YALE-NEW HAVEN HOSPITAL	TITLE: C2729 Tosoh A1A 900 **Soft Code: C2729**		***DEPT OF LAB MEDICINE*** **Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories** DOCUMENT NO: IMM202
REVIEW BY PREP/SUPVR: Susan Goettlich, MT ASCP) & Jimmy Debevoise MT (ASCP)	EFFECTIVE DATE: January 24, 2013	REVISION DATE: N/A	Page 1 of 11
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I. Name and Intended Use

ST AIA-PACK 27.29 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of breast cancer antigen (CA 27.29) in human serum on TOSOH AIA-900 System analyzer.

II. Introduction

Breast carcinoma-associated antigen coded by the human MUC-1 gene is identified by several names including MAM 6, milk mucin, 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 is similar, if not identical, to CA 15-3. This antigen is a glycoprotein (molecular weight 300 – 450 kDa) which contains 20 amino acid tandem repetitive sequence of the mucin core. Carbohydrate comprises more than half the molecule. 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. ST AIA-PACK 27.29 was developed with a monoclonal antibody that recognizes an 8 amino acid sequence in the tandem repeat portion of the polypeptide chain. 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.

III. 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 immobilized on a magnetic solid phase and enzyme-labeled monoclonal

antibody in the AIA-PACK. The magnetic beads are washed to remove unbound materials 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.

IV. Specimen Collection

The test should be performed on serum only. Separate serum by centrifugation @ 3000 RPM for 15 minutes. Repeated freeze-thaw cycles should be avoided.

Standard aliquot volume = 1 mL
Minimum aliquot volume = 500 µL
Grossly Hemolyzed: Reject
Grossly Lipemic: Spin at 10,000 RPM for 20 minutes
Stability: 24 hours refrigerated, 60 days frozen (-20°C)

V. Warnings and Precautions

- 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.
- 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. 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.

VI. Materials

A. Reagents

i. Substrate Solution

Composition: AIA Pack Substrate Reagent II (lyophilized) contains 4-methylumbelliferyl phosphate, stabilizers and sodium azide as a preservative. AIA Pack Substrate Reconstituent II (liquid) contains buffer with sodium azide as a preservative (Cat. # 0020968).

Preparation: Bring all reagents to room temperature (18°-25°C) before preparing the working Substrate. Add the entire 100mL of substrate reconstituent liquid to the

lyophilized substrate reagent. Mix and allow solid material to fully dissolve before using.

Stability & Storage: Substrate reconstituent fluid and lyophilized reagent are stable until expiration date on label and stored at 2-8°C. When reconstituted, the working Substrate is stable for 7 days at 2-8°C.

ii. Wash Solution

Composition: Buffer solution with detergent and bacteriostatic reagent (Cat. # 0020955)

Preparation: Add two 100mL bottles of wash concentrate into the wash tank of the instrument. QS up to the five liter mark with CLRW. Cap and invert the bottle 6-8 times to mix.

Stability & Storage: Wash concentrate is stable until expiration date on label at 2-8°C. Prepared wash solution is stable for 30 days at room temperature.

iii. Diluent Solution

Composition: Buffer solution with detergent (Cat. # 0020956)

Preparation: Add one 100mL bottle of diluent concentrate into the diluent tank of the instrument. QS up to the five liter mark with CLRW. Cap and invert the bottle 6-8 times to mix.

Stability & Storage: Diluent concentrate is stable until expiration date on label at 2-8°C. Prepared diluent solution is stable for 30 days at room temperature.

iv. 27.29 Sample Diluting Solution

Composition: Buffered bovine serum albumin containing no detectable concentration of CA 27.29 with sodium azide as a preservative (Cat # 0020502)

Preparation: No preparation needed. Ready for use.

Stability & Storage: If unopened, stable until the expiration date on label if stored at 2-8°C. Once opened, store at 2-8°C and do not use past 7 days.

v. Calibrators

Composition: Contains buffered bovine serum albumin with assigned levels of CA 27.29 (Cat # 0020302)

Preparation: The calibrator set for CA 27.29 contains 6 calibrators. No reconstitution needed, they are ready for use.

Stability & Storage: Stable unopened at 2-8°C until expiration date on label. Once opened, calibrators are only stable for 1 day. Allow calibrators to come to room temperature prior to use.

vi. AIA-PACK 27.29 Test Cups

Plastic test cups containing lyophilized twelve magnetic beads coated with mouse anti-CA 27.29 monoclonal antibody and 100µL of mouse anti-CA 27.29 monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative. (Cat # 0025202)

Stability & Storage: Stable until expiration date if stored at 2-8°C or up to 1 day at 18-25°C

- ❖ NOTE: It is mandatory to pretest new lot of test cups by using five previously run samples that span the calibration curve. The difference between current and new lot result cannot exceed 15% for each sample.

vii. Controls

Lyphochek® Tumor Marker Control Level 1	Biorad Cat #367
Lyphochek® Tumor Marker Control Level 2	Biorad Cat #368
Lyphochek® Tumor marker Control Level 3	Biorad Cat #369

Composition: Controls are prepared from human serum with added constituents of human and animal origin, chemicals, and stabilizers. They are provided in lyophilized form for increased stability.

Preparation: Reconstitute lyophilized controls with 2mL of CLRW using a 2mL volumetric pipet. Allow to stand for 15 minutes at room temperature with occasional swirling. The controls are then aliquoted in 300µL amounts in Nunc Cryovials and frozen at -20°C for 30 days.

Stability & Storage: Lyophilized controls are stable until expiration date when stored at 2-8°C. Reconstituted controls are stable for 30 days at -20°C. After thawing, controls can be used for up to 3 days at 2-8°C.

B. Consumables

Refer to AIA-900 instrument procedure for a list of daily consumables and their usage.

VII. Calibration

A. Reasons to calibrate

1. Every 90 days
2. New lot of AIA-Pack 27.29 Test Cups
3. Quality control violations occur (e.g. 2-2s rule)
4. Certain service procedures are performed; consult TOSOH for more information

B. Calibration Procedure

1. After allowing calibrators to reach room temperature, request a CA 27.29 calibration on the AIA-900 (See Section V. of AIA-900 instrument procedure for setting up calibration)

2. Calibrators are run undiluted on the AIA-900 and each standard is run in triplicate. The concentration range of the calibration curve is displayed in 1/21 of the assay range in serum samples. The concentration of the patient samples are calculated by multiplying the concentrations obtained on the calibration curve with the dilution factor.

C. Calibration Review

1. The mean rate for Calibrator 1 should be <3.0nmol/(L·s)
2. The rate should increase as the concentration increases due to direct relationship
3. The replicate values should be within a 10% range.
4. Review the calibration curve carefully
5. Edit calibration if necessary, then accept (See Section V. of AIA-900 instrument procedure for more notes on calibration)

VIII. Quality Control

A. Frequency

1. Three levels per shift or every 8 hours
2. Immediately after calibration to verify curve acceptability
3. As needed following certain service procedures

B. QC Guidelines

1. Westgard rules of 10x, 2-2s and 1-3s are followed
2. QC charts for daily input are found in L Drive under QC CHARTS folder
3. For more information on quality control monitoring refer to Immunology Laboratory Guidelines for Quality Control (Doc # Imm 38.)

C. New Lots of Quality Control Material

1. New lots are pretested until at least 30 data points are collected to determine an in-house control range of +/- 3 standard deviations.

2. If a new lot of control must be put into use before 30 points are collected, the manufacturer's range will be used until 30 points are collected.

IX. Assay Procedure

1. Call a Soft pending list by test code (C2729). Refer to Soft Immunology Procedure (Doc# IMM 120) on how to use Resulting Worklist.

2. See AIA-900 instrument procedure for detailed instructions on loading and programming barcoded or non-barcoded samples.

3. Samples are automatically diluted at 1:21 by the AIA-900 with 27.29 Sample Diluting Solution.

4. Any samples that are above the Analytical Measuring Range (AMR) on the initial dilution of 1:21 are indicated by >**H flag** and no concentration value. See next section for programming higher dilutions.

X. Dilution Request

1. Do not load any other samples while handling a dilution request.

2. Refer to **Table 1** on next page as a guide on which dilution factors to request.

3. From HOME screen, press RESULT button.

4. Find sample number which needs higher dilution, indicated by >**H flag** and no concentration value, using arrow keys. Press SELECT button twice followed by FUNCTION button. Press RESCHEDULE then OK.

5. Go back into HOME screen and press ORDER(BARCODE) or ORDER(NON-BAR) depending on which mode sample was originally assayed in. The sample number which has been rescheduled should be present. Press ANALYTE button.

6. Delete the request for 1:21 and use PANEL button to request a different dilution. Press OK. The new dilution factor has replaced the old in ORDER screen. To order another dilution, press REPEAT button at top. This will duplicate the sample number and ANALYTE button can be pressed again to delete one dilution and request another.

7. The newly requested dilutions should appear on ORDER screen. Use FUNCTION button to print a Worklist.

8. Load samples according to worklist arrangement. If running two dilutions, split sample into correct rack positions.

9. Press ASSAY START and confirm END OF REQUEST number is correct.

10. Press START.

Table 1. Requesting Higher Dilutions (above 1:21) for CA 27.29

If previous result is:	Then request following dilutions:
No previous result	42 and 84
400 – 800	42
801 – 1600	42 and 84
1601 – 3200	84 and 168
3201 – 6400	168 and 336
6401 – 12,800	336 and 672
> 12,800	672 and (manual 1:2 on 672)

***The dilution scheme provided above takes into account that patient is being treated and concentration is getting lower.**

***Always assign a unique sample ID when making a manual 1:2 dilution to be run on 672. This will make distinction to operator that concentration value printed on AIA-900 must be multiplied by factor of 2 before reporting.**

***If final value is higher than 20,000 then report as "> 20,000 U/mL"**

XI. Result Verification

1. The AIA-900 automatically calculates final concentration value of CA 27.29 in U/mL.

2. If sample concentration is above measuring range, a **>H flag** prints without a value. Follow previous section for requesting higher dilutions. Report as >20,000 U/mL.

3. If sample concentration is below measuring range, a **<L flag** prints without a value. Check sample for bubbles and/or fibrin before reporting as <4 U/mL.

4. When result is available, it must be manually transmitted to LIS (Soft). To do this, press RESULT button.

5. Find sample ID and press SELECT. To release more than one result, use arrows to choose other sample IDs. When finished, press SELECT again. The "+" symbol will convert to ">" symbol.

6. Press FUNCTION, then TRANSMIT followed by OK when asked "TRANSMIT Are you sure?"

7. Results will be released to Soft where they can be manually posted in Instrument Menu.

XII. Analytical Measuring Range (AMR)

Since standard concentrations may vary from lot to lot, the AMR is an approximate range. Therefore, the Clinical Reportable Range has been fixed to avoid exceeding any lot specific AMR.

CA 27.29:

AMR: 2.1-400 U/mL

Maximum allowable instrument dilution: 1:672

Maximum allowable assay dilution: 1:1344 when running manual 1:2 at instrument dilution of 1:672

Minimum allowable dilution: 1:21

CRR (Clinical Reportable Range): 4-20,000 U/mL

*AMR verification does not need to be performed every 6 months because the standard curve used to calibrate contains more than 3 points.

XIII. Reference Range

Serum: <35 U/mL

XIV. Limitations of the Procedure

1. 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.).

2. The exact linearity of the ST AIA-PACK 27.29 depends on the particular lot of calibrator in use. Although the approximate value of the highest calibrator is 21 U/mL, the exact concentration may be slightly different.

3. 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. REJECT GROSSLY HEMOLYZED SPECIMENS.

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

5. 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 27.29.

6. Specimens from patients taking medicines and/or medical treatment may show erroneous results.

XV. YNHH Method Validation Summary

Accuracy and Linearity:

Accuracy and linearity studies were performed by running Tosoh controls for CA 27.29.

Error limits were set as follows:

Allowable Total Error (TEa): 20%

Systematic Error Budget: 50%

Allowable Systematic Error (SEa): 10%

The accuracy test passed. The maximum deviation for a mean recovery from 100% was 0.8%. 3 of 3 mean recoveries were accurate within the Allowable Systematic Error (SEa) of 10%. 6 of 6 results were accurate within the Allowable Total Error (TEa) of 20%. The results are linear.

Correlation:

Correlation was performed by comparing 40 patient results for CA 27.29 between the Tosoh AIA-360 and the AIA-900. The CA 27.29 value on each patient was originally reported using the AIA-360. Regression Analysis was done with EP Evaluator® and acceptability was determined within 95% confidence intervals for slope and intercept. Those intervals are given in parentheses below. The acceptable correlation coefficient (R) cutoff was 0.95.

X Method: Tosoh AIA-360 Y Method: Tosoh AIA-900

Slope 1.028 (1.003 to 1.053), Intercept -0.782 (-2.738 to 1.175)

Correlation Coefficient (R) = 0.9972

Precision:

Intra-run Precision: Intra-assay performance was evaluated by assaying three levels of Lyphochek® Tumor Marker Plus controls 4 times each within a single run. The acceptable Coefficient of Variation (CV) limit for intra-run precision is 10%. All three controls had a CV of <5%.

Inter-run Precision: Inter-assay performance was evaluated by assaying three levels of Lyphochek® Tumor Marker Plus controls over 4 successive days. The acceptable CV limit for inter-run precision
is 20%. All three controls had a CV of <10%.

Carryover:

Specimen to specimen carryover was determined by assaying Tumor Marker level three control followed by Tumor Marker level one control. Using the Carryover module by EP Evaluator®, the following analysis data was given: High-Low mean = 0.83318, Low-Low mean = 0.83246.

Carryover = (High-Low mean) – (Low-Low mean) = 0.00072

The carryover test passed since Carryover was less than the Error Limit of 0.06445.

Reference Range Verification:

Reference range for CA 27.29 was established using 5 years' worth of YNHH historical data. The data was analyzed using EP Evaluator® and reviewed by the medical director who then determined the range below:

CA 27.29: <35 U/mL

CAP Proficiency Results:

Survey TMB 2011 was tested for CA 27.29 and all results were acceptable when compared to other Tosoh AIA-PACK 27.29 users.

XVI. References

1. Tosoh AIA-900 Operator's Manual, Revision C. Tosoh Bioscience, Tokyo, Japan.

2. ST AIA-Pack 27.29 (Package Insert) Tosoh Bioscience, Tokyo, Japan. Rev. 10/2011

3. AIA Substrate Set II, (Package Insert), Tosoh Bioscience, Tokyo, Japan, Rev. 10/2011.

4. AIA-Pack Wash Concentrate, (Package Insert), Tosoh Bioscience, Rev. 10/2011.

5. AIA-Pack Diluent Concentrate, (Package Insert), Tosoh Bioscience, Tokyo, Japan.

Rev. 10/2011.

6. AIA-Pack 27.29 Sample Diluting Concentrate (Package Insert) Tosoh Bioscience, Tokyo, Japan. Rev. 10/2011

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TEODORICO LEE	LAB MANAGER			NEW		1/24/13
BRIAN SMITH	LAB DIRECTOR			NEW		1/24/13

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