ACCESS

Access CEA

Immunoassay Systems

Instructions For Use

Carcinoembryonic Antigen

REF **33200**

FOR PROFESSIONAL USE ONLY
Rx Only
FOR USE WITH TEST NAME: CEA2

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

WARNING

The concentration of CEA in a given specimen determined with different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CEA assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining CEA values is changed, additional sequential testing should be carried out to confirm baseline values.

Caution: For U.S.A. only, Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to by or on the order of a physician.

INTENDED USE

The Access CEA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of Carcinoembryonic Antigen (CEA) levels in human serum, using the Access Immunoassay Systems. CEA measured by the Access Immunoassay Systems is used as an aid in the management of cancer patients in whom changing CEA concentrations have been observed.

SUMMARY AND EXPLANATION

Carcinoembryonic antigen (CEA), first described by Gold and Freedman in 1965, was isolated from extracts of liver metastases of colon adenocarcinomas and normal fetal digestive tract.[1,2] It is considered one of the most extensively investigated human tumor associated antigens. An immunologically heterogenous group of glycoproteins, CEA is approximately 200,000 daltons with 50-85% carbohydrates by weight.[3] CEA is a member of the immunoglobulin superfamily and appears to have functions of an intercellular adhesion molecule.[4] In addition, molecules structurally related to CEA (i.e. NCA, NCA-2, NFA) have been reported in normal adult tissues.[5,6,7]

The measurement of serum CEA has shown substantial benefit in the prognosis and management of patients with malignant diseases, especially colorectal cancer.[8,9,10,11,12,13] Serial measurements can be used to monitor patients for progression, regression or recurrence of cancer following treatment. A persistent elevation of CEA following therapeutic or surgical intervention signals residual disease or recurrence, whereas decreasing levels to within the normal range is indicative of successful intervention.[14]

CEA is also elevated in the serum of patients with non-malignant diseases and in heavy smokers, therefore CEA should not be used in the diagnosis of cancer or for screening asymptomatic patients.

METHODOLOGY

The Access CEA assay is a two-site immunoenzymatic “sandwich” assay using two mouse monoclonal anti-CEA antibodies (MAb) which react with different epitopes of CEA. A sample is added to a reaction vessel, along with the first anti-CEA MAb-alkaline phosphatase conjugate and the second anti-CEA MAb bound to paramagnetic particles. The incubation is followed by a magnetic separation and washing. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is proportional to the concentration of CEA in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibrator curve.

SPECIMEN

Specimen Collection and Preparation

1. Serum is the recommended sample.

2. Observe the following recommendations for handling, processing, and storing blood samples:[15]

- Collect all blood samples observing routine precautions for venipuncture.
- For serum, allow samples to clot adequately before centrifugation.
- Keep tubes stoppered at all times.
- Physically separate serum from contact with cells as soon as possible.
- Store samples, tightly stoppered, at room temperature (15 to 30°C) for no longer than eight hours.
- If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
- If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.

3. Use the following guidelines when preparing specimens:

- Ensure residual fibrin and cellular matter has been removed prior to analysis.
- Follow blood collection tube manufacturer's recommendations for centrifugation.

4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

REAGENTS

Product Information

Access CEA Reagent Pack

Cat. No. 33200: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate packs at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.

- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- After initial use, the pack is stable at 2 to 10°C up to 28 days.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

R1a:	Solid phase: Paramagnetic particles coated with mouse anti-CEA MAb, suspended in TRIS buffered bovine serum albumin (BSA), with < 0.1% sodium azide and 0.1% ProClin* 300.
R1b:	Diluent: Phosphate buffer, protein (bovine, murine) with < 0.1% sodium azide and 0.1% ProClin 300.
R1c:	Conjugate: Mouse anti-CEA MAb bound to alkaline phosphatase (bovine), diluted in phosphate buffer, protein (bovine), < 0.1% sodium azide and 0.1% ProClin 300.

*ProClin™ is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- **For** *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS, GHS HAZARD CLASSIFICATION and EU HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

PMP (Compartment R1a) WARNING

H317	May cause an allergic skin reaction.
P280	Wear protective gloves, protective clothing and eye/face protection.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.

	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Diluent (Compartment R1b)	DANGER	
		
		
	H317	May cause an allergic skin reaction.
	H318	Causes serious eye damage.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Polyoxyethylated Octyl Phenol 1 - 5%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Conjugate (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.

P280	Wear protective gloves, protective clothing and eye/face protection.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before use.
	reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

EUROPEAN HAZARD CLASSIFICATION

PMP (Compartment R1a)	Xi;R43	
	R43	May cause sensitization by skin contact.
	S28	After contact with skin, wash immediately with plenty of soap and water.
	S37	Wear suitable gloves.
Diluent (Compartment R1b)	R52/53	
	Xi;R43	
	R43	May cause sensitization by skin contact.
	R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
	S28	After contact with skin, wash immediately with plenty of soap and water.
	S37	Wear suitable gloves.
	S61	Avoid release to the environment. Refer to special instructions/Safety data sheets.
Conjugate (Compartment R1c)	Xi;R43	
	R43	May cause sensitization by skin contact.

S28	After contact with skin, wash immediately with plenty of soap and water.
S37	Wear suitable gloves.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access CEA Calibrators
   Provided at zero and approximately 1, 10, 100, 500 and 1,000 ng/mL.
   Cat. No. 33205
2. Access CEA QC or other commercially available control material.
   Cat. No. 33209
3. Access CEA Diluent
   Cat. No. 33206
4. Access Substrate
   Cat. No. 81906
5. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

Equipment and Materials

R1	Access CEA Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access CEA assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access“ format rather than a “batch” format, quality control materials should be included in each 24-hour period.[16] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a description of installation, theory of operation, performance characteristics, system processing (i.e., operating instructions), calibration information and procedures, maintenance, and troubleshooting. The operational limitations and precautions and hazards are

included where appropriate throughout the manuals.

2. Mix contents by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs-mix reagents by swirling gently.
3. Use thirty-five (35) µL of sample for each determination in addition to the sample container and system dead volume. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is ng/mL.

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software using a weighted four-parameter logistic (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. CEA concentrations were measured in human serum samples from 301 apparently healthy blood donors (including smokers and non-smokers), using the Access CEA assay:

	n	0.0-3.0 (ng/mL)	3.1-5.0 (ng/mL)	5.1-10.0 (ng/mL)	> 10.0 (ng/mL)
Non-smokers	151	95.4%	3.9%	0.7%	0.0%
Smokers	150	82.0%	8.7%	8.0%	1.3%
Total	301	88.7%	6.3%	4.3%	0.7%

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the reportable range of the lower limit of detection and the highest calibrator value (approximately 0.1-1,000 ng/mL). If a sample contains more than the stated value of the highest Access CEA Calibrator (S5), report the result as greater than that value (i.e. > 1,000 ng/mL). Alternatively, dilute one volume of the sample with nine volumes of Access CEA Calibrator S0 (zero) or Access CEA Diluent. After assaying the diluted sample, multiply the obtained value by the dilution factor, ten (10). If a sample contains less than the lower limit of detection for the assay, report the result as less than the lower limit of detection (< 0.1 ng/mL). Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[17,18]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access CEA results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information. Elevated levels of CEA may occur in non-neoplastic conditions, therefore, the Access CEA assay is not intended for the diagnosis of, or for screening of cancer.
4. Access CEA assay does not demonstrate any hook effect up to 100,000 ng/mL.

PERFORMANCE CHARACTERISTICS

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ACCURACY

CORRELATION

Comparison of serum CEA values using the Access CEA assay on the Access Immunoassay Analyzer and a commercially available automated immunoassay kit gives the following statistical data using Deming calculations:

n	Range of Observations (ng/mL)	Intercept	Slope	r
288	0.26-920.36	0.52	0.97	0.96

SPIKING RECOVERY

Known amounts of CEA were added to three patient samples. The concentration of CEA was determined before and after the addition of exogenous CEA and the percent recovery was calculated:

Sample 1 CEA Added (ng/mL)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
0.0	-	8.51	-
2.5	11.01	11.03	100.2
5.0	13.51	14.04	103.9
7.5	16.01	16.98	106.1
10.0	18.51	20.35	109.9
		Mean % Recovery	**105.0**

Sample 2 CEA Added (ng/mL)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)

Sample 2 CEA Added (ng/mL)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
0.0	-	30.98	-
2.5	33.48	32.98	98.5
5.0	35.98	35.95	99.9
7.5	38.48	37.59	97.7
10.0	40.98	42.38	103.4
		Mean % Recovery	**99.9**

Sample 3 CEA Added (ng/mL)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
0.0	-	722.22	-
25.0	747.22	736.26	98.5
50.0	772.22	785.54	101.7
75.0	797.22	836.11	105.5
100.0	822.22	879.31	106.9
		Mean % Recovery	**103.2**

DILUTION RECOVERY (LINEARITY)

Dilution (non-serial) of three patient samples containing CEA with the Access CEA Calibrator S0 (zero) resulted in the following data:

Sample 1	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	-	89.21	-
1/1.5	59.47	60.40	101.6
1/2.5	35.68	36.04	101.0
1/6.0	14.87	14.68	98.7
1/15.0	5.95	6.08	102.2
1/48.0	1.86	1.85	99.5
		Mean % Recovery	**100.6**

Sample 2	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	-	37.93	-
3/4	28.45	28.98	101.9
1/2.0	18.97	19.72	104.0
1/4.0	9.48	9.97	105.2
1/8.0	4.74	5.07	107.0
1/24.0	1.58	1.65	104.4
		Mean % Recovery	**104.5**

Sample 3	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	-	662.67	-
1/5.0	132.53	125.17	94.4
1/10.0	66.27	63.15	95.3
1/50.0	13.25	12.71	95.9
1/100	6.63	6.23	94.0
1/300	2.21	2.09	94.6
		Mean % Recovery	**94.8**

IMPRECISION

This assay exhibits total imprecision of less than or equal to 9% at concentrations greater than 2.1 ng/mL, and total SD of less than or equal to 0.19 ng/mL at concentrations less than or equal to 2.1 ng/mL. The assay imprecision was evaluated by testing 3 levels of controls in triplicate for a maximum of 2 runs per day for a total of 20 runs. The mean, SD and percent CV were calculated by analysis of variation (ANOVA):[19]

		Mean Concentration (ng/mL)	Within Run		Total	
Sample	n		SD	%CV	SD	%CV
Low Level	60	5.37	0.20	3.69	0.22	4.04
Medium Level	60	51.59	1.55	3.01	1.96	3.80
High Level	60	523.17	20.78	3.97	23.61	4.51

ANALYTICAL SENSITIVITY

The lowest detectable level of CEA distinguishable from zero (Access CEA Calibrator S0) with 95% confidence is 0.1 ng/mL. This value is determined from a total of ten assays across five Access Analyzers. Each assay consisted of a complete six point calibration curve, tri-level controls and ten replicates of the zero calibrator. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the fitted zero calibrator signal.

SPECIFICITY

Antigens related to CEA were added to the zero (S0) calibrator at concentrations up to 1,000 ng/mL. Results of these samples evaluated with the Access CEA assay are expressed as CEA concentrations.

Level of added antigen (ng/mL)	NCA-50 apparent CEA level (ng/mL)	NCA-2 apparent CEA level (ng/mL)	NCA apparent CEA level (ng/mL)	NFA-1 apparent CEA level (ng/mL)
0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0
100	0.1	0.0	0.0	0.0
500	0.5	0.0	0.0	0.0
1,000	0.9	0.0	0.0	0.0

INTERFERENCE

Hemoglobin, triglycerides, bilirubin, human serum albumin and rheumatoid factor, tested up to the following concentrations, respectively, 500 mg/dL, 1,800 mg/dL, 30 mg/dL, 5 g/dL and 500 IU/mL, do not interfere with the Access CEA assay.

The following therapeutic agents were tested at the concentrations listed and the percent recovery determined. There was no significant interference from these therapeutic agents.

Substance	Concentrations Added	Mean Recovery (%)
Bleomycin	0.1 IU/mL	102.92
Cisplatin	1.5 µg/mL	100.16
Cyclophosphamide	3,000 µg/mL	102.62
Doxorubicin	100 µg/mL	99.65
Fluorouracil	360 µg/mL	102.59
Leucovorin	60 µg/mL	102.44
Methotrexate	4,500 µg/mL	101.40
Mitomycin	60 µg/mL	98.88
Tamoxifen	133 µg/mL	102.68
Vinblastine	1.2 µg/mL	98.41

Substance	Concentrations Added	Mean Recovery (%)
Vincristine	0.7 µg/mL	99.93

ADDITIONAL INFORMATION

Symbols Key

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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