



CA 19-9 ELISA

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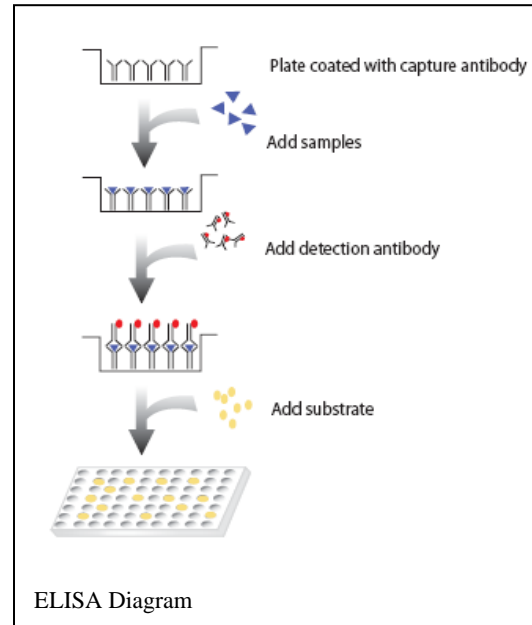
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Introduction

CA 19-9 represents the most important and basic carbohydrate tumor marker (1). The immunohistologic distribution of CA 19-9 in tissues is consistent with the quantitative determination of higher CA19-9 concentrations in cancer than in normal or inflamed tissues. Recently reports indicates that the serum CA19-9 level is frequently elevated in the serum of subjects with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA, elevated CA 19-9 is suggestive of gallbladder neoplasm in the setting of inflammatory gallbladder disease. Research studies demonstrate that serum CA 19-9 values may have utility in monitoring subjects with the above-mentioned diagnosed malignancies. It has been shown that a persistent elevation in serum CA19-9 value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising serum CA 19-9 value may be associated with progressive malignant disease and poor therapeutic response. A declining CA 19-9 value may be indicative of a favorable prognosis and good response to treatment (2-7).

Principle of the assay

The CA 19-9 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA 19-9 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA 19-9 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in CA 19-9 being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA 19-9 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kits:

- Murine monoclonal anti-CA19-9 coated 96 well microtiter plate
- CA19-9 reference standards, containing 0, 25, 75, 150, 300, and 600 U/ml CA19-9, liquid, 1 ml each, ready to use. 1 set
- CA 19-9 Assay Buffer, 13 ml
- Enzyme Conjugate (12 X), 1.1 ml.
- CA 19-9 Conjugate Diluent, 13 ml
- Wash Buffer (20x), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

Preparation for Assay

- All reagents should be taken to room temperature (18-25°C) before use.
- Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

Assay Procedure

1. Add 10 µl of standard, specimens, and controls into appropriate wells
2. Add 100 µl of CA 19-9 Assay buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at 37° for 90 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X). Strike the wells sharply onto paper towel to remove all residual water droplets.
7. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X).
11. Strike the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

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4. Malesci, A., Tommasini, M.A., Bonato, C. et al. Determination of CA19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. *Gastroenterology* 1987; 92:60-7.
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6. Steinberg, W. The clinical utility of CA 19-9 tumor associated antigen. *American J. of Gastroenterology* 1990; 85:350-355.
7. Steinberg, W.M., Gelfand, R., Anderson, K.K., et al. Comparison of the sensitivity and specificity of the CA 19-9 and carcinoembryonic antigen assays in detecting cancer of the antigen assays in detecting cancer of the pancreas. *Gastroenterology* 1986; 90:343-9.

Example of standard curve

CA19-9 (U/ml)	Absorbance (450 nm)
0	0.058
25	0.347
75	0.845
150	1.322
300	2.020
600	2.830

