Tumor ichroma™ CA19-9

INTENDED USE

ichroma™ CA19-9 is a fluorescence immunoassay (FIA) for the quantitative determination of CA19-9 in human serum/plasma. It is useful as an aid in management and monitoring of cancer patients.

For *in vitro* diagnostic use only.

INTRODUCTION

CA19-9 is a high molecular weight glycolipid and called sialyl Lewis antigen A. It is normally synthesized in the pancreas and biliary tract, stomach, colon, endometrium, and salivary gland epithelial cells. CA 19-9 is transported by mucoglycoprotein, the normal reference range of CA 19-9 is less than 37 U/mL in serum. Previously, CA 19-9 was used in colorectal cancer and gastrointestinal malignancies, but now it is most used in pancreatic cancer[1]. CA19-9 is increased in about 70% of pancreatic cancer patients and has a sensitivity of 79% and a specificity of 82% compared to other carcinomas[2,3]. however CA 19-9 value does not rise in patients with early pancreatic cancer or Lewis antigen negative. It can also be elevated by jaundice, digestive system cancer, Liver disease, gallbladder cancer[4,5]. This fact has made it difficult to use serum CA19-9 determination as a confirmed diagnosis. Through many studies, it was found that CA19-9 was associated with the pancreatic cancer treatment and the recurrence[6]. Therefore, the role of CA19-9 lies in monitoring post-treatment clinical conditions and post-treatment evaluation of patients, repetitive measures can be useful as early indicators of benefit

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-streptavidin on test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show CA19-9 concentration in the sample.

COMPONENTS

ichroma™ CA19-9 consists of 'cartridges', 'detector Tubes', and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line, and rabbit IgG at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they further packaged in a box.
- The detector has 2 granules containing anti human CA19-9 fluorescence conjugate, anti human CA19-9 biotin conjugate, anti Rabbit IgG fluorescence conjugate, bovine serum albumin (BSA), sucrose as a stabilizer, bromophenol blue, Mouse IgG as blocker and sodium azide as a preservative in phosphate buffered saline (PBS). All detectors are packed in a pouch.
- The detector diluent contains tween20 as a surfactant, NaCl and sodium azide as a preservative in Tris-HCl Buffer, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent, capillary tube and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- Detector tube and detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in ichroma™ CA19-9 when biotin concentration in the sample was below 100 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ CA19-9** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ CA19-9** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Sodium heparin

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes the antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 - 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
		20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-139

Components of **ichroma™ CA19-9**

- Cartridge Box:
  - Cartridge 25
  - Detector Tube 25
  - Detector diluent 1
  - ID Chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ CA19-9**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
  - **ichroma™-50** REF FPRR022
  - **ichroma™-50 PLUS** REF FPRR036
- **Boditech CA19-9 Control** REF CFPO-358

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ CA19-9** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ CA19-9**: Sealed cartridges, detection tubes, detector diluent, an ID chip and an instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID Chip.
- If the sealed cartridge, the detector tube, and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.

TEST PROCEDURE

▶ **ichroma™ II, ichroma™ M2**

Multi test mode / Read now mode

1) Take 150 µL of the detector diluent using a pipette and dispense it to the detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer. (The detection buffer must be used within 30 seconds.)
2) Take sample 10 µL of sample (serum/ plasma/control) using a pipette and dispense it to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times. (The sample mixture must be used within 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Leave the cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) To scan the sample-loaded cartridge, insert it into the

cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
7) Tap the 'START' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M2 is tested automatically after inserting.)
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode/ Walk away mode

1) The test procedure is same with the 'Multi test mode 1) – 4)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Tap the 'START' button on the instrument for ichroma™ tests.
(ichroma™ M2 is tested automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with the 'Single test mode'.

▶ **ichroma™-50, ichroma™-50 PLUS**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tubes in place.
3) Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4) Insert the cartridge magazine with the cartridges into the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of test cartridge by tapping.
8) Tap the button located in the upper side of the No. of reagent region to select ID chip what you want to use.
9) When the selected cartridge slot is activated, set the number of detector tube by tapping.
10) Set the number of pipette tips by tapping.
11) Tap the 'START' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays CA19-9 concentration of the test sample in terms of U/mL.
- Cut-off: 37 U/mL
- Working range : 8-1000 U/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided on demand with **ichroma™ CA19-9**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
(Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LOB)	2.73 U/mL
Limit of Detection (LOD)	3.75 U/mL
Limit of Quantitation (LOQ)	8.00 U/mL

- **Analytical specificity**
  - Interference
  Interferents listed in the following table were added to the test sample at the concentration mentioned below. the **ichroma™ CA19-9** test results did not show any significant interference with these materials.

Interference material	Conc.
Hemoglobin	1000 mg/dL
Bilirubin, unconjugated	0.7 mM/L
Triglycerides	50 g/L
Ascorbic acid	0.3 mM/L
Glucose	1000 mg/dL
Cholesterol	400 mg/dL
Biotin	100 ng/mL

  - Cross-reactivity
  Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. ichroma™ CA19-9 test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactivity material	Conc. (/mL)
CEA	500 ng/ml
Cyfra21-1	500 ng/ml
CA125	5000 U/ml
PSA	400 ng/ml
AFP	800 ng/ml

- **Precision**
  - Single-site study
  Repeatability (within-run precision)
  within-laboratory precision (Total precision)
  Lot to lot precision
  3 Lots of **ichroma™ CA19-9** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.
  - Multi-site study
  Reproducibility

1 Lot of **ichroma™ CA19-9** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Single-site study						
CA19-9 [U/mL]	Repeatability		Within-laboratory precision		Lot to lot precision	
	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
10	9.99	6.05	10.01	5.97	9.99	5.80
30	29.85	5.91	30.11	5.68	29.98	5.73
500	498.99	6.46	501.96	5.98	501.42	5.57

Multi-site study			
CA19-9 [U/mL]	Reproducibility		
	AVG	SD	CV(%)
10	10.05	0.57	5.64
30	29.89	1.66	5.56
500	500.83	27.90	5.57

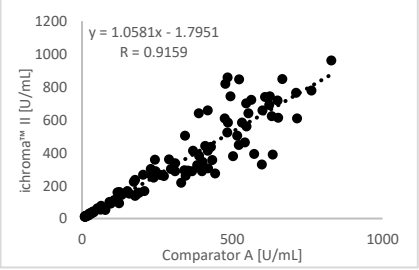
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ CA19-9**. The tests were repeated 10 times at each concentration of the control standard.

CA19-9 [U/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
500.00	472.94	484.15	466.42	474.50	95%
402.00	384.05	395.50	398.69	392.74	98%
353.00	342.56	347.93	343.76	344.75	98%
157.00	145.38	158.88	157.14	153.80	98%
402.00	412.31	391.29	375.84	393.15	98%
10.00	9.49	9.58	9.42	9.49	95%

- **Comparability**

CA19-9 concentrations of 100 clinical samples were quantified independently with **ichroma™ CA19-9 (ichroma™ II)** and comparator A as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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