AIA-360 Assay Specifications
ST 27.29 Test Code 091

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 U/mL
3	Cal 2	0.2 U/mL (example)
4	Cal 3	2.5 U/mL (example)
5	Cal 4	5.0 U/mL (example)
6	Cal 5	10.0 U/mL (example)
7	Cal 6	20.0 U/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	U/mL
11	Smpl. Vol	20
12	Dil. Vol	100
13	Assay L	0.10
14	Assay H	19.05
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#2729
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	4
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	21.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-900 Assay Specifications
ST 27.29 Test Code 091

No.	Item	Data
1	Code	91
2	ACT	1
3	Analyte	#27.29
4	Lot	***Enter current cal. lot no.
5	CAL 1	0 U/mL
6	CAL 2	0.2 U/mL (example)
7	CAL 3	2.5 U/mL (example)
8	CAL 4	5.0 U/mL (example)
9	CAL 5	10.0 U/mL (example)
10	CAL 6	20.0 U/mL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	U/mL
14	Decimal	2
15	Assay Low	0.1
16	Assay High	19.05
17	Reference Low	0
18	Reference High	0
19	Reschedule Low	2.1
20	Reschedule High	400
21	Factor A	1
22	Factor B	0
23	Sample Volume	20
24	Diluent Volume	100
25	2 Step reagent dispensing volume	0
26	Calibration code	90
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	4
30	CAL. CV	90
31	DIL. SP1	21
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	21
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	90
39	DIL. NAME	27.29
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	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST 27.29 Test Code 091

No.	Item	Data
1	Unit	U/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	2.1
6	Reschedule high	400
7	Assay range low	0.10
8	Assay range high	19.05
9	Specimen diluent code	90
10	Specimen diluent name	27.29
11	Dilution factor for Sp. 1	21
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	21
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	4
19	Calibrator replicates	3
20	Calibrator lot	***Enter cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 U/mL
23	Cal - 02	0.2 U/mL (example)
24	Cal - 03	2.5 U/mL (example)
25	Cal - 04	5.0 U/mL (example)
26	Cal - 05	10.0 U/mL (example)
27	Cal - 06	20.0 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	20
39	Diluent volume	100
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	090
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

27.29
ST AIA-PACK 27.29

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 27.29 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 27.29 is changed during the course of monitoring patients with serial CA 27.29 levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and Intended Use

ST AIA-PACK 27.29 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of CA 27.29 in human serum or plasma on Tosoh AIA systems. ST AIA-PACK 27.29 is to be used as an aid in monitoring response to therapy for patients with Stage IV (metastatic) breast cancer as well as determining early recurrence in Stage II and Stage III breast cancer patients who were previously treated and free of disease.

Serial testing for patient CA 27.29 assay values should be used in conjunction with other clinical methods used for monitoring response to therapy in patients with Stage IV metastatic breast cancer and for detecting early recurrence in Stage II and Stage III disease.

Summary and Explanation of Test

Breast carcinoma-associated antigen coded by the human MUC-1 gene is identified by several names including MAM 6, milk mucin, polymorphic epithelial mucin, PEM, CA 27.29 and CA 15-3. As indicated by epitope mapping, inhibition of antibody binding, tracer exchange and clinical correlation studies, CA 27.29 and CA 15-3 epitopes overlap. This antigen is a glycoprotein (molecular weight 300 – 450 kDa) which contains a 20 amino acid tandem repetitive sequence of the mucin core. Carbohydrates comprise more than half of the molecule.[1] The number of tandem repeats and the degree of glycosylation is variable between individuals, so that CA 27.29 is very heterogeneous in structure. In malignant cells, CA 27.29 is over expressed on the entire cell surface, and increasing amounts are shed into the circulation.

Tumors involving glandular organs, such as the breast, can produce high concentration of CA 27.29 in serum, making it useful as a tumor marker.

Monoclonal antibodies that recognized distinct epitopes on CA 27.29 and CA 15-3 antigen molecules have been used to develop immunoassays.[2-4] ST AIA-PACK 27.29 was developed with a monoclonal antibody that recognizes an 8 amino acid section in the tandem repeat portion of the polypeptide chain.[5]

Since the antigen levels correlated closely with disease regression or progression, CA 27.29 proved reliable in the follow-up of patients with advanced breast cancer.[6,7]

Principle of the Assay

The ST AIA-PACK 27.29 is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. CA 27.29 present in the test sample is bound with monoclonal antibody (Biomira, Inc.) immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody (Biomira, Inc.) 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 27.29 concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK 27.29, Cat. No. 0025202)

5 trays x 20 test cups (ST AIA-PACK 27.29 Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with mouse anti-CA 27.29 monoclonal antibody (B27.29) and mouse monoclonal antibody (to human CA 27.29) 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 27.29 analysis using the ST AIA-PACK 27.29 (Cat. No.0025202). They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-360	0019945
AIA-900	0022930
AIA-900 9 Tray Sorter	0022931
AIA-900 19 Tray 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 27.29 CALIBRATOR SET	0020302
CALIBRATOR #1 0 U/mL	
CALIBRATOR #2 0.2 U/mL (approx.)	
CALIBRATOR #3 2.5 U/mL (approx.)	
CALIBRATOR #4 5.0 U/mL (approx.)	
CALIBRATOR #5 10.0 U/mL (approx.)	
CALIBRATOR #6 20.0 U/mL (approx.)	
AIA-PACK 27.29 SAMPLE DILUTING SOLUTION	0020502
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 27.29 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 27.29 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 27.29 has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. Samples with CA 27.29 concentrations between 0.1 U/mL and 953 U/mL on the 1:21 dilution will read > 400 U/mL. This translates to an upper limit on the original sample of 20,013 U/mL. The “hook effect” is tested in routine QC release procedures for each lot of ST AIA-PACK 27.29 using a sample with CA 27.29 concentration exceeding 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.
- 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 27.29	0025202
AIA-PACK 27.29 CALIBRATOR SET	0020302
AIA-PACK 27.29 SAMPLE DILUTING SOLUTION	0020502
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 27.29 test cups may be stored for up to 24 hours at a room temperature of 18 - 25 °C. Calibrators are stable for up to 7 days after opening, if kept tightly sealed and refrigerated at 2 - 8 °C.

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK 27.29 SAMPLE DILUTING SOLUTION is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for 7 days when used for automatic dilution provided it is not on the instrument (18 - 25 °C) for more than 7 hours per day and the bottles are tightly sealed and refrigerated at 2 - 8 °C immediately after use.

- The sample diluting solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the bottles are kept tightly sealed and refrigerated immediately after use.
- For auto-dilution on the AIA-900 the 27.29 SDS can be transferred to a 5 mL bottle from the 100 mL bottle. It is stable for 7 days after the transfer as long as it is tightly sealed and stored at 2 - 8 °C after use.

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.

ST AIA-PACK 27.29 assay requires a 1:21 dilution of the serum with 27.29 Sample Diluting Solution prior to analysis. AIA-900 and AIA-2000 software can be configured to perform the dilutions automatically. Diluted serum or plasma should be used within 24 hours and should not be stored.

The sample required for analysis is 20 µL of diluted serum or heparinized plasma (1:21).

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

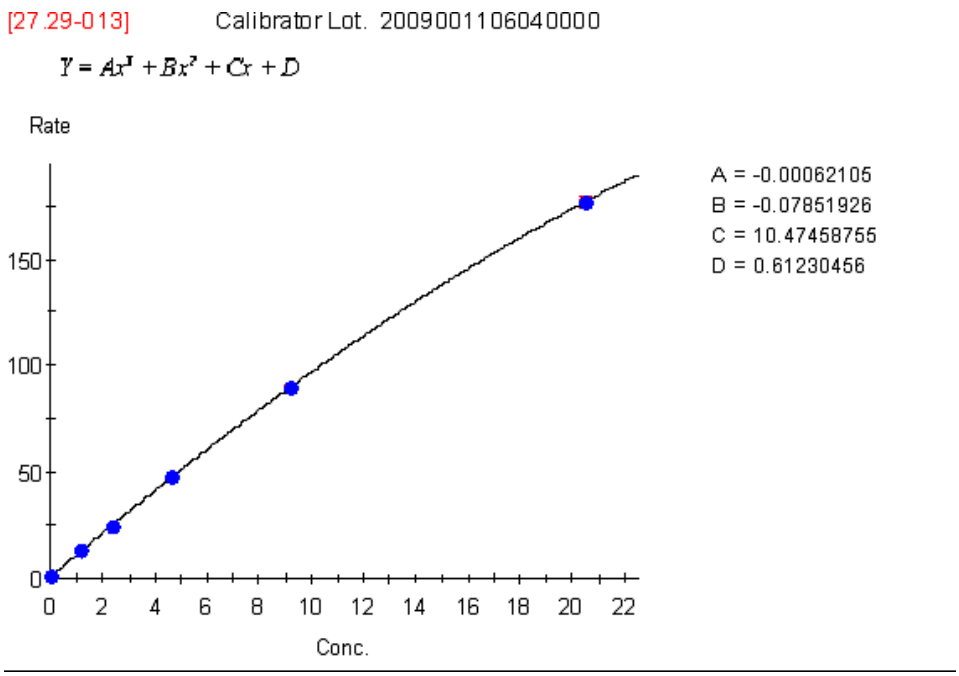
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK 27.29 are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the ST AIA-PACK 27.29 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 27.29 come pre-diluted (ready for use). No further dilution is necessary for any of the AIA systems.

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. (All quality control material requires a 1:21 dilution.) 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 27.29 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 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 CA 27.29 concentration is found to be greater than the linearity limit of the assay, 19.05 U/mL (400 U/mL after x21 dilution factor) the specimen should be diluted with the AIA-PACK 27.29 SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for samples containing greater than 19.05 U/mL (400 U/mL after x21 dilution factor) is 1:10 or 1:100. It is desirable to further dilute the serum or plasma sample so that the diluted sample reads between 2 and 400 U/mL after multiplying with x21 dilution factor. This second dilution factor should be entered into the software for AIA-900 and AIA-2000. For further information on the dilution of specimens, refer to the AIA System Operator's Manual.
4) All AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK 27.29 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 091.

Calculation of Results

AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to CA 27.29 concentration in U/mL.

For samples above linearity, the AIA-900 and AIA-2000 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. Dilution factors may be entered into the Test File, or pre-defined dilution factors may be selected in Specimen Processing.

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 controls 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

ST AIA-PACK 27.29 SHOULD NOT BE USED AS A SCREENING TEST.

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 27.29, the highest concentration of CA 27.29 measurable without secondary dilution is 19.05 U/mL (400 U/mL after x21 dilution factor), and the lowest measurable concentration is 0.1 U/mL (2.0 U/mL after x21 dilution factor) (assay sensitivity). All serum or plasma samples are diluted 1:21 with Sample Diluting Solution prior to initial assay either automatically on the AIA systems or manually if so desired.

Although the approximate value of the highest calibrator is approximately 20 U/mL (420 U/mL after x21 dilution factor), the exact concentration may be slightly different depending upon the lot. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 19.05 U/mL (400 U/mL after x21dilution factor).

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 or occasionally falsely decreased values when tested for CA 27.29 using ST AIA-PACK 27.29.

A CA 27.29 result below the upper reference limit of normal does not indicate the absence of breast cancer because patients with histopathologic evidence of breast carcinoma may have CA 27.29 assay values within the range of normal individuals. Conversely, a CA 27.29 assay value exceeding the upper limit reference limit of normal does not necessarily indicate the presence of breast malignancy since 1-2% of healthy individuals and individuals with non-malignant conditions may have elevations in CA 27.29 assay results. A CA 27.29 assay value should not be interpreted as absolute evidence for the presence or absence of malignant disease.

The performance of this test has not been established for male breast cancer patients.

The performance of this test with patients with central nervous system metastases has not been established.

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.

Expected Values

Each laboratory should determine a reference interval corresponding 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.[8]

Clinical Performance Studies

Reference Limit for Apparently Healthy Individuals

Serum samples from 117 women (51 pre-menopausal, 66 post-menopausal) and 96 men, all apparently disease free, were analyzed. Values for AIA-PACK 27.29 and a commercially available RIA assay for CA 27.29 were determined in duplicate, and a cumulative distribution was established to determine a value for the 99% cutoff. The table below demonstrates the ninety-ninth order statistic with ninety-five percent confidence intervals in each cohort. Confidence intervals were obtained by resampling each cumulative distribution 10,000 times. The 2.5th and 97.5th percentile in each sampling distribution were used as the lower and upper bound for each confidence interval.

Examination of the confidence intervals indicates that the stated normal cutoff for the reference method of 37.7 U/mL is contained within each interval obtained with AIA-PACK 27.29. This indicates that in healthy groups, the distribution of normals with the AIA-PACK 27.29 and the reference method are equivalent at the 99th order statistic and an upper cutoff of 37.7 U/mL is justified for AIA-PACK 27.29.

99th Order Statistic – Apparently Healthy Individuals

	AIA-PACK 27.29			Comparative Device CA 27.29 RIA		
	U/mL	95% Confidence Interval		U/mL	95% Confidence Interval	
Patient Group	**99th Order Statistic**	**Lower Bound**	**Upper Bound**	**99th Order Statistic**	**Lower Bound**	**Upper Bound**
Female Pre-Menopausal	39.8	28.6	44.3	34.9	26.2	38.0
Female Post-menopausal	41.9	38.9	43.4	38.5	32.2	41.5
All Female	41.9	34.8	44.3	37.0	32.1	41.5
Male	63.8	36.0	84.8	52.5	34.5	60.6

Prospective Clinical Studies

Five hundred and forty-nine serum samples each from a different patient from six clinical sites in the United States were collected in a prospective study to determine the efficacy of the Tosoh AIA-PACK 27.29 for the evaluation of patients with breast cancer. These cross-sectional samples were collected under IRB approval with signed informed consent documents obtained from each patient enrolled in the study. Each sample was tested by AIA-PACK 27.29 as well as by a comparative device previously cleared by the FDA which measured CA 27.29 by RIA. The table below shows the mean, median, standard error of the mean, and the 95% confidence limits for the three parameters: age, RIA device results (U/mL), and AIA-PACK 27.29 results (U/mL) by cohort.

AIA-PACK 27.29 Statistics by Clinical Cohort

Patient Cohort	Number of Patients	Variable	Mean U/mL	Median U/mL	Standard Error of Mean	95% Confidence Interval (Mean)	
Healthy F Pre-Menopausal	51	Age in years	38.4	39.7	1.3	35.9	41.0
		AIA-PACK 27.29	18.3	18.4	1.0	16.3	20.3
		RIA CA 27.29	18.0	18.3	0.9	16.2	19.8
Healthy F Post-Menopausal	66	Age in years	63.7	62.8	1.2	61.4	66.0
		AIA-PACK 27.29	21.1	19.7	1.1	18.9	23.2
		RIA CA 27.29	20.8	20.1	0.7	19.4	22.3
Healthy Male	96	Age in years	56.7	61.0	1.5	53.8	59.7
		AIA-PACK 27.29	22.2	21.3	1.2	19.9	24.5
		RIA CA 27.29	22.2	21.5	0.9	20.5	23.9
Benign Diseases	76	Age in years	64.0	65.8	1.4	61.3	66.8
		AIA-PACK 27.29	21.8	22.6	1.0	19.9	23.7
		RIA CA 27.29	21.4	20.0	0.8	19.8	23.0
Other Cancers	22	Age in years	67.5	67.6	1.9	63.8	71.2
		AIA-PACK 27.29	24.0	24.4	1.9	20.2	27.7
		RIA CA 27.29	26.4	25.8	1.8	22.8	30.0
Benign Breast Disease	101	Age in years	51.5	50.9	1.2	49.1	54.0
		AIA-PACK 27.29	20.1	19.7	0.7	18.7	21.5
		RIA CA 27.29	19.3	19.1	0.6	18.1	20.4
Treated Breast Cancer	137	Age in years	59.5	58.6	1.1	57.4	61.6
		AIA-PACK 27.29	23.4	20.8	1.2	21.1	25.7
		RIA CA 27.29	22.9	20.2	1.0	21.0	24.8

Results from the AIA-PACK 27.29 and RIA CA 27.29 assays on the 549 serum samples described above were bifurcated at the stated cut-off value of 37.7 U/mL corresponding to the ninety-ninth percentile for apparently healthy samples. The distribution of bifurcated results from the two assays is shown below. Out of 549 samples tested only 16 discrepant results were found and these discrepancies resulted in clinically insignificant differences relative to the cutoff. When the assay imprecision of the predicate device was taken into consideration, results agreed in all but one case in the benign breast disease category and one in the treated breast cancer group.

Concordance by Clinical Cohort

Clinical Cohort			AIA-PACK 27.29		Total	McNemar Exact 2-Sided p
			≤37.7 U/mL	>37.7 U/mL		
Healthy F Pre-Menopausal	RIA CA 27.29	≤37.7 U/mL	50	0	50	1.000
		>37.7 U/mL	0	1	1	
		Total	50	1	51	
Healthy F Post-Menopausal	RIA CA 27.29	≤37.7 U/mL	62	3	65	0.250
		>37.7 U/mL	0	1	1	
		Total	62	4	66	
Healthy Male	RIA CA 27.29	≤37.7 U/mL	91	2	93	0.500
		>37.7 U/mL	0	3	3	
		Total	91	5	96	
Benign Diseases	RIA CA 27.29	≤37.7 U/mL	73	1	74	1.000
		>37.7 U/mL	0	2	2	
		Total	73	3	76	
Other Cancers	RIA CA 27.29	≤37.7 U/mL	19	0	19	1.000
		>37.7 U/mL	1	2	3	
		Total	20	2	22	
Benign Breast Disease	RIA CA 27.29	≤37.7 U/mL	99	1	100	1.000
		>37.7 U/mL	0	1	1	
		Total	99	2	101	
Treated Breast Cancer	RIA CA 27.29	≤37.7 U/mL	122	6	128	0.289
		>37.7 U/mL	2	7	9	
		Total	124	13	137	

Clinical Studies from Retrospective Serial Samples

A retrospective study was performed in which CA 27.29 concentrations were measured by both the AIA-PACK 27.29 assay and an FDA cleared CA 27.29 RIA method in serial serum specimens collected after primary therapy from 60 patients diagnosed with breast cancer. The cohort consisted of 66.7% Caucasian, 15% African American, with the remaining 18.4% of other ethnic origin. One-third of the patient population was pre/peri-menopausal. One-third of the group was post-menopausal. The remaining women had undergone hysterectomy. The following table shows the stage of cancer at diagnosis for this group of patients:

Stage of Disease at Diagnosis

Stage at Diagnosis	Frequency	Percent
IIA	23	38.33
IIB	7	11.67
IIIA	5	8.33
IIIB	15	25.00
IV	10	16.67
Total	60	100.00

There were 255 samples from these sixty patients. Forty-four patients contributed four samples each. There were twelve patients with five samples, two with three samples and two with greater than five samples. The median number of samples per patient was four. The following table demonstrates the clinical status of each patient's disease at the time of the first sample draw:

Clinical Status of Disease At Time of First Sample

Status of Disease	Frequency	Percent
Active	3	5.00
Stable	20	33.33
Progressive	13	21.67
Responding	2	3.33
NED	22	36.67
Total	60	100

Changes in the values of the AIA-PACK 27.29 and the CA 27.29 RIA assay were computed from sample to sample in chronological order of specimen draw for each patient. These changes were then classified as follows:

- Positive: An increase of more than 10%(50% for CA 27.29 RIA) in the result from the previous value or an increase from below the cutoff to above the cutoff.
- Neutral: Any change less than +/- 10% (+/- 50% for CA 27.29 RIA) of the previous value unless the value changed above or below the cutoff.
- Negative: A decrease of more than 10% (50% for CA 27.29 RIA) in the value of the device from the previous value or a decrease from above the cutoff to below the cutoff.

The table below shows classification of the AIA-PACK 27.29 and the CA 27.29 RIA assay changes by current clinical status using the change classification listed above. Results of the exact binomial tests for the extension of the McNemar test indicate that the AIA-PACK 27.29 and the CA 27.29 RIA assay are equivalent.

Concordance of Test and Predicate Devices

By Current Clinical Status

Current Status			Change in CA 27.29 RIA			Total	Exact 2-tail p for McNemar Test Extension
			Negative	Neutral	Positive		
NED	AIA-PACK 27.29	Negative	0	21	0	21	Not computed
		Neutral	0	39	3	42	
		Positive	0	18	4	22	
	Total		0	78	7	85	
Stable	AIA-PACK 27.29	Negative	7	13	0	20	1.000
		Neutral	0	15	1	16	
		Positive	0	13	12	25	
	Total		7	41	13	61	
Responding	AIA-PACK 27.29	Negative	1	5	0	6	0.219
		Neutral	0	1	0	2	
		Positive	0	2	3	4	
	Total		1	8	3	12	
Active/ Progressive	AIA-PACK 27.29	Negative	2	7	0	9	0.549
		Neutral	0	5	1	6	
		Positive	0	4	18	22	
	Total		2	16	19	37	

Performance Characteristics

The following performance characteristics were determined using the Tosoh AIA Immunoassay Analyzers.

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of CA 27.29 and assayed before and after spiking.

Sample	Initial Value (U/mL)	CA 27.29 Added (U/mL)	Expected Value (U/mL)	Measured Value (U/mL)	Percent Recovery (%)
Serum A1	5.4	69.3	74.7	76.5	102.4
	5.4	138.6	144.0	148.5	103.1
	5.4	277.1	282.5	285.3	101.0
Serum B1	13.7	69.1	82.8	86.7	101.7
	13.7	138.3	152.0	158.6	104.3
	13.7	276.5	290.2	295.3	101.8
Serum C1	5.6	70.2	75.8	75.1	99.1
	5.6	140.5	146.1	140.1	95.9
	5.6	280.9	286.5	280.8	98.0

1b) Dilution:

Three serum samples containing high concentrations of CA 27.29 which had been diluted by 21 fold were serially diluted with 27.29 Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (U/mL)	Measured Value (U/mL)	Percent Recovery (%)
Serum A2	None		212.1	100.0
	7.5/10	159.1	154.6	97.2
	5.0/10	106.1	105.3	99.3
	2.5/10	53.0	52.7	99.4
	1.0/10	21.2	20.9	98.5
Serum B2	None		315.5	100.0
	7.5/10	236.6	238.7	100.9
	5.0/10	157.7	155.3	98.5
	2.5/10	78.9	75.9	96.2
	1.0/10	31.5	29.4	93.2
Serum C2	None		246.3	100.0
	7.5/10	184.7	179.0	96.9
	5.0/10	123.1	123.6	100.4
	2.5/10	61.6	61.4	99.7
	1.0/10	24.6	24.2	98.1

2) Precision

2a) Intra-assay Precision

The intra-assay (within run) precision coefficient of variation was evaluated in two control samples by 10 replicate determinations.

Sample	Number of Replicates	Mean (U/mL)	Standard Deviation (U/mL)	Coefficient of Variation (%)
Control L	10	41.3	0.9	2.2
Control H	10	300.9	4.1	1.4

2b) Inter-assay Precision

The inter-assay (between run) precision coefficient of variation was evaluated at two different concentrations by analyzing control samples by 21 replicate determinations.

Sample	Number of Replicates	Mean (U/mL)	Standard Deviation (U/mL)	Coefficient of Variation (%)
Control L	21	40.4	0.9	2.2
Control H	21	301.0	6.5	2.2

Specificity

The following substances were tested for cross-reactivity by adding to human serum containing CA 27.29 (266 U/mL). The cross-reactivity (%) is the percent of the compound identified as CA 27.29. If these compounds are present in the specimen at the same concentration as CA 27.29, the final result will be increased by these percentages.

Material Added	Amount Added	Recovery %
CEA	300 ng/mL	99.6
AFP	300 ng/mL	98.6
CA 19-9	300 ng/mL	101.3
CA 125	300 ng/mL	100.2

Sensitivity

The minimal detectable concentration (MDC) of CA 27.29 is estimated to be 0.10 U/mL which corresponds to 2 U/mL in the original specimen (dilution factor 21). The MDC is defined as that concentration of CA 27.29 corresponding 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 436 mg/dL) does not interfere with the assay.
- Added free bilirubin (up to 16.5 mg/dL) and conjugated bilirubin (up to 18.4 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1667 mg/dL), does not interfere with the assay.
- Protein, as indicated by added albumin (concentrations up to 50 mg/mL), does not interfere with the assay.
- Ascorbic acid (concentrations up to 20 mg/dL) does not interfere with the assay.
- Citric acid (concentrations up to 20 mg/mL) does not interfere with the assay.
- EDTA (concentrations up to 10.0 mg/mL) does not interfere with the assay.
- Heparin (concentrations up to 100 U/mL) does not interfere with the assay.
- The following pharmaceutical agents were tested and found not to cause analyte recovery outside 10%: 5'-fluorouracil (Adrucil), acetaminophen, adriamycin (Doxorubicin HCl), coumarin, cyclophosphamide (Cytoxan), paclitaxel, tamoxifen citrate, amethopterin hydrate (Methotrexate), mitoxanntrone (Novatrone), folinic acid (Leucovorin)
- 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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8) Young, D., 1990, Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, Washington, DC, American Association for Clinical Chemistry Press.

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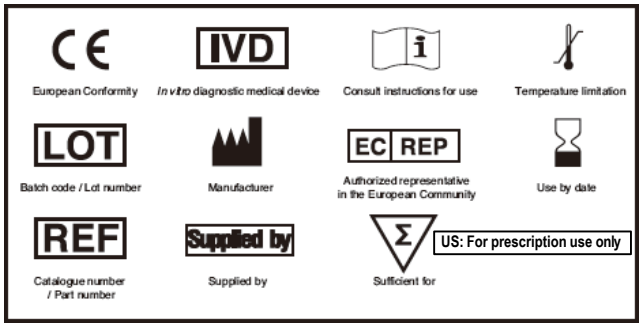


AIA-PACK
27.29 CALIBRATOR SET

Intended Use

The AIA-PACK 27.29 CALIBRATOR SET is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK 27.29 (CA 27.29) Assay.

Summary and Explanation

The AIA-PACK 27.29 CALIBRATOR SET contains bovine serum albumin with assigned levels of CA 27.29. 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. 0020302)

2 x 1 mL Zero Calibrator

Bovine serum albumin containing no detectable concentration of CA 27.29 (0 U CA 27.29/mL), and 0.1% sodium azide as preservative (ready for use).

2 x 1 mL

Calibrator No. 2	0.2	U/mL (approx.)
Calibrator No. 3	2.5	U/mL (approx.)
Calibrator No. 4	5.0	U/mL (approx.)
Calibrator No. 5	10.0	U/mL (approx.)
Calibrator No. 6	20.0	U/mL (approx.)

Bovine serum albumin containing the assigned concentration of CA 27.29 (described on each vial), and 0.1% sodium azide as a preservative (ready for use).

Warnings and Precautions

- The AIA-PACK 27.29 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

- Calibrators for the AIA-PACK 27.29 assay come ready to use. Note: 27.29 calibrators are prediluted (1:21) and need no further dilution. Mix the calibrators gently but thoroughly prior to performing the calibration.
- Bring calibrators 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 27.29 CALIBRATOR SET is stable until the expiration date on the label. After opening, the calibrators should be used within 7 days.

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 Procedure

1) Assure that the calibrator concentrations listed on the vial labels as well as the calibrator lot numbers have been correctly entered into the analyzer software.
2) Calibrators for ST AIA-PACK 27.29 are provided ready to use. No dilution is necessary for any of the AIA systems.
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 27.29 CALIBRATOR SET contains assigned concentrations of CA 27.29. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 2.0 to 400.0 U/mL of CA 27.29 (dilution 1:21). The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Results

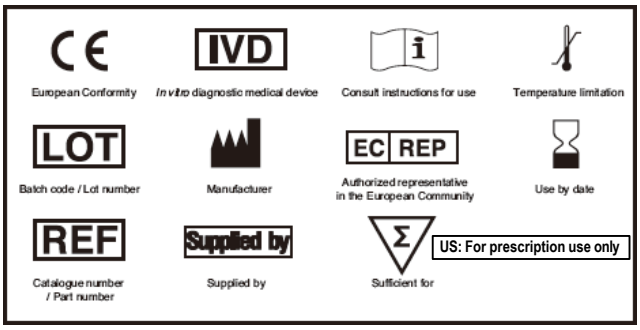
- The mean rate for the zero calibrator should be <3.0 nM/sec.
- Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- The replicate values should be within a 10% range.

Limitations

The AIA-PACK 27.29 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 Analyzer Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.



AIA-PACK
27.29 SAMPLE DILUTING SOLUTION

Intended Use

The AIA-PACK 27.29 SAMPLE DILUTING SOLUTION is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of CA 27.29 above the linear range of the assay.

Summary and Explanation

The AIA-PACK 27.29 SAMPLE DILUTING SOLUTION contains bovine serum albumin with no detectable concentration of CA 27.29. This Sample Diluting Solution is to be used only with samples that are being tested for CA 27.29 concentrations using the ST AIA-PACK 27.29 (CA 27.29) assays.

Material Provided (Cat. No. 0020502)

4 x 100 mL	Sample Diluting Solution	Bovine serum albumin containing no detectable concentration of CA 27.29 (0 U CA 27.29/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK 27.29 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 27.29 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 27.29 SAMPLE DILUTING SOLUTION is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for 7 days when used for automatic dilutions provided it is not on the instrument (18 - 25 °C) for more than 7 hours per day and the bottles are tightly sealed and refrigerated at 2 - 8 °C immediately after use.

- The sample diluting solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the bottles are kept tightly sealed and refrigerated immediately after use.
- For auto-dilution on the AIA-900 the 27.29 SDS can be transferred to a 5 mL bottle from the 100 mL bottle. It is stable for 7 days after the transfer as long as it is tightly sealed and stored at 2 - 8 °C after use.

Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution. ST AIA-PACK 27.29 is always run using a 1:21 dilution of the serum or plasma with AIA-PACK 27.29 SAMPLE DILUTING SOLUTION. AIA-900 and AIA-2000 can be programmed to perform the dilution and the mathematical calculations automatically.

1) If a specimen is found to contain greater than the linearity limit of 19.05 U/mL (400 U/mL after x21 dilution factor), the specimen should be further diluted with the Sample Diluting Solution and assayed according to the instructions in the AIA System Operator's Manual.
2) The recommended dilution for serum or plasma containing greater than 19.05 U/mL (400 U/mL after x21 dilution factor) is 1:10 or 1:100. However, it is desirable to dilute the serum or plasma samples that contain more than 19.05 U/mL (400 U/mL after x21 dilution factor) so that the diluted sample reads between 2 and 400 U/mL after multiplying with x21 dilution factor.

Results

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

Limitations

The AIA-PACK 27.29 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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