AIA-360 Assay Specifications
ST CA 125 Test Code 026

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 U/mL
3	Cal 2	10 U/mL (example)
4	Cal 3	30 U/mL (example)
5	Cal 4	125 U/mL (example)
6	Cal 5	500 U/mL (example)
7	Cal 6	1000 U/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	U/mL
11	Smpl. Vol	100
12	Dil. Vol	50
13	Assay L	2.0
14	Assay H	1000
15	Ref. L	
16	Ref. H	
17	Decimal	1
	Visible only in Test Mode	
18	Test Name	#125
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	4
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	**3.850000**
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 CA 125 Test Code 026

No.	Item	Data
1	Code	26
2	ACT	1
3	Analyte	#CA125
4	Lot	***Enter current cal lot no.
5	CAL 1	0 U/mL
6	CAL 2	10 U/mL (example)
7	CAL 3	30 U/mL (example)
8	CAL 4	125 U/mL (example)
9	CAL 5	500 U/mL (example)
10	CAL 6	1000 U/mL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	U/mL
14	Decimal	1
15	Assay Low	2.0
16	Assay High	1000
17	Reference Low	0
18	Reference High	0
19	Reschedule Low	2.0
20	Reschedule High	1000
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	8
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	4
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	8
39	DIL. NAME	CA125
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)	0
46	Protocol	1
47	SYS. F_A	3.85
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST CA 125 Test Code 026

No.	Item	Data
1	Unit	U/mL
2	Decimal places	1
3	Reference low	
4	Reference high	
5	Reschedule low	2.0
6	Reschedule high	1000
7	Assay range low	2.0
8	Assay range high	1000
9	Specimen diluent code	008
10	Specimen diluent name	CA125
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	004
19	Calibrator replicates	3
20	Calibrator lot	2000800106040001
21	Calibrator Concentration	
22	Cal - 01	0 U/mL
23	Cal - 02	10 U/mL (example)
24	Cal - 03	30 U/mL (example)
25	Cal - 04	125 U/mL (example)
26	Cal - 05	500 U/mL (example)
27	Cal - 06	1000 U/mL (example)
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	**3.85**
49	Calibration Code Check	008
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

CA 125
ST AIA-PACK CA 125

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 CA 125 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 CA 125 is changed during the course of monitoring patients with serial CA 125 levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and Intended Use

ST AIA-PACK CA 125 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of CA 125 in human serum or heparinized plasma on specific Tosoh AIA System analyzers. ST AIA-PACK CA 125 is to be used as an aid in monitoring response to therapy for patients with epithelial ovarian cancer. Serial testing for patient CA 125 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

Summary and Explanation of Test

CA 125 determinant, identified in 1981 by Bast et al, is associated with a high molecular weight (220K dalton) glycoprotein complex. The precise immunological and physiological function of the glycoprotein complex expressing CA 125 is unknown[1]. However, the test has been used in the evaluation of women for ovarian cancer since it was first discovered[2,3]. Elevated serum levels of CA 125 are associated with greater than 80 percent of nonmucinous epithelial ovarian neoplasms. Other gynecological neoplasms, primarily endometrial and fallopian tube carcinomas and certain tumors of the pancreas, liver, colon, breast, and lung are also associated with elevated serum levels of CA 125[4]. CA 125 has been most useful in the detection of recurrent disease confirmed by laparoscopy after initial surgical debulking and adjuvant chemotherapy[5]. It has also been useful in the prediction of survival[6]. In 1992, a new generation of CA 125 antibody tests was introduced. This has spawned renewed interest in this assay for use in the evaluation of women with ovarian cancer[7]. Studies also suggest that long term monitoring of CA 125 in patients with known ovarian carcinomas has utility in detecting early recurrence, response to chemotherapy and disease progression[8].

Principle of the Assay

The ST AIA-PACK CA 125 is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. CA 125 present in the test sample is bound with monoclonal antibody (Biomira, Inc. B27.1) immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody (Biomira, Inc. B43.13) 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 CA 125 concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK CA 125, Cat. No. 0025288)

5 trays x 20 test cups (ST AIA-PACK CA 125 Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti-CA 125 mouse monoclonal antibody and mouse monoclonal antibody (to human CA 125) conjugated to bovine alkaline phosphatase with 0.1% sodium azide (lyophilized) as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform CA 125 analysis using the ST AIA-PACK CA 125 (Cat. No. 0025288) 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 CA 125 Calibrator Set		0020388
Calibrator #1	0 U/mL	
Calibrator #2	10 U/mL (approx.)	
Calibrator #3	30 U/mL (approx.)	
Calibrator #4	125 U/mL (approx.)	
Calibrator #5	500 U/mL (approx.)	
Calibrator #6	1000 U/mL (approx.)	
AIA-PACK CA 125 Sample Diluting Solution		0020588
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)		996010
Preloaded Pipette Tips (96 Tips X 5 Racks)		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 CA 125 is intended for in vitro diagnostic use only.
- ℞ 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 CA 125 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 CA 125 has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with CA 125 concentrations between 1,000 and 20,000 U/mL will read > 1,000 U/mL. The "hook effect" phenomenon may occur only at CA 125 concentrations > 20,000 U/mL.
- 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.

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 CA 125	0025288
AIA-PACK CA 125 Calibrator Set	0020388
AIA-PACK CA 125 Sample Diluting Solution	0020588
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 CA 125 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, the Sample Diluting Solution is stable for up to 90 days when 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 uL.

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

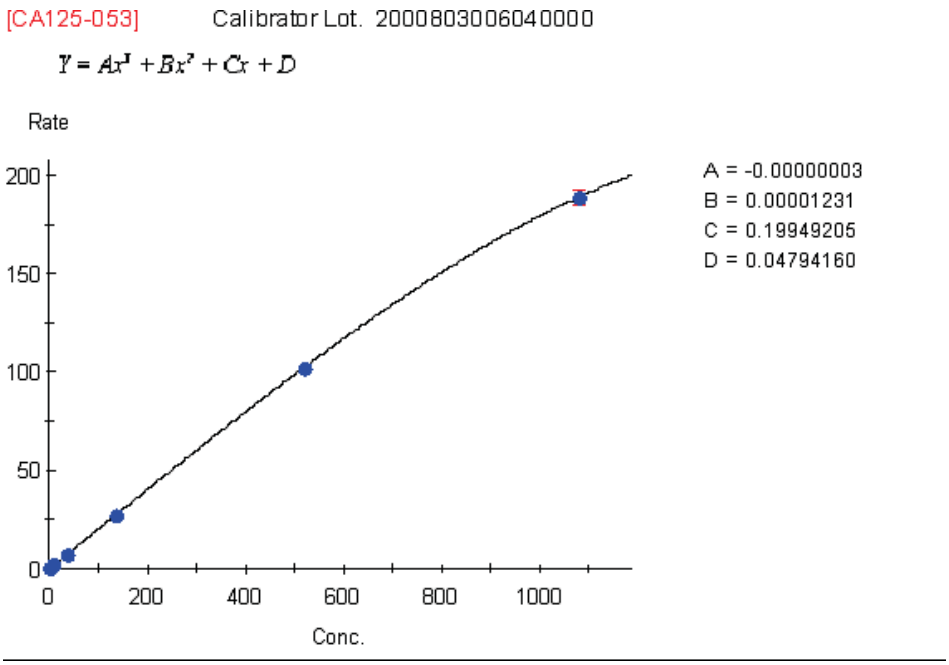
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK CA 125 are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the ST AIA-PACK CA 125 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 both the calibrator lot and concentration numbers have been correctly entered into the software.

ii) The ST AIA-PACK CA 125 CALIBRATOR (1) is provided ready for use.

iii) The ST AIA-PACK CA 125 CALIBRATOR levels (2) - (6) are lyophilized. Lyophilized calibrators should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.

iv) 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) Review the calibration curve carefully, using the criteria listed above.

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 CA 125 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 specimen CA 125 concentration is found to be greater than the linearity limit of the assay, 1000 U/mL, the specimen should be diluted with the AIA-PACK CA 125 Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for samples containing greater than 1000 U/mL is 1:10 or 1:100. It is desirable to dilute the sample so that the diluted sample reads between 10 and 1000 U/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 CA 125 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 026.

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 CA 125 concentration in U/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 CA 125, the highest concentration of CA 125 measurable without dilution is 1000 U/mL, and the lowest measurable concentration is 2.0 U/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 1000 U/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, 1000 U/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 CA 125.

A CA 125 result below 35 U/mL does not indicate the absence of residual ovarian cancer because patients with histopathologic evidence of ovarian carcinoma may have CA 125 assay values within the range of normal individuals. Conversely, a CA 125 assay value exceeding 35 U/mL does not necessarily indicate the presence of ovarian malignancy since 1-2% of healthy individuals and individuals with non-malignant conditions may have elevations in CA 125 assay results. A CA 125 assay value should not be interpreted as absolute evidence for the presence or absence of malignant disease. THE ST AIA-PACK CA 125 ASSAY SHOULD NOT BE USED AS A SCREENING TEST.

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.[9]

The following clinical data was collected using the AIA-PACK CA 125. The ST AIA-PACK CA 125 demonstrates equivalent performance. (See the Comparative Analysis.)

One hundred and fifty samples from women with a history of ovarian cancer were tested with the AIA-PACK CA 125 assay and another FDA cleared CA 125 method. A model I regression analysis (ordinary least squares) was performed on the values of CA 125 in this cohort. The regression was performed on three ranges of results for the predicate device as shown in the table below:

Parameter Estimates for Ordinary Least Squares Regression by Range

Range	N	a	95% CI	b	95% CI	R^2
0-100 U/mL	124	-4.14	-6.45,-1.83	1.16	1.09, 1.23	0.89
0-1000 U/mL	144	-4.05	-10.06,1.96	1.34	1.30, 1.38	0.97
Full Range	150	-36.5	-92, 19.6	1.55	1.50, 1.60	0.96

Passing-Bablok regression was also performed on these data. The table below presents the parameter estimates for the ranges defined above.

Parameter Estimates for Passing-Bablok Regression by Range

Range	N	a	95% CI	b	95% CI
0-100 U/mL	124	-4.55	-5.3, -3.5	1.22	1.15, 1.29
0-1000 U/mL	144	-5.28	-6.12, -4.51	1.32	1.27, 1.35
Full Range	150	-5.49	-6.27, -4.61	1.33	1.28, 1.37

Values of the AIA-PACK CA 125 and another CA 125 assay previously cleared by the FDA were bifurcated at the stated cut-off value of 35 U/mL which corresponds to the ninety-ninth percentile for the normal healthy samples. The distribution of bifurcated results from the two assays is shown below. Out of 150 samples tested only four discrepant results were found. Three of these were cases in which the AIA-PACK CA 125 result was just over the cutoff while the other method was just below which resulted in clinically insignificant differences.

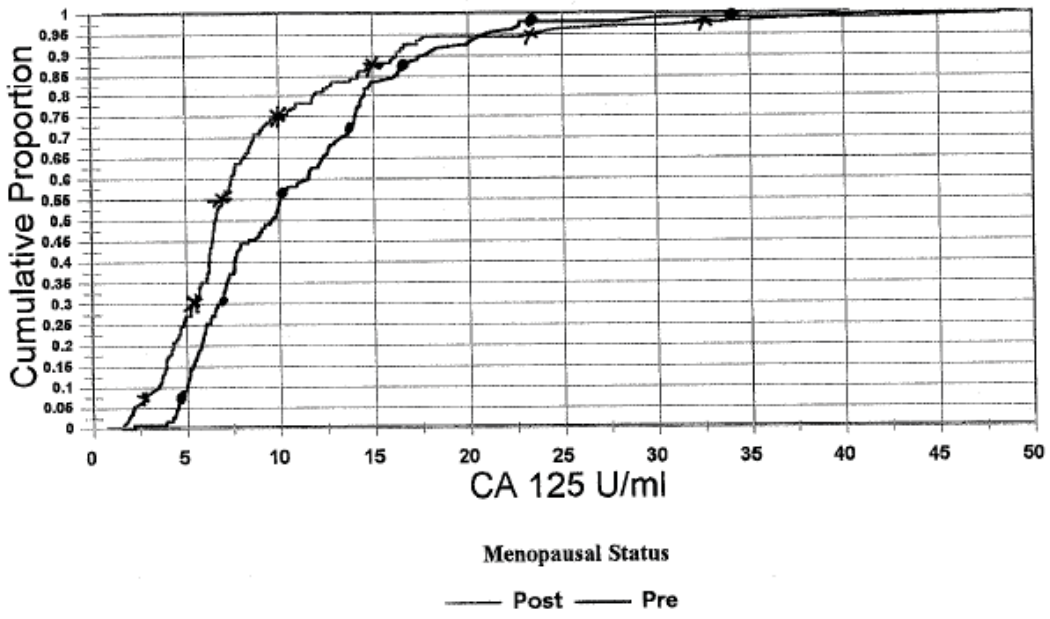
Bifurcated Values of the Predicate and Test Devices

	Predicate Device		
Test Device	**≥ 35 U/mL**	**< 35 U/mL**	**Total**
≥ 35 U/mL	50	3	53
< 35 U/mL	1	96	97
Total	51	99	150

Reference Ranges

To determine a cutoff value for AIA-PACK CA 125, a sample of 220 apparently disease free women (110 pre-menopausal, 110 post-menopausal) was used. The average age of the pre-menopausal cohort was 36.5 years. In the post-menopausal group, the average age was 66.2 years. Values for this assay were determined in duplicate and a cumulative distribution was established the two groups of women. The chart below shows this cumulative distribution for the AIA-PACK CA 125 assay performed on the pre- and post-menopausal women.

Chart 4.1
Cumulative Distribution of CA 125 as Measured by the Test Assay (Tosoh AIA-PACK™) by Normal Cohort Menopausal Status (Pre-, Post-)



The ninety-ninth order statistic in each cohort was determined to be:

Pre-menopausal: 29.5 U/mL (95% CI: 21, 41)

Post-menopausal 37.5 U/mL (95% CI: 23, 48)

Since there is significant overlap in the confidence intervals in these two groups, there is confidence that the 99% order statistics in each cohort are the same value. Therefore, the universal cutoff value for AIA-PACK CA 125 has been established at 35 U/mL regardless of

menopausal status. This value is well within the 95% confidence interval bounds for both menopausal states studied.

Clinical Performance Studies

A retrospective study was performed in which CA 125 concentrations were measured by the AIA-PACK CA 125 assay and another FDA cleared CA 125 method in serial serum specimens collected after primary therapy from 54 women diagnosed with epithelial ovarian cancer. A change of 25% was considered a significant increase or decrease in the test result. Changes smaller than this were considered to represent a stable status. There were no clinically significant differences between the two assays in either stable, responding or progressive disease. The trend in CA 125 values for the three disease groups (responding, progressive and stable/NED) are summarized in the Clinical Outcomes chart below. One representative graphical series from the responding group and one from the progressive group are also displayed.

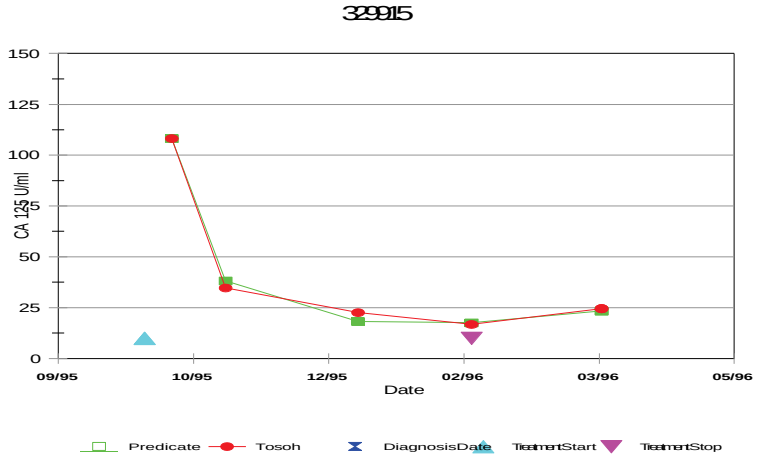
Clinical Outcome

Change in CA 125*	Progression(n=16)	Stable/NED(n=19)	Responding(n=19)
Increase ≥ 25%	13	1	3
Decrease ≥ 25%	2	1	7
Stable	1	17	9 *.*.

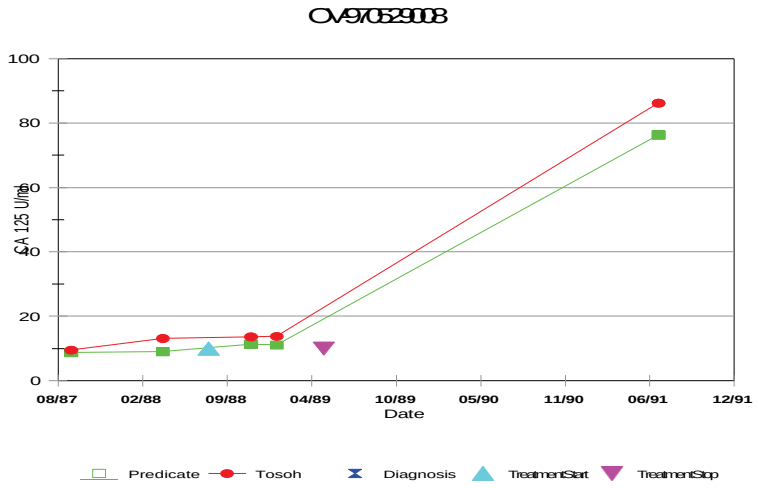
* Minimum change was defined as ± 5 U/mL.

** Of the 9 patients responding to therapy, 7 had stable values for the last three points. Dramatic decreases were observed only after the first point in a series.

Responding Disease Pattern



Progressive Disease Pattern



Mean Values for AIA-PACK CA 125 in Various Patient Populations

Clinical Status	N	Mean Value U/mL
Normal Healthy Female, Pre-menopausal	110	10.74
Normal Healthy Female, Post-menopausal	110	8.81
Benign Diseases – Urogenital	7	8.41
Hypertension (Females)	57	10.47
Chronic Heart Disease (Females)	56	9.53
Breast Cancer (Treated)	50	9.58
Cervical Cancer (Treated)	31	72.35
Colon Cancer (Treated)	25	18.70
Endometrial Cancer (Treated)	8	9.57
Lung Cancer (Treated)	32	158.97
Other GU Cancer (Treated)	5	495.50

Performance Characteristics

The following performance characteristics were established using the Tosoh AIA NexIA Automated Immunoassay Analyzer.

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of CA 125 and assayed before and after spiking.

Sample	Initial Value (U/mL)	CA 125 Added (U/mL)	Expected Value (U/mL)	Measured Value (U/mL)	Percent Recovery (%)
Serum A1	5.6 5.6 5.6	63.5 126.9 253.8	69.0 132.5 259.4	76.0 147.4 282.7	110.1 111.2 109.0
Serum B1	5.8 5.8 5.8	67.3 134.5 269.1	73.0 140.3 274.8	84.6 162.1 291.4	115.9 115.5 106.0
Serum C1	7.3 7.3 7.3	67.3 134.5 269.1	74.6 141.8 276.4	79.6 140.2 271.3	106.8 98.9 98.2

1b) Dilution:

Three serum samples containing high concentrations of CA 125 were serially diluted with AIA-PACK CA 125 Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (U/mL)	Measured Value (U/mL)	Percent Recovery (%)
Serum A2	None		896.8	100.0
	7.5/10	672.6	606.3	90.1
	5.0/10	448.4	403.6	90.0
	2.5/10	224.2	190.5	85.0
	1.0/10	89.7	79.7	88.9
Serum B2	None		912.9	100.0
	7.5/10	684.7	617.2	90.1
	5.0/10	456.5	430.2	94.3
	2.5/10	228.2	218.9	95.9
	1.0/10	91.3	87.6	95.9
Serum C2	None		876.3	100.0
	7.5/10	657.2	620.3	94.4
	5.0/10	438.1	397.1	90.6
	2.5/10	219.1	213.0	97.2
	1.0/10	87.6	86.1	98.3

1c) Comparative Analysis:

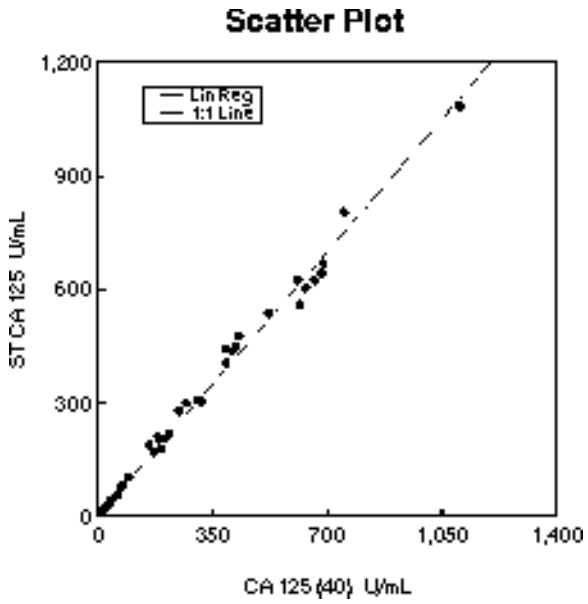
Samples were collected at random. Results from the ST AIA-PACK CA 125 (y) were compared to those obtained using the AIA-PACK CA 125 (x). A total of 48 serum samples were test in the study.

Slope	0.994 (95% CI: 0.970, 1.018
y-intercept	5.4492 95% CI: -3.1132, 14.0116)
Correlation Coefficient	0.9967
Range of Samples (x)	10.098 – 1086.3
Number of Samples (x)	48

Figure 1

Comparative Analysis

ST AIA-PACK CA 125 v. AIA-PACK CA 125 (40 min)



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 (CV).

Sample	Mean (U/mL)	Pooled SD (U/mL)	Coefficient of Variation (%)
Serum A3	21.5	0.701	3.3
Serum B3	102.5	2.64	2.6
Serum C3	657.6	22.8	3.5

2b) Inter-assay precision

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

Sample	Number of Replicates	Mean (U/mL)	Standard Deviation (U/mL)	Coefficient of Variation (%)
Serum A4	20	59.9	2.2	3.7
Serum B4	20	756	24.3	3.2

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 (CV).

Sample	Mean (U/mL)	Pooled SD (U/mL)	Coefficient of Variation (%)
Serum A3	21.5	1.00	4.7
Serum B3	102.5	5.33	5.2
Serum C3	657.6	32.0	4.9

Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percent of the compound which will be identified as CA 125. If these compounds are present in the specimen at the same concentration as CA 125, the final result will be increased by these percentages.

Compound	Cross-reactivity (%)
CA 125	100.0
CA 19-9	Not detected
CEA	Not detected
AFP	Not detected

Sensitivity

The minimal detectable concentration (MDC) of CA 125 is estimated to 2.0 U/mL. The MDC is defined as that concentration of CA 125 which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 10 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 19 mg/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 human albumin concentrations (up to 5 g/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Therapeutic concentrations of the common drugs and/or chemo-therapeutic agents listed below do not interfere with ST AIA-PACK CA 125 Assay results. Observed mean values were within 10% of the expected mean when these medications were added to samples of known concentration of CA 125.

1. 5'- fluorouracil/Adrucil
2. Acetaminophen/Tylenol
3. Acetylsalicylic acid/aspirin
4. Adriamycin/Doxorubicin
5. Amethopterin, Methotrexate
6. Amikacin/Amikin
7. Amitriptyline
8. Cisplatin
9. Cortisone/Hydrocortisone
10. Cyclosporin A
11. Digoxin
12. Gentamycin
13. Kanamycin
14. Lidocaine/Xylocaine
15. Lithium carbonate
16. Mitomycin C
17. Phenytoin/Dilantin
18. Propranolol/Inderal
19. Quinidine gluconate/Caffeine
20. Salicylate/salicylic a.
21. Streptomycin
22. Theophylline
23. Tobramycin
24. Valproic acid

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2. Bast, R.C., Klug, T, John, E., et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. New England Journal of Medicine. 309; 883-7 (1983).
3. Sevelda, P., Salzer, H, Dittrich, C.H., Pateisky, N., Spona, J., The diagnostic utility of the tumor marker CA 125 in ovarian cancer patients. Tumor Dign Ther.. 8; 115-20 (1987).
4. Kabawat, S.E., Bast, R.C. et al. Tissue Distribution of a Colemic-Epithilium-Related Antigen Recognized by the Monoclonal Antibody OC125. Int. J. Gyn. Path. 2; 275-285 (1983).
5. Niloff, J.M., Bast, R.C., Schaetzi, E.M., Knapp, R.C. Predictive value of CA125 antigen levels in second look procedures for ovarian cancer. American Journal of Obstetrics and Gynecology. 151; 981-6 (1985).
6. Rossman, M., Thiel, R.P., et al. Prognostic factors for poor risk epithelial ovarian cancer. Cancer. 74; 1322-28 (1994).
7. Nagle, F., Petru, E., Medi, M., Kainz, C., Graf, A.H., Sevelda, P., Preoperative CA 125 as an independent prognostic factor in patients with stage 1 epithelial ovarian cancer. Obstetrics and Gynecology. 86; 259-64 (1995).
8. Hogberg, T. and Kagedal, B. Long Term Follow-up of Ovarian Cancer with Monthly Determinations of Serum CA 125. Gynecol. Oncol. 46; 191-198 (1992).
9. Young, D., 1990, Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, Washington, DC, American Association for Clinical Chemistry Press.

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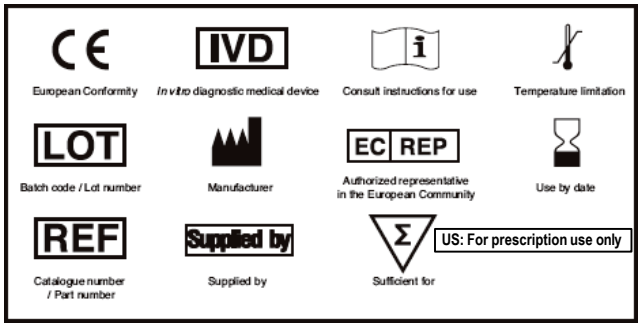


AIA-PACK
CA 125 Calibrator Set

Intended Use

The AIA-PACK CA125 Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK CA 125 Assays.

Summary and Explanation

The AIA-PACK CA 125 Calibrator Set contains bovine serum albumin with assigned levels of CA 125. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Material Provided (Cat. No. 0020388)

2 x 1 mL	Zero Calibrator	
	Bovine serum albumin containing no detectable concentration of CA 125 (0 U CA 125/mL), and 0.1% sodium azide as preservative. (Ready for Use)	
2 x 1 mL	Calibrator No. 2	10 U/mL (approx.)
	Calibrator No. 3	30 U/mL (approx.)
	Calibrator No. 4	125 U/mL (approx.)
	Calibrator No. 5	500 U/mL (approx.)
	Calibrator No. 6	1000 U/mL (approx.)
	Bovine serum albumin containing the assigned concentration of CA 125 (described on each vial), and 0.1% sodium azide as a preservative. (Lyophilized)	

Warnings and Precautions

- The AIA-PACK CA 125 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.
- No human sera is used in the preparation of this product, but 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

Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized calibrators accurately with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.. Allow the lyophilized material to fully dissolve, then mix the calibrators gently but thoroughly prior to performing the calibration.

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.

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK CA 125 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 CA 125 Calibrator Set contains assigned concentrations of CA 125. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0.0 to 1000.0 U/mL of CA 125. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

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.

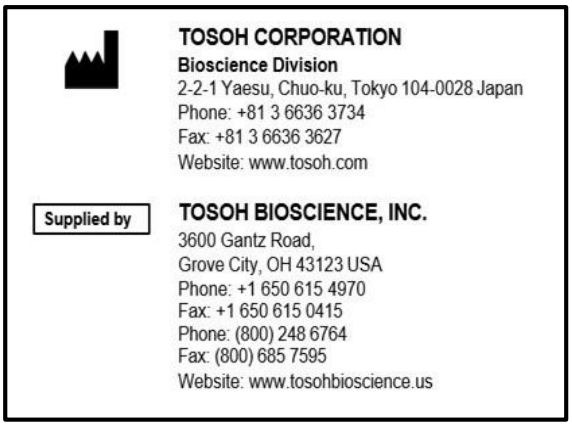
The replicate values should be within a 10% range.

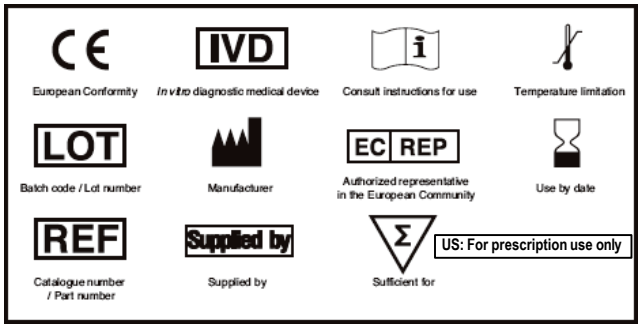
Limitations

The AIA-PACK CA 125 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.





AIA-PACK
CA 125 Sample Diluting Solution

Intended Use

The AIA-PACK CA 125 Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of CA 125 above the linear range of the assay.

Summary and Explanation

The AIA-PACK CA 125 Sample Diluting Solution contains bovine serum albumin with no detectable concentration of CA 125. This Sample Diluting Solution is to be used only with samples that are being tested for CA 125 concentrations using the ST AIA-PACK CA 125 Assays.

Material Provided (Cat. No. 0020588)

4 x 4 mL	Sample Diluting Solution Bovine serum albumin containing no detectable concentration of CA 125 (0 U CA 125/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK CA 125 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, flush with large volumes of water to prevent azide build-up.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK CA 125 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 CA 125 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

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen is found to contain greater than the linearity limit of 1000 U/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.
2. 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.
3. The recommended dilution for serum containing greater than 1000 U/mL is 1:10 or 1:100. However, it is desirable to dilute the serum samples that contain more than 1000 ng CA 125/mL so that the diluted sample reads between 10 and 1000 U CA 125/mL.

Results

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

Limitations

The AIA-PACK CA 125 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.

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