplate shaker-thermostat , able to maintain temperature $+37\pm 1^{\circ}\text{C}$ and shaking speed 400 to 600 rpm;
- automatic microplate reader;
- glass vials (10- 15)ml.
- 500 ml volumetric cylinder;
- 1000 ml volumetric beaker;
- distilled water.

6. REAGENT PREPARATION FOR ASSAY

6.1. All the kit components and serum samples should be kept at room temperature (18-25) for at least 30 minutes prior to use.

6.2. CA 19-9 calibrators and control are ready to use. Once opened, store at $+2... 8^{\circ}\text{C}$ for no more than 1 month.

6.3. Microtitration strips. Before opening keep the bag at room temperature ($+18...25^{\circ}\text{C}$) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at $+2...8^{\circ}\text{C}$ until expiry date stated on the label.

6.4. wash buffer: if crystals appear in the concentrated wash buffer, warm up the vial at $30-40^{\circ}\text{C}$ until the complete dissolution of the crystals and mix thoroughly. prepare the necessary volume of wash buffer by dilution of buffer P with distilled water. store prepared wash buffer firmly closed at 2-8 for no more than 3 days. The rest of buffer P should be stored firmly closed at 2-8 until expiry date.

6.5. working solution of conjugate: immediately before the assay mix the appropriate volumes of conjugate E and conjugate diluent . Once prepared , the working solution of conjugate should be stored at room temperature $18-25^{\circ}\text{C}$ for not more than 3 hours.

6.6. TMB substrate solution. Immediately before the assay mix the appropriate volumes of concentrated TMB solution and TMB diluent. Once prepared, the TMB substrate solution should be stored protected from light at room temperature $18-25^{\circ}\text{C}$ for not more than 3 hours.

6.7. sample diluent and stop reagent: are ready to use. Once opened, store at $2-8^{\circ}\text{C}$ until expiry date.

7. ASSAY PROCEDURE

7.1. All the components and samples should be brought to room temperature and stirred thoroughly before the assay.

7.2. Mark the wells as follows:

A1, A2— №1 for calibrator №1; E1, E2— №5 for calibrator №5;
B1, B2— №2 for calibrator №2; F1, F2— №6 for calibrator №5;
C1, C2— №3 for calibrator №3; G1, G2— №7 for control;
D1, D2— №4 for calibrator №4; The rest of wells are used for patient samples.

7.3. Perform each assay in duplicate for both calibrators and unknowns.

7.4. pipette 50 of sample diluent into each well.

7.5. Pipette 50 µl of the CA 19-9 calibrators and serum specimens into the corresponding wells. Total time of sample dispensing should not exceed 15- 20 minutes.

7.6.cover the strips with the adhesive sealer included in the kit and incubate on shaker-thermostat at 37°C and 400 rpm for 30 minutes.

7.7 remove the plate sealer, aspirate the liquid from the wells and wash the strips five times with wash buffer,prepare as described in 6.4. each time add 250µl of wash buffer per well. After each washing cycle invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

7.8. pipette 100 of working solution of conjugate into each well.

7.9. cover the strips with the adhesive sealer included in the kit and incubate on shaker-thermostat at 37°C and 400 rpm for 60 minutes.

7.10. remove the plate sealer, aspirate the liquid from the wells and wash the strips 5 times with wash buffer, prepare as described in 6.4. each time add 250 µl of wash buffer per well. After each washing cycle invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

7.11. immediately add 100 µl of TMB Substrate solution into each well.

7.12 cover the strips with the adhesive sealer. Incubate at room temperature 18-25°C in the dark for 10-15 minutes, depending on the color intensity.

7.13 Remove the plate sealer. Add 100 µl of stop solution to all the wells and shake thoroughly for 10-15 seconds. Stop solution should be dispensed into the wells exactly in the same order as TMB substrate solution was on stage 7.11. during the incubation the yellow coloring appears.

7.14 Wait 2-3 minutes and read the optical density in the microwells at a wavelength of 450 nm.the time interval between shopping of reaction and OD measurement should not exceed 20 minutes.

7.15 Calculate the mean absorbency for each duplicate. Draw the calibration curve in linear coordinates by plotting absorbency values for calibrators against corresponding CA 19-9 concentrations. Determine CA 19-9 concentrations in the unknown samples and control.

TMB solution, μ l	100	100	100	100	100	100	100	100
Incubation №3	10-15 minutes in the dark at room temperature							
Stop-reagent, μ l	100	100	100	100	100	100	100	100
Stirring	10-15 seconds on shaker at room temperature							
OD measuring	Microplate reader (wavelength 450 nm)							
Calculations	Corresponding software (recommended)							

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