Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

CE

AccuDiag™ CA-19-9 ELISA Kit

REF 6909-16

IVD See External Label 2-8°C 96 Tests

CA-19-9 ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-240U/mL
Sample	50 µL Serum
Specificity	97%
Sensitivity	5 U/ml
Incubation Time	140 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The CA-19-9 assay kit is intended to be used as a monitoring and screening test. An abnormal result (i.e. an elevated serum CA-19-9) suggests the need for further clinical management. This test has been found useful for patients in clinical remission, as post-operative serum CA-19-9 values which fail to return to normal strongly suggest the presence of residual tumor and tumor recurrence is often accompanied by a rise of serum levels before progressive disease is clinically evident.

SIGNIFICANCE AND SUMMARY

A group of mucin type glycoprotein Sialosyl Lewis Antigens (SLA), such as CA-19-9 and CA-19-5, have come to be recognized as circulating cancer associated antigens for gastrointestinal cancer.

CA-19-9 represents the most important and basic carbohydrate tumor marker. The immunohistologic distribution of CA-19-9 in tissues is consistent with the quantitative determination of higher CA-19-9 concentrations in cancer than in normal or inflamed tissues. Recent reports indicate that the serum CA-19-9 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. This tumor-associated antigen may also be elevated in some non-malignant conditions. 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 CA-19-9 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.

ASSAY PRINCIPLE

The CA-19-9 ELISA 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 CA-19-9 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 colorimetric reactions. The intensity of the color formed is proportional to the concentration of CA-19-9 present in the sample.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

MATERIALS AND COMPONENTS

Materials provided with the test kit

1. Murine monoclonal anti-CA-19-9 coated 96 well microtiter plate.
2. Assay buffer, 12 ml.
3. Enzyme conjugate reagent, 12 ml.
4. CA-19-9 reference standards (liquid, one set), containing 0, 15, 30, 60, 120, and 240 U/ml CA-19-9, Ready for use.
5. TMB Substrate, 12 ml.
6. Stop solution, 12 ml.
7. Wash Buffer Concentrate (50x), 15 ml.

Materials required but not provided

1. Precision pipettes and tips, 0.04~0.2ml, 1.0ml.
2. Distilled water.
3. Vortex mixer.
4. Absorbent paper or paper towel.
5. Graph paper.

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6. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at a wavelength of 450nm.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stroed at 2-8°C.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Bufferconcentrate (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50** µL of CA-19-9 standards, specimens, and controls into appropriate wells.
2. Dispense **100** µL of Assay Buffer to each well. 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.
5. Rinse and empty the microtiter plate 5 times with washing buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Dispense **100** µL of enzyme conjugate reagent into each well. Mix well.
7. Incubate at 37°C for another 60 minutes.
8. At the end of the 60 min. incubation, remove the contents and wash the wells as described in step 4 above.
9. Dispense **100** µL of the TMB substrate reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes without shaking.
11. Stop the reaction by adding **100** µL of Stop Solution to each well.
12. Gently mix for 10 seconds. It is very important that the blue color Completely changes to yellow.
13. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note:

14. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
15. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting 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 pipetting is available.
16. Duplication of all standards and specimens, although not required, is recommended.

RESULTS

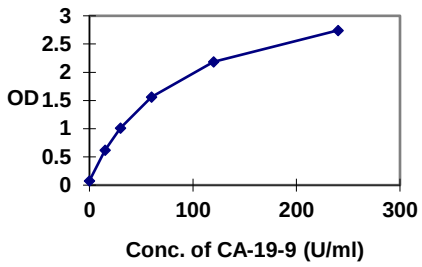
Calculate the mean absorbance value for each set of CA-19-9 reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CA-19-9 in units per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of typical standard run with optical density reading at 450nm shown in the Y axis against CA-19-9 concentrations shown in the X axis.

CA-19-9 Values (U/ml)	Absorbance (450nm)
0	0.078
15	0.620
30	1.009
60	1.562
120	2.182
240	2.742

This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.



EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA-19-9 assay values **below 35 U/ml**. The **minimum detectable concentration** of CA-19-9 in this assay is estimated **to be 5 U/ml.**

LIMITATIONS OF THE PROCEDURE

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air.

2. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
3. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
4. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

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REF 6909-16	**AccuDiag™ - CA-19-9 ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu**
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