

AIA-360 Assay Specifications

ST CEA Test Code 006

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	50 ng/mL (example)
4		
5		
6		
7		
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	100
12	Dil. Vol	50
13	Assay L	0.5
14	Assay H	100.0
15	Ref. L	
16	Ref. H	
17	Decimal	1

Visible only in Test Mode

18	Test Name	#CEA
19	Calib. No	2
20	Calib. Mul	3
21	Calib. Equ	1
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	1.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-900 Assay Specifications

ST CEA Test Code 006

No.	Item	Data
1	Code	6
2	ACT	1
3	Analyte	#CEA
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/mL
6	CAL 2	50 ng/mL (example)
7		
8		
9		
10		
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	1
15	Assay Low	0.5
16	Assay High	100
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.5
20	Reschedule High	100
21	Factor A	1
22	Factor B	0
23	Sample Volume	100
24	Diluent Volume	50
25	2 Step reagent dispensing volume	0
26	Calibration code	1
27	CAL. No.	2
28	CAL. MUL.	3
29	CAL. EQU.	1
30	CAL. CV	90
31	DIL. SP1	1
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	1
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	1
39	DIL. NAME	CEA
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	0
42	PRE. 1VOL (Vol. of pretreatment sol-1)	0
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	0
44	PRE. CODE (pretreated sol. code)	0
45	PRE. NAME (pretreated sol. name)	
46	Protocol	1
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications

ST CEA Test Code 006

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	1
3	Reference low	
4	Reference high	
5	Reschedule low	0.5
6	Reschedule high	100.0
7	Assay range low	0.5
8	Assay range high	100.0
9	Specimen diluent code	1
10	Specimen diluent name	CEA
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	100
15	Default multiplier for >H	10
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	1
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	50 ng/mL (example)
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	100
39	Diluent volume	50
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1
49	Calibration Code Check	001
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Carcinoembryonic Antigen

ST AIA-PACK CEA

CAUTION: The sale and distribution of this device is restricted by United States federal law to, by, or on the order of a physician. In addition, the use of this device is restricted to, by, or on the order of a physician. Because of differences in reagent specificity and assay methods, the concentration of CEA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for CEA is changed during the course of monitoring patients with serial CEA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and Intended Use

ST AIA-PACK CEA is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Carcinoembryonic Antigen (CEA) in human serum and plasma to aid in the management of cancer patients in whom changing concentrations of CEA are observed on specific Tosoh AIA System analyzers.

Summary and Explanation of Test

Carcinoembryonic Antigen or CEA is a protein-polysaccharide β -globulin with a molecular weight of approximately 200,000 daltons and a sedimentation coefficient of 7S-8S.¹⁻³ It is normally present at very low concentrations in the blood of healthy adults. Although elevated serum CEA levels were originally thought to be specific for colorectal cancer,^{4,5} a variety of neoplastic and other disease processes cause significant elevation of CEA.⁶⁻¹³ Thus, CEA is used as a tumor marker in the management of patients with colorectal, breast, lung, prostatic, pancreatic, ovarian and other carcinomas, as long as these tumors secrete the CEA moiety.

Preoperative CEA levels may be of prognostic value since the level of elevation is correlated with body burden of tumor.^{8,14,15} Persistent elevation or increasing levels of CEA may indicate the presence of residual or disseminated malignancy.¹⁶ Since CEA concentration frequently declines during successful therapy and increases during disease progression, monitoring of serum CEA levels in patients with tumors which produce CEA has been shown to facilitate the clinical management of the disease. Measurement of serum CEA is not recommended as a screening procedure since elevated CEA levels are also seen in a variety of non-neoplastic and benign diseases such as alcoholic cirrhosis, hepatitis, ulcerative colitis, and Crohn's disease^{17,18} as well as in heavy cigarette smokers.¹⁹

Likewise, since not all tumors are CEA producing, a serum CEA measurement within the reference intervals found in apparently healthy subjects does not rule out the possibility of the presence of malignant disease.

Principle of the Assay

The ST AIA-PACK CEA is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. CEA present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the CEA concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK CEA, Cat. No. 0025254)

5 trays x 20 test cups (ST AIA-PACK CEA Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti-CEA mouse monoclonal antibody and mouse monoclonal antibody (to human CEA) conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform Carcinoembryonic Antigen analysis using the ST AIA-PACK CEA (Cat. No. 0025254) on specific Tosoh AIA Systems. They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-360	0019945
AIA-900	0022930
AIA-900 9tray Sorter	0022931
AIA-900 19tray Sorter	0022932
AIA-2000 ST	0022100
AIA-2000 LA	0022101
AIA-PACK:	
AIA-PACK Substrate Set II	0020968
AIA-PACK Substrate/Reconstituent	
AIA-PACK CEA Calibrator Set	0020354
Calibrator Zero 0 ng/mL	
Positive 50 ng/mL (approx.)	
AIA-PACK CEA Sample Diluting Solution	0020554
AIA-PACK Wash Concentrate Set	0020955
AIA-PACK Diluent Concentrate Set	0020956
AIA-PACK Detector Standardization Test Cups	0020970
AIA-PACK Sample Treatment Cups	0020971
Sample Cups	0018581
ADDITIONAL REQUIREMENTS: (Except AIA-360)	
Pipette Tips (1000/Pkg)	019215
Tip Rack (Empty)	019216

Preloaded Pipette Tips (96 Tips X 50 Racks)
Preloaded Pipette Tips (96 Tips X 5 Racks)

996010
996005

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The ST AIA-PACK CEA is intended for in vitro diagnostic use only.
- **Rx** only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots should not be mixed within a tray.
- The ST AIA-PACK CEA contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera is not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- Do not use beyond the expiration date.
- The ST AIA-PACK CEA has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. Samples with CEA concentrations between 100 and 20,000 ng/mL will read > 100 ng/mL. The “hook effect” phenomenon may occur at CEA concentrations > 20,000 ng/mL.
- The Tosoh AIA-600 or AIA-1200 Series Analyzers CANNOT be used to perform the ST AIA-PACK CEA assay.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
Refrigerator Temperature (2 - 8 °C):	
ST AIA-PACK CEA	0025254
AIA-PACK CEA Calibrator Set	0020354

AIA-PACK CEA Sample Diluting Solution	0020554
AIA-PACK CEA Calibration Verification/Linearity Test Set	0020654
AIA-PACK Substrate Set II	0020968
AIA-PACK Wash Concentrate	0020955
AIA-PACK Diluent Concentrate	0020956

Room Temperature (18 - 25 °C):

AIA-PACK Detector Standardization Test Cups	0020970
AIA-PACK Sample Treatment Cups	0020971

ST AIA-PACK test cups may be stored for up to 24 hours at a room temperature of 18 - 25 °C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators should be used within 24 hours. After opening, Sample Diluting Solution is stable for up to 90 days refrigerated at 2 - 8 °C. Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 2 - 8 °C. Working diluent and wash solutions are stable for 30 days at room temperature (18 - 25 °C). Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum or heparinized plasma is required for the assay. EDTA and citrated plasma **SHOULD NOT BE USED**.

No special patient preparation is necessary. When using serum, a venous blood sample is collected aseptically without additives. Store at 18 - 25 °C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay.

To use heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Samples may be stored at 2 - 8 °C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20 °C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18 - 25 °C) and mix gently.

The sample required for analysis is 100 µL.

Procedure

1) Reagent Preparation

1a) Substrate Solution

Bring all reagents to room temperature (18 - 25 °C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

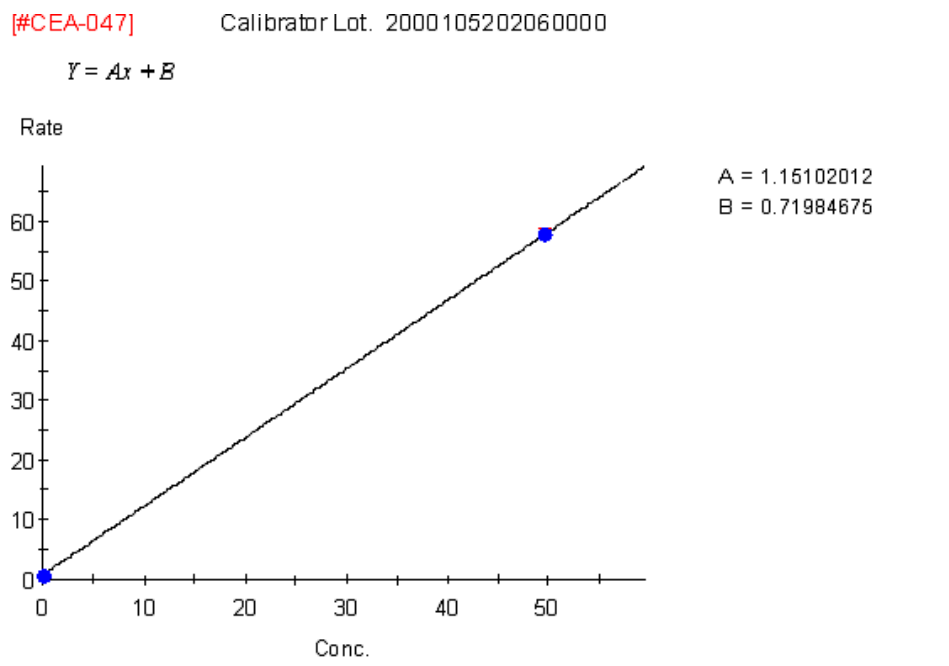
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK CEA have been standardized against WHO 1st IRP 73/601 (1975).

The calibration curve for the ST AIA-PACK CEA is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change). For further information regarding instrument operation, consult the AIA System Operator's Manual.

The sample calibration curve below shows the algorithm used to calculate the results. This is an example only. Actual results will vary depending on the Instrument type and lot number used.



2b) Calibration Procedure

Refer to the appropriate AIA System Operator's Manual for procedural instructions.

- i) Verify that the calibrator concentrations listed on the vial labels as well as the calibrator lot numbers have been correctly entered into the software.

- ii) Calibrators for ST AIA-PACK CEA are provided ready to use.
- iii) Tosoh recommends that all calibrators be run in triplicate.

2c) Calibration Acceptability criteria

- i) The mean rate for the zero calibrator should be < 3.0 nM/sec.
- ii) Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- iii) The replicate values should be within a 10% range.

2d) Calibration Review and Acceptance

- i) Using the criteria above, review the calibration curve carefully.
- ii) Edit the calibration if necessary, then accept the calibration.
- iii) For further information regarding calibration, consult the specific AIA System Operator's Manual.

3) Quality Control

3a) Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two (2) levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

3b) Quality Control Procedure

- i) Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the AIA System Operator's Manual for detailed instructions on defining and editing the files.
- ii) Quality control material to be run with this assay is defined by individual laboratory policy.

4) Specimen Processing

4a) Preparation

Following specific instructions in the Operator's Manual for the analyzer, place samples on the instrument appropriately.

4b) Assay Procedure

- i) Ensure a sufficient quantity of ST AIA-PACK CEA test cups for the number of samples to be run.
- ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-900 and AIA-2000 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.
3. If a serum or plasma specimen Carcinoembryonic Antigen concentration is found to be greater than the linearity limit of the assay, 100 ng/mL, the specimen should be diluted with the AIA-PACK CEA Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for samples containing greater than 100 ng/mL is 1:10 or 1:100. It is desirable to dilute the serum or plasma sample so that the diluted sample reads between 5 and 100 ng/mL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the AIA System Operator's Manual.
4. The AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK CEA Test cups can be used during the same run.
5. If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 006.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to Carcinoembryonic Antigen concentration in ng/mL.

For samples requiring dilution, the AIA 900 and AIA-2000 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the appropriate Operator's Manual.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

- After calibration, two levels of controls are run in order to accept the calibration curve.
- The two levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, therapy, etc.).

Using ST AIA-PACK CEA, the highest concentration of Carcinoembryonic Antigen measurable without dilution is 100 ng/mL, and the lowest measurable concentration is 0.5 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 50 ng/mL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 100 ng/mL.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated values when tested for Carcinoembryonic Antigen.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.²⁰

Reference Ranges

The interval given here was determined in serum samples from apparently healthy individuals. A total of 352 specimens were tested. Based on smoking history, there were 31 smokers, 173 never smoked, 85 previous smokers and 63 specimens with unknown smoking history. The never smoked, previous smokers and unknown smoking history specimens were combined into one category of "non-smokers". The 99th percentile was used to calculate the cut-off limits for smokers and non-smokers.

	Non-smoker*	Smoker
Number of Samples (n)	321	31
Reference Interval (99 th percentile)	7.2 ng/ml	14.5 ng/ml
*Includes never smoked, previous smokers and unknown smoking history		

The following data was collected using the AIA-PACK CEA. The ST AIA-PACK CEA demonstrates equivalent performance. (See Comparative Analysis.)

Distribution of Serum AIA-PACK CEA Concentrations in Healthy Subjects, Malignant and Benign Disease States

	Number of patients	0 - 3.0 ng/mL	3.1- 6.0 ng/mL	6.1- 10.0 ng/mL	10.1- 20.0 ng/mL	>20.0 ng/mL
Healthy Subjects						
Nonsmokers	103	88%	10%	2%	0%	0%
Smokers	101	49%	37%	11%	3%	0%
Total	204	69%	23%	6%	1%	0%
Carcinoma						
Colorectal	98	13%	16%	11%	19%	40%
Gastric	20	35%	35%	15%	5%	10%
Mammary	47	34%	13%	13%	11%	30%
Ovarian	20	65%	15%	0%	5%	15%
Pancreatic	20	20%	30%	35%	10%	5%
Pulmonary	50	24%	18%	16%	12%	30%
Other*	20	50%	25%	15%	5%	5%
TOTAL	275	19%	19%	14%	13%	27%
Nonmalignant Diseases						
Cirrhosis	19	21%	47%	26%	5%	0%
Polyp	20	75%	20%	5%	0%	0%
Pulmonary	20	58%	26%	11%	0%	5%
Pancreatitis	23	48%	35%	9%	9%	0%
Ulcerative colitis	20	45%	30%	25%	0%	0%
Other (breast)	21	71%	24%	0%	5%	0%
Total	123	53%	30%	12%	3%	1%

*The "Other" category of the Carcinoma classification includes the following:

Testicular	Uterine	Cervical	Liver (HCG)	Renal	Prostatic	Bladder
5	3	2	4	2	2	2

Performance Characteristics

The following performance characteristics were established using the Tosoh AIA NexIA Automated Immunoassay Analyzer. All AIA systems demonstrate equivalent performance.

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of CEA and assayed before and after spiking.

Sample	Initial Value (ng/mL)	CEA Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A1	1.45	24.18	25.63	25.14	98.1
	1.45	48.36	49.81	50.12	100.6
	1.45	96.72	98.17	97.00	98.8
Serum B1	1.95	24.18	26.13	26.79	102.5
	1.95	48.36	50.31	52.39	104.1
	1.95	96.72	98.67	99.96	101.3
Serum C1	2.75	24.18	26.93	28.31	105.1
	2.75	48.36	51.11	53.85	105.4
	2.75	96.72	99.47	103.40	104.0

1b) Dilution:

Three serum samples containing high concentrations of CEA were serially diluted with CEA Sample Diluting Solution and assayed.

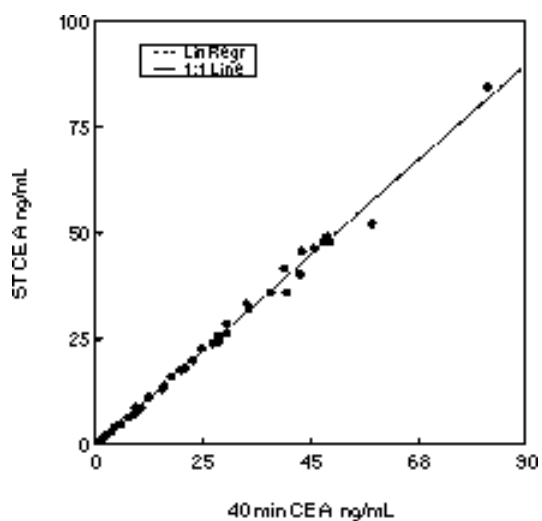
Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A2	none		100.37	
	7.5/10	75.28	74.37	98.8
	5.0/10	50.18	49.40	98.4
	2.5/10	25.09	24.24	96.6
	1.0/10	10.04	10.02	99.8
Serum B2	none		94.28	
	7.5/10	70.71	74.94	106.0
	5.0/10	47.14	49.35	104.7
	2.5/10	23.57	24.66	104.6
	1.0/10	9.43	10.11	107.2
Serum C2	none		95.55	
	7.5/10	71.66	70.68	98.6
	5.0/10	47.78	47.96	100.4
	2.5/10	23.89	23.88	99.9
	1.0/10	9.56	9.78	102.3

1c) Comparative Analysis:

Samples were collected at random. Results from the ST AIA-PACK CEA assay (y) were compared to those obtained using the AIA-PACK CEA assay (x). A total of 50 serum samples were tested in the study.

Slope 1.000 (95% CI: 0.980 to 1.022)
y-intercept: -0.280 (95% CI: -0.891 to 0.330)
Correlation Coefficient 0.997
Range of Samples (x) 1.227 – 81.926
Number of Samples 50

Figure 1
Comparative Analysis
ST AIA-PACK CEA vs AIA-PACK CEA (40 min)



$$y = 1.00x - 0.280$$
$$r = 0.997$$

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision was determined using three controls in a total of 20 runs. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation.

Sample	Mean (ng/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Serum A3	3.28	0.080	2.5
Serum B3	10.71	0.213	2.0
Serum C3	76.99	1.539	2.0

2b) Inter-assay precision

The inter-assay (between run) precision coefficient of variation was evaluated at three different concentrations by analyzing samples in 20 separate runs.

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A3	20	3.20	0.162	5.1
Serum B3	20	10.54	0.322	3.1
Serum C3	20	75.50	2.802	3.7

2c) Total precision

Total precision was determined by the duplicate assay of three controls in 20 separate runs. The means of each run were used to calculate the standard deviation (SD) and coefficient of variation.

Sample	Mean (ng/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Serum A3	3.28	0.149	4.6
Serum B3	10.71	0.399	3.7
Serum C3	76.99	2.876	3.7

Sensitivity

The minimal detectable concentration (MDC) of Carcinoembryonic Antigen is estimated to be 0.5 ng/mL. The MDC is defined as that concentration of CEA which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 20 replicate determinations of a zero calibrator.

Interference

- Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration.
- Added hemoglobin (up to 390 mg/dL), free bilirubin (up to 17 mg/dL), and conjugated bilirubin (up to 19mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1,660 mg/dL), does not interfere with the assay.
- Protein, as indicated by added albumin (up to 5 g/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Chemotherapeutic Agents: Therapeutic concentrations of the following chemotherapeutic agents added to serum samples which were then assayed, do not interfere with ST AIA-PACK CEA Assay: Cisplatin, Velban, Bleomycin, Adriamycin, Vincristine, Methotrexate, 5-Fluorouracil, and Mitomycin C.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

References

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	TOSOH CORPORATION Bioscience Division 2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan Phone: +81 3 6636 3734 Fax: +81 3 6636 3627 Website: www.tosoh.com
	Supplied by TOSOH BIOSCIENCE, INC. 3600 Gantz Road, Grove City, OH 43123 USA Phone: +1 650 615 4970 Fax: +1 650 615 0415 Phone: (800) 248 6764 Fax: (800) 685 7595 Website: www.tosohbioscience.us

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AIA-PACK

CEA Calibrator Set

Intended Use

The AIA-PACK CEA Calibrator Set is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK CEA Assays.

Summary and Explanation

The AIA-PACK CEA Calibrator Set contains human serum with assigned levels of Carcinoembryonic Antigen. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set have been standardized against WHO 1st IRP 73/601 (1975).

Material Provided (Cat. No. 0020354)

2 x 1 mL	Zero Calibrator
	Human serum containing no detectable concentration of CEA (0 ng CEA/mL), and 0.1% sodium azide as preservative. (Ready for Use)
2 x 1 mL	Positive Calibrator
	Human serum containing the assigned concentration of CEA (approximately 50 ng CEA/mL, described on each vial) and 0.1% sodium azide as preservative. (Ready for Use)

Warnings and Precautions

- The AIA-PACK CEA Calibrator Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK CEA calibrators are provided ready to use.
- Bring calibrator to room temperature (18 - 25 °C) for use.
- Always store the Calibrator Set in an upright position at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK CEA Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours.

Procedure

Refer to the CALIBRATION PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

Calibration

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The AIA-PACK CEA Calibrator Set contains assigned concentrations of Carcinoembryonic Antigen. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of 0.0 to 100.0 ng/mL of Carcinoembryonic Antigen. The calibrators in this set have been standardized against WHO 1st IRP 73/601 (1975).

Results

- The mean rate for the zero calibrator should be <3.0 nM/sec.
- Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- The replicate values should be within a 10% range.

Limitations

The AIA-PACK CEA Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

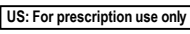
- 1) AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
- 2) AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.



TOSOH CORPORATION
Bioscience Division
2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan
Phone: +81 3 6636 3734
Fax: +81 3 6636 3627
Website: www.tosoh.com

Supplied by

TOSOH BIOSCIENCE, INC.
3600 Gantz Road,
Grove City, OH 43123 USA
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AIA-PACK

CEA Sample Diluting Solution

Intended Use

The AIA-PACK CEA Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of Carcinoembryonic Antigen above the linear range of the assay.

Summary and Explanation

The AIA-PACK CEA Sample Diluting Solution contains human serum with no detectable concentration of Carcinoembryonic Antigen. This Sample Diluting Solution is to be used only with samples that are being tested for Carcinoembryonic Antigen concentrations using the ST AIA-PACK CEA assays.

Materials Provided (Cat. No. 0020554)

4 x 4 mL Sample Diluting Solution

Human serum containing no detectable concentration of Carcinoembryonic Antigen (0 ng CEA/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK CEA Sample Diluting Solution is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK CEA Sample Diluting Solution is provided ready to use.
- Always store the Sample Diluting Solution in an upright position at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK CEA Sample Diluting Solution is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for up to 90 days when refrigerated at 2 - 8 °C.

Procedure

1. Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.
2. If a specimen is found to contain greater than the linearity limit of 100 ng/mL, the specimen should be diluted with the Sample Diluting Solution and assayed according to the procedure in the AIA-PACK section of the analyte application..
3. The AIA 900 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
4. The recommended dilution for serum or plasma containing greater than 100 ng/mL is 1:10 or 1:100. However, it is desirable to dilute the serum or plasma samples that contain more than 100 ng CEA/mL so that the diluted sample reads between 5 and 100 ng CEA/mL.

Results

When an auto-dilution is performed, the AIA instrument will calculate the final result.

Limitations

The AIA-PACK CEA Sample Diluting Solution is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

**TOSOH CORPORATION****Bioscience Division**

2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan

Phone: +81 3 6636 3734

Fax: +81 3 6636 3627

Website: www.tosoh.com**Supplied by****TOSOH BIOSCIENCE, INC.**

3600 Gantz Road,

Grove City, OH 43123 USA

Phone: +1 650 615 4970

Fax: +1 650 615 0415

Phone: (800) 248 6764

Fax: (800) 685 7595

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AIA-PACK

CEA Calibration Verification/Linearity Test Set

Intended Use

The AIA-PACK CEA Calibration Verification/Linearity Test Set is intended for IN VITRO diagnostic use only for the verification of the calibration and linearity of the ST AIA-PACK CEA Assays.

Summary and Explanation

The AIA-PACK CEA Calibration Verification/Linearity Test Set can be used as part of a quality assurance program to assist in complying with various regulatory requirements under which an institution may operate. Specific guidelines for the acceptability of data are determined by the institution in conformance with these requirements. Good laboratory practices stipulate using different materials for calibration and quality control purposes. Calibration Verification Materials should be analyzed as unknown test samples according to the directions stated in the AIA System Operator's Manual.

Materials Provided (Cat. No. 0020654)

2 x 4 mL	Sample Diluting Solution (SDS) Human serum containing no detectable concentration of CEA (0 ng CEA/mL), and 0.1% sodium azide as a preservative.
2 x 2 mL	Calibration Verification Material (CVM) Human serum containing the assigned concentration of CEA (approximate upper linearity limit of 100 ng CEA/mL described on each vial) and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK CEA Calibration Verification/Linearity Test Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such materials, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The Calibration Verification Material and Sample Diluting Solution are provided ready to use.
- The materials should be at room temperature (18 - 25 °C) prior to use.
- Always store the Calibration Verification/Linearity Test Set in an upright position at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK CEA Calibration Verification/Linearity Test Set is stable until the expiration date on the label. After opening, the materials should be used within 24 hours.

Procedure

The frequency of performing calibration verification and linearity of the AIA-PACK CEA assay is established by each institution in accordance with its quality assurance program and appropriate regulatory requirements. Refer to the ASSAY PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding auto dilution, refer to the AIA System Operator's Manual.

1. Make the desired dilutions of the Calibration Verification Material with Sample Diluting Solution and mix well. A sufficient amount should be made to assay each diluted sample in triplicate.
2. Program the instrument to run each of the prepared dilutions three times.
3. Add the appropriate amounts of each diluted sample to sample cups (refer to the instrument worklist for the amount required in each sample cup).
4. Verify the appropriate amount of test cups for the assay.
5. Load sample cups on the instrument as instructed in the AIA System Operator's Manual.
6. Start the assay.
7. Record the values obtained on the appropriate forms and calculate the desired statistics.

Assignment of Values

The Calibration Verification Material contains an assigned concentration of Carcinoembryonic Antigen. The assigned value is determined on a lot-to-lot basis and is designed to approximate the upper linear range of the assay. The Calibration Verification Material has been standardized against WHO 1st IRP 73/601 (1975).

Results

- Make a determination of the acceptability of the data according to the specific guidelines established by your institution which satisfy the regulatory requirements under which your institution operates. If results do not meet your specifications, please initiate corrective action, as appropriate or contact Tosoh Bioscience, Inc. for assistance.
- Retain test records in the laboratory in the designated location for future reference.

Limitations

The AIA-PACK CEA Calibration Verification/Linearity Test Set is designed solely for use with AIA-PACK assay procedures. The AIA-PACK CEA Calibration Verification/Linearity Test Set will only verify linearity between the established sensitivity of the assay and the value of analyte level listed on the Calibration Verification Material label.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.



TOSOH CORPORATION
Bioscience Division
2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan
Phone: +81 3 6636 3734
Fax: +81 3 6636 3627
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