
CARBOHYDRATE ANTIGEN 19-9 (CA19-9) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0211-2

INTENDED USE

The Autobio carbohydrate antigen 19-9 (CA19-9) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of CA19-9 concentration in human serum.

INTRODUCTION

The CA19-9 is a carbohydrate antigen predominantly associated with gastrointestinal (GI) malignancies; pancreatic, colorectal, gastric and hepatic carcinomas¹⁻³. The main application of CA19-9 is in the management of diagnosed pancreatic and colorectal cancers⁴⁻⁶. Immunochemically, CA 19-9 antigen is the sialated form of the blood group antigen Lewis⁷⁻⁸. Clinical studies indicate that CA 19-9 assay levels have been found elevated in the serum with carcinomas of the exocrine pancreas, the colon and rectum, the stomach and lung cancer⁹⁻¹¹. It has been shown that a persistent elevation in CA19-9 assay value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising CA19-9 assay value may be associated with progressive malignant disease and poor therapeutic response. A declining CA19-9 assay value may be indicative of a favorable prognosis and a good response to treatment.¹²⁻¹⁸ Increased serum CA19-9 assay values have also been observed in patients with nonmalignant conditions such as hepatitis, cirrhosis, pancreatitis, and other gastrointestinal disease.¹⁹⁻²⁶ The CA 19-9 concentration should always be interpreted taking into consideration other clinical data and should not be used as a cancer screening test.

PRINCIPLE OF THE TEST

The CA19-9 CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The CA19-9 molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of CA19-9 present in the sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to carbohydrate antigen 19-9 (anti-CA19-9 MAB) (1 plate, 48 wells/96wells)
2. Sample diluent: (1 vial, 6.0ml/11.0 ml).
3. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-CA19-9 MAB in Stabilizing Buffer (1 vial, 6.0ml/11.0ml)
4. Reference Standards: 0, 15, 30, 60, 120, and 240U/ml CA19-9 in Stabilizing Buffer (6 vials, 0.5ml/ea)
5. Substrate A: (1 vial, 3.5ml/6.0ml)
6. Substrate B: (1 vial, 3.5ml/6.0ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200 μ l, 100-1000 μ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2~8°C upon receipt. The test kit may be used throughout the expiration date of the kit (6 months from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted standards should be used within 14 days and be frozen at -20°C for long term storage. Avoid repeated freezing and thawing of the standards. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least two months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18~25°C) prior to use.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2~8°C

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50µl of Sample Diluent to each well.
2. Dispense 50µl of CA19-9 standards, specimens, and controls into appropriate wells. Mix gently for 30 seconds.
3. Incubate at 37°C for 60 minutes.
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300µl.
5. Dispense 100µl of Enzyme Conjugate Reagent into each well. Mix well.
6. Incubate at 37°C for another 60 minutes.
7. At the end of the 60 minute incubation, remove the contents and wash the wells as described in step 4 above.
8. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.

- Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

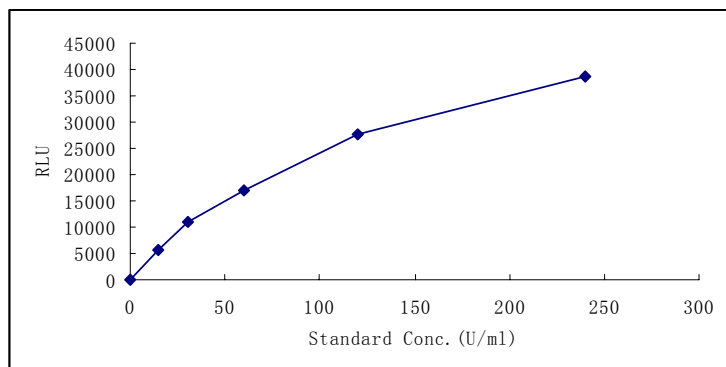
CALCULATION OF RESULTS

- Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
- On linear graph paper plot the RLU (ordinate) obtained from each reference standard against the corresponding concentration of CA19-9 in U/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
- Read the concentration for each control and sample by interpolating on the calibration curve.
- Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a point to point function curve fitting is recommended.
- Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

CA19-9 (U/ml)	RLU
0	87.231
15	5827.615
30	10944.8
60	16983.9
120	27584.2
240	38732.65



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. Approximately 95% of the normal healthy population has CA19-9 levels less than 35U/ml.

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of Standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio CA19-9 CLIA kit is not higher than 1.0U/ml.

B. Specificity

No interference was detected with the performance of Autobio CA19-9 CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
human albumin	100mg/ml
CEA	500ng/ml
CA125	400U/ml
CA15-3	500U/ml

C. Precision

a. Intra-assay Precision

Intra-assay Precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	45.34	3.26	7.19
High titer	20	122.07	6.79	5.56

b. Inter-assay Precision

Inter-assay Precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	43.86	3.12	7.11
High titer	10	125.64	5.86	4.66

D. Accuracy

For 88 specimens in the range of 0U/ml to 240U/ml, the correlation between the Autobio CA19-9 CLIA kit and Roche Elecsys assay was as follows:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Roche® (ECLIA)	88	$y = 0.9916x + 0.9052$	0.955

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER
	CONSULT INSTRUCTIONS FOR USE

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