



CA-19-9
Product Code: 3925-300

Intended Use: The Quantitative Determination of Cancer Antigen (CA 19-9) Concentration in Human Serum by a Microplate Immunoassay

SUMMARY AND EXPLANATION OF THE TEST

A mucin type Sialyl Lewis Antigens group of glycoproteins (SLA) such as CA 19-9, 19-5 have been recognized as circulating cancer associated antigens for gastrointestinal cancer. The discovery of a monoclonal antibody clone (1116NS 19-9), which exhibited selective reactivity with human gastrointestinal carcinomas through the recognition of a carbohydrate determinant (CA 19-9) defined as a sialyl lacto-N-flucoperoxase II, resulted in the successful purification and thus, determination of human gastrointestinal tumor associated glycoprotein antigen expressing CA 19-9 from colorectal carcinoma cell lines. Recently reports indicate that serum CA 19-9 level is frequently elevated in the circulation of patients with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA elevated CA 19-9 is suggestive of gallbladder disease. The tumor associated antigen may also be associated in some malignant conditions. Research studies demonstrate that serum CA 19-9 values may have utility in monitoring subjects with the above mentioned diagnosed malignancies.

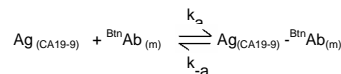
In this method, CA19-9 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for CA19-9) is added and the reactants mixed. Reaction between the CA19-9 antibodies and native CA19-9 forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to CA19-9 is added to the wells. The enzyme labeled antibody binds to the CA19-9 already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the CA19-9 in the sample.

PRINCIPLE

Immunoassay (TYPE 4):

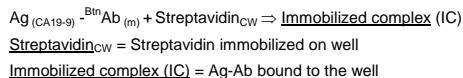
The essential reagents required for an immunoassay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA19-9 antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

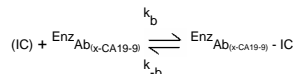


$B^{in}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 $Ag_{(CA19-9)}$ = Native Antigen (Variable Quantity)
 $Ag_{(CA19-9)} - B^{in}Ab_{(m)}$ = Antigen-antibody complex (Variable Quantity)
 k_a = Rate Constant of Association
 k_a = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.



$Enz_{Ab_{(k-CA19-9)}}$ = Enzyme labeled Antibody (Excess Quantity)
 $Enz_{Ab_{(k-CA19-9)}} - IC$ = Antigen-Antibodies Complex

k_b = Rate Constant of Association
 k_b = Rate Constant of Disassociation

REAGENTS

Materials Provided:

- A. Human Serum References -- 1.0 ml/vial - Icons A-F**
Six (6) vials of human serum based reference calibrators at concentrations of 0 (A), 10 (B), 50 (C), 100 (D), 250 (E) and 500 (F) U/ml. Store at 2-8°C. A preservative has been added.
Note: The standards, human serum based, were made using a >99% pure affinity purified preparation of CA 19-9. The preparation was calibrated against Centocor CA 19-9 IRMA test.
- B. CA19-9 Biotin Reagent -- 13 ml/vial** ▽
One (1) vial of Anti-Human CA19-9 (MoAb)-Biotin reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.
- C. CA19-9 Enzyme Reagent-- 13 ml/vial - Icon** ⓔ
One (1) vial of Anti-Human CA19-9-HRP conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.
- D. Streptavidin Plate-- 96 wells - Icon** ↓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
- E. Wash Solution -- 20ml - Icon** ⬇
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.
- F. Substrate A --7.0ml/vial - Icon S^A**
One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.
- G. Substrate B -- 7.0ml/vial - Icon S^B**
One (1) bottle containing hydrogen peroxide (H₂O₂) in acetate buffer. Store at 2-8°C.

H. Stop Solution -- 8.0ml/vial - Icon ⓧ
One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.
Note 3: Above reagents are for a single 96-well microplate

Required But Not Provided:

1. Pipette capable of delivering 25 & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

PRECAUTIONS

*For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals*

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

REAGENT PREPARATION:

- 1. Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.
- 2. Working Substrate Solution**
Pour the contents of vial labeled Solution 'A' into the vial labeled Solution 'B'. Place the yellow cap on the mixed reagent for easy identification. Mix and label accordingly. Store at 2-8°C.

Note: Do not use the working substrate if it looks blue.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the biotinylated labeled antibody to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the Ca19-9 Enzyme Reagent labeled antibody to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 60 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

CALCULATION OF RESULTS

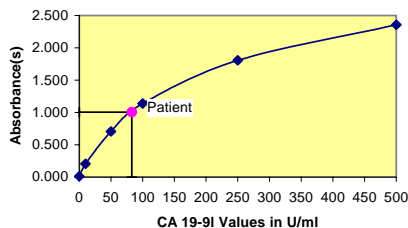
A dose response curve is used to ascertain the concentration of CA19-9 in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding CA19-9 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
- Draw the best-fit curve through the plotted points.
- To determine the concentration of CA19-9 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.004) intersects the dose response curve at 82.9U/ml CA19-9 concentration (See Figure 1).

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.013	0.014	0
	B1	0.014		
Cal B	C1	0.210	0.208	10
	D1	0.212		
Cal C	E1	0.754	0.708	50
	F1	0.662		
Cal D	G1	1.128	1.140	100
	H1	1.152		
Cal E	A2	1.850	1.805	250
	B2	1.760		
Cal F	C2	2.310	2.355	500
	D2	2.400		
Patient	A3	1.009	1.004	82.9
	B3	0.999		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

- The absorbance (OD) of calibrator F should be ≥ 1.3 .
- Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF PROCEDURE

- It is important that the time of reaction in each well is held constant for reproducible results.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- Use components from the same lot. No intermixing of reagents from different batches.
- Patient specimens with CA 19-9 concentrations above 500U/ml may be diluted (for example 1/10 or higher) with CA19-9 zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).

B. Interpretation

- If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
- CA 19-9 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA 19-9 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

EXPECTED RANGES OF VALUES

The serum CA 19-9 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salpingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for the CA 19-9 Elisa Test System

Healthy and non-pregnant subjects ≤ 40 U/ml

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the CA 19-9 AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

TABLE 3
Between Assay Precision* (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.34	9.2%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

B. Sensitivity

The CA 19-9 procedure has a sensitivity of 1.0 U/ml.

C. Accuracy

The Monobind CA 19-9 AccuBind™ ELISA procedure was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 136. The least square regression equation and the correlation coefficient were computed for the CA 19-9 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4
Least Square Regression Analysis

Method	Mean	Correlation Coefficient
This Method (X)	18.62	$x = 1.4577 + 0.8837(y)$
Reference (Y)	19.43	0.955

D. Specificity

In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reactions for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Concentration	Percent (%) Cross Reaction
CA 19-9	-	100
CA 125	10000 U/ml	0.001
CA 15-3	1000 U/ml	ND*
PSA	5000 ng/ml	ND*
AFP	10000 ng/ml	ND*
CEA	10000 ng/ml	ND*
HCG	10000 mIU/ml	ND*
RF	1000 kIU/ml	ND*

REFERENCES

- Zamcheck, N, *Adv Intern Med*, **19**, 413 (1974).
- Rayncao G, Chu TM, *JAMA*, **220**, 381 (1972).
- Harrison, *Principles of Internal Medicine*, McGraw Hill Book Company, New York, 12th Ed.
- Wild D, *The Immunoassay Handbook*, Stockton Press, 444 (1994).
- Hasholzner U, Steiber P, Baumgartner L, Pahl H, Meier W, Fateh-Moghadam A, "Methodological and clinical evaluation of three automated CA 19-9 assays compared with CA 19-9 II RIA (Centocor)", *Tumor Diagnosis & Ther*, **15**, 114-117(1994).
- Hasholzner U, Steiber P, Baumgartner L, Pahl H, Meier W, Fateh-Moghadam A, "Clinical significance of the tumor markers CA 19-9 II and CA 72-4 in ovarian carcinoma", *Int J Cancer*, **69**, 329-34 (1996).
- Ovarian Cancer – NIH Consensus Conference, *JAMA*, **273**, 491-497 (1995).
- Daoud E, Bodor G, Weaver C, Landenson JH and Scott MG, "CA 19-9 concentrations in malignant and non-malignant disease", Washington University Case Conference, *Clin Chem*, **37**, 1968-74 (1991).
- De Bruijn HWA, Van Der Zee AGJ & Alders JG, "The value of Cancer Antigen 125 (CA 19-9) during treatment and follow up of patients with ovarian cancer", *Curr Opin Gynecol*, **9**, 8-13 (1997).

Revision: B

Date: 102506

Cat #: 3925-300

Size	96(A)	192(B)
Reagent (fill)	A)	1ml set
	B)	1 (13ml) 2 (13ml)
	C)	1 (13ml) 2 (13ml)
	D)	1 plate 2 plates
	E)	1 (20ml) 1 (20ml)
	F)	1 (7ml) 2 (7ml)
	G)	1 (7ml) 2 (7ml)
	H)	1 (8ml) 2 (8ml)

Interesting products and services.

For Orders and Inquiries, please contact

Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92630 USA

Tel: 949-951-2665
Fax: 949-951-3539
Email: info@monobind.com
On the Web: www.monobind.com

Please visit our website to learn more about our other interesting products and services.



Instruments & Applications

Monobind's immunoassay products are designed to work in both manual and automated lab environments. AccuBind™ and AccuLite™ are compatible with any open-ended instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument, please visit the instrument section of our website, or contact techsupport@monobind.com

Monobind offers several instruments, including the Impulse 2 Luminometer CLIA Plate Reader designed hand-in-hand with our products and capable of 2-point calibration. Visit our website for more information.

[Please visit our website for more interesting products]