YALE-NEW HAVEN HOSPITAL	TITLE: B2 Microglobulin Tosoh A1A 900 **Soft Code: B2M**		***DEPT OF LAB MEDICINE*** **Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories** DOCUMENT NO: IMM201
REVIEW BY PREP/SUPVR: Susan Goettlich, MT ASCP) & Jimmy Debevoise MT (ASCP)	EFFECTIVE DATE: January 24, 2013	REVISION DATE: N/A	Page 1 of 10
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I. Name and Intended Use

ST AIA-PACK BMG is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of β2 microglobulin (BMG) in human serum on TOSOH AIA-900 System analyzer.

II. Introduction

Human β2 microglobulin (BMG) is a single polypeptide of 100 amino acids, identified in 1968 by Berggard et al. The molecular weight of BMG is 11,800 daltons and represents the light-chain moiety of the major histocompatibility complex class antigens. It is worth noting that BMG shows approximately 35% sequence homology with immunoglobulin domains. BMG is shed from the cell surface of all nucleated cells during growth and differentiation, and cleared by glomerular filtration in the kidneys. Since it is reabsorbed by the proximal tubular cells and degraded, only trace amounts are detected in urine from healthy individuals, whereas marked elevation is seen in patients with proximal tubular dysfunctions due to exposure to aminoglycosides, anti-cancer or inflammatory drugs and heavy metals.

Determination of serum BMG is of diagnostic value in a variety of disorders. It can be used to assess glomerular function in advanced renal disease and after renal transplantation. It can provide a measure of tumor burden and prognosis in patients with multiple myeloma, B cell lymphoma and chronic lymphocytic leukemia. It is useful to monitor disease activity in chronic inflammatory disorders, such as rheumatoid arthritis, systemic lupus erythematosis, Sjogren's syndrome, and Crohn's disease. More recently, BMG has been reported to be of value in monitoring patients with HIV infection.

III. Principle of the Assay

The ST AIA-PACK BMG is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. β2 microglobulin present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled

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 BMG 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 BMG 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 expiration date.
- The ST AIA-PACK BMG has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. On the recommended 51 fold dilution, serum samples with BMG concentrations between 20.4 and 102 mg/L will read > 0.400 mg/L. Using the recommended initial dilution, the "hook effect" phenomenon may occur at BMG serum concentrations > 102 mg/L.

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. BMG Sample Diluting Solution

Composition: Buffered bovine serum albumin containing no detectable concentration of β2 microglobulin with sodium azide as a preservative (Cat. # 0020559)

Preparation: Add 90mL of CLRW to the BMG Sample Diluting Concentrate (10mL) and mix well.

Stability & Storage: Concentrate is stable until expiration date on label at 2-8°C. Working diluting solution is stable for 7 days at 2-8°C.

v. Calibrators

Composition: Contains buffered bovine serum albumin with assigned levels of β2 microglobulin (Cat. # 0020359)

Preparation: The calibrator set for BMG contains 6 calibrators. Calibrator 1 is ready for use while Calibrators 2-6 are lyophilized. Reconstitute Calibrators 2-6 with 1mL of CLRW using a volumetric pipet. Allow all calibrators to fully dissolve and mix by inverting each several times before using. All calibrators should be at room temperature prior to use.

Stability & Storage: Calibrators stable until expiration date on label if unopened and stored at 2-8°C. After reconstituting, stability is for 7 days at 2-8°C. Do not freeze.

vi. AIA-Pack BMG Test Cup

Plastic test cups contain lyophilized twelve magnetic beads coated with anti-β2 microglobulin mouse monoclonal antibody and 135µL of anti-β2 microglobulin rabbit polyclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative. (Cat. # 0025259)

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

a) Every 90 days
b) New lot of AIA-Pack BMG Test Cups
c) Quality control violations occur (e.g. 2-2s rule)
d) Certain service procedures are performed; consult TOSOH for more information

B. Calibration Procedure

1. After the calibrators have been reconstituted and are at room temperature, request a B2M 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 1/51 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

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

VIII. Quality Control

A. Frequency

a) Three levels per shift or every 8 hours
b) Immediately after calibration to verify curve acceptability
c) As needed following certain service procedures

B. QC Guidelines

- Westgard rules of 10x, 2-2s and 1-3s are followed
- QC charts for daily input are found in L Drive under QC CHARTS folder
- 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 (B2M). 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 1:51 by the AIA-900 with BMG Sample Diluting Solution.

4. Any samples that are above the analytical measuring range (AMR) on the initial dilution of 1:51 will not print a numerical result but will give a >H flag. A higher dilution(s) must be requested for these samples.

X. Dilution Request

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

2. From HOME screen, press RESULT button.

3. Find sample number which needs higher dilution using arrow keys. Samples above AMR are indicated by >H and do not have a value. Press SELECT button twice followed by FUNCTION button. Press RESCHEDULE.

4. Go back into HOME screen and press ORDER(BARCODE). The sample number which has been rescheduled has been added. Press SAMP.ID button.

5. Delete the request for 1:51 and use PANEL button to request BMG 1:102 dilution. Press OK.

6. The newly requested dilution should now be added to ORDER screen. Use FUNCTION button to print a Worklist.

7. Find primary tube and load according to worklist setup.

8. Press ASSAY START(BAR) and confirm END OF REQUEST number is correct.

9. Press START.

10. If 1:102 dilution is still high, repeat process on 1:204 dilution.

XI. Result Verification

1. The AIA-900 automatically calculates and prints final concentration value of β2 microglobulin in mg/L.

2. If sample concentration is above measuring range, a >H flag prints without a value. Follow previous section for requesting higher dilutions. Report as >50 mg/L.

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 <0.10 mg/L.

4. When result is final on AIA-900, 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 include other sample IDs. When finished, press SELECT again. This will enable the FUNCTION button to be used.

6. After pressing FUNCTION, press TRANSMIT followed by OK when asked "TRANSMIT Are you sure?"

7. Results will be released to Soft and autoverified.

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.

β2 microglobulin (BMG):
AMR: 0.10-20 mg/L
Maximum allowable dilution: 1:204
Minimum allowable dilution: 1:51

CRR (Clinical Reportable Range): 0.10-50 mg/L

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

IX. Reference Range

Serum <2.65 mg/L

X. 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.).

- The exact linearity of the ST AIA-PACK BMG depends on the particular lot of calibrator in use. Although the approximate value of the highest calibrator is 0.400 mg/L, the exact concentration may be slightly different.

- 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.

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

- 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 β2 microglobulin.

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

XI. YNHH Method Validation Summary

Accuracy and Linearity:

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

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 0.9%. 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 BMG between the Tosoh AIA-360 and the AIA-900. The BMG 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 0.903 (0.824 to 0.983), Intercept 0.28502 (-0.06799 to 0.63803)
Correlation Coefficient (R) = 0.9639

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 <1%.

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 <12%.

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 BMG 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:

β2 microglobulin (BMG): <2.65 mg/L

CAP Proficiency Results:
Survey TMB 2011 was tested for BMG and all results were acceptable when compared to other Tosoh AIA-PACK BMG users.

XII. References

1. Tosoh A1A 900 Operator's Manual, Revision C. Tosoh Bioscience, Tokyo, Japan.

2. ST A1A-Pack BMG (Package Insert). Tosoh Bioscience, Tokyo, Japan. Rev. 7/2009
3. A1A Substrate Set II, (Package Insert), Tosoh Bioscience, Tokyo, Japan, Rev. 10/2011.
4. A1A-Pack Wash Concentrate, (Package Insert), Tosoh Bioscience, Rev. 10/2011.
5. A1A-Pack Diluent Concentrate, (Package Insert), Tosoh Bioscience, Tokyo, Japan. Rev. 10/2011.
6. A1A-Pack BMG Sample Diluting Concentrate, (Package Insert), Tosoh Bioscience, Tokyo, Japan. Rev. 7/2009.

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Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER			NEW		1/24/13
BRIAN SMITH	LAB DIRECTOR		1/24/13	NEW		1/24/13