VIDAS® β2 Microglobulin (B2M)

IVD

VIDAS β2 Microglobulin is an automated quantitative test for use on the VIDAS family instruments for the quantitative measurement of β2 Microglobulin in human serum or plasma (lithium heparin or EDTA) and in urine using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

β2 Microglobulin (β2 M) is a low molecular weight polypeptide (11,800 Da) (1, 2, 3, 4). It is synthesized by most nucleated cells. Its turnover corresponds to a production of approximately 150 mg/24h (2, 3, 4). Half the plasma β2 microglobulin, which is renewed daily, originates from lymphocyte cells. Circulating β2 microglobulin is filtered through renal glomeruli, and then reabsorbed and catabolized by the proximal tubule (1).

Elevated plasma β2 microglobulin levels indicate:

- either decreased glomerular filtration,
- or increased synthesis (1, 2).

Plasma β2 microglobulin levels can therefore be elevated in patients with diseases other than renal disorders, and in particular, those which affect the immune system (2, 3). A significant increase can be seen in diseases such as erythematosus lupus, rheumatoid arthritis, Sjögren's syndrome, malignant diseases of the lymphoid system (multiple myeloma, B-cell lymphoma), certain viral diseases (hepatitis or AIDS), and in hemophiliacs. It is one of the markers of unfavorable prognosis in cases of HIV infection (4, 2, 1).

It is the most effective test for the detection of proximal tubular dysfunction (2). The determination of urinary β2 microglobulin levels is useful for monitoring renal transplant patients (1).

Numerous assay methods have been described, including: radial-immunodiffusion, electro-immuno-diffusion, immunonephelometry, lymphocyto-toxicity inhibition and RIA and ELISA techniques. The latter two techniques are most commonly used.

PRINCIPLE

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After diluting the sample, the β2 microglobulin in the sample binds with the specific monoclonal antibody coating the interior of the SPR. Unbound components are eliminated during the washing steps. The β2 microglobulin retained is revealed by an alkaline phosphatase-labeled polyclonal anti-human β2 microglobulin antibody (sheep). Unbound conjugate is eliminated during the washing phase.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of β2 microglobulin present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (30 TESTS):

30 B2M strips	STR	Ready-to-use.
30 B2M SPRs	SPR	Ready-to-use. Interior of SPRs coated with anti-β2 M BE 104 mouse monoclonal antibody.
B2M calibrator 1 x 1 ml (liquid)	S1	Ready-to-use. TRIS buffer (0.05 mol/l) pH 7.4 containing β2 microglobulin of human origin + bovine albumin + 1 g/l sodium azide. MLE data indicate the concentration in mg/L ("Calibrator1 (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range").
B2M low positive control 1 x 1 ml (liquid)	C1	Ready-to-use. TRIS buffer (0.05 mol/l) pH 7.4 containing β2 microglobulin of human origin + bovine albumin + 1 g/l sodium azide. MLE data indicate the confidence interval in mg/L ("Control C1 Dose Value Range").
B2M high positive control 1 x 1ml (liquid)	C2	Ready-to-use. TRIS buffer (0.05 mol/l) pH 7.4 containing β2 microglobulin of human origin + bovine albumin + 1 g/l sodium azide. MLE data indicate the confidence interval in mg/L ("Control C2 Dose Value Range").
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

The SPR

The interior of the SPR is coated during production with anti-β2 M BE104 mouse monoclonal antibody. Each SPR is identified by the B2M code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The Reagent Strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the B2M strip:

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (0.05 mol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l sodium azide (600 µl).
3	Pre-wash buffer: TRIS buffer (0.05 mol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l sodium azide (600 µl).
4 - 5 - 7 - 8	Wash solution: TRIS buffer (0.05 mol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l sodium azide (600 µl).
6	Conjugate: alkaline phosphatase-labeled anti-β2 microglobulin antibody (sheep) + 1 g/l sodium azide (400 µl).
9	Sample diluent: TRIS buffer (0.05 mol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l sodium azide (400 µl).
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + Diethanolamine (DEA*) (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 100 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- VIDAS family instrument.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only**
- **For professional use only**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.

- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS β2 Microglobulin kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type and collection:

1. Use serum or plasma (validated anticoagulants: lithium heparin, EDTA).

– Do not use contaminated or inactivated sera (after 30 mins at 56°C, the concentration of β2 microglobulin in the sample is reduced by 18 to 50%).
– Samples containing impurities must be centrifuged before analysis.

None of the following factors have been found to significantly influence this assay:
– hemolysis (after spiking samples with hemoglobin: 0 to 300 µmol/l (monomer)),
– lipemia (after spiking samples with lipids: 0 to 5 mmol/l equivalent in triglycerides),
– bilirubinemia (after spiking samples with bilirubin: 0 to 513 µmol/l).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

2. **Urine:** measure the pH of the urine to be tested as soon as possible after collection.

– If the pH is higher than 5.5, the sample can either be tested without treatment, but used rapidly due to the existence of proteolytic enzymes in the urine, or neutralized (5).
– If the pH is less than 5.5, neutralize the urine, since the urine protease reaches maximum activity at a pH of less than 5.5, and β2 microglobulin is unstable at this pH.

Specimen stability:

– Serum and plasma samples can be stored at 2-8°C in stoppered tubes for up to 5 days; if longer storage is required, freeze the sera or plasma at - 25 ± 6°C (avoid successive freezing and thawing). A study performed on frozen samples over a period of 12 months showed that the quality of results is not affected.
– Neutralized urine samples can be stored frozen at -25 ± 6°C.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.

IT IS POSSIBLE TO ENTER MLE DATA MANUALLY OR AUTOMATICALLY **DEPENDING ON THE INSTRUMENT (REFER TO THE USER'S MANUAL).**

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested **in duplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "B2M" strip and one "B2M" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "B2M" code on the instrument. The calibrator must be identified by "S1", and tested in **duplicate**. If the low positive control is to be tested, it should be identified by "C1". If the high positive control needs to be tested, it should be identified by "C2".
4. Mix the calibrator, controls and samples using a vortex-type mixer (for serum, plasma or urine separated from the pellet).
5. **For this test, the calibrator, control, and sample test portion is 100 µl.**
6. Insert the "B2M" SPRs and "B2M" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
8. Restopper the vials and return them to 2–8°C after pipetting.
9. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistics model) and are expressed in mg/l.

Note: 1 IU = 1.4 x 10^{-2} µg (1st international standard preparation).
Samples with β2 microglobulin concentrations greater than 4 mg/l should be retested after being diluted by 1/10 in physiological saline solution.
If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result by the dilution factor to obtain the sample concentration.
Interpretation of VIDAS β2 Microglobulin test results should be made taking into consideration the patient history, and in addition to other diagnostic methods, according to the pathologies.

QUALITY CONTROL

One low positive and one high positive control are included in each VIDAS β2 Microglobulin kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified by C1 and C2. Results cannot be validated if the control values deviate from the expected values.

Note
It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient history, and the results of any other tests performed.

RANGE OF EXPECTED VALUES

These figures are given as a guide; it is recommended that each laboratory establishes its own reference values from a rigorously selected population.

First study:

The reference value for serum or plasma β2 microglobulin concentrations was found to be 1.50 mg/l. This average was obtained from 100 presumably healthy adults (blood donors), aged between 18 and 60. The reference range corresponding to the 95th percentile value varies from 0.81 to 2.19 mg/l. In urine, an average value of 0.07 mg/l was found for a population of 52 presumably healthy young adults. The reference range corresponding to the 95th percentile value varies from 0.00 to 0.15 mg/l.

Second study:

95 sera and 47 urine samples were tested. These samples were obtained from hospitalized patients, for whom no disease which could cause an increase in the β2 microglobulin, had been identified.

* Sera:

Age range	No. of patients	Mean value (mg/l)	Highest value (mg/l)
20 to 39 years	45	1.77	3.47
40 to 59 years	31	1.59	3.17
60 to 80 years	19	2.28	3.75

* Urine:

Age range	No. of patients	Most frequent value (mg/l)	Highest value (mg/l)
20 to 39 years	24	0.01	1.11
40 to 59 years	20	0.05	1.8
60 to 80 years	3	-	2.33

PERFORMANCE

Studies performed using VIDAS β2 Microglobulin gave the following results:

Measurement range

The measurement range of the VIDAS β2 microglobulin kit is: 0.004 to 4 mg/l.

Analytical detection limit

Defined graphically as the smallest concentration of β2 microglobulin which is significantly different from the zero concentration with a probability of 95%: 0.004 mg/l.

Precision

Within-run reproducibility (intra-assay):

5 samples were tested 30 times in the same run.

Sera	Mean concentration (mg/l)	CV (%)
S 1	1.4	9.5
S 2	2.3	11.4

Urine	Mean concentration (mg/l)	CV (%)
U 1	0.01	23.8
U 2	0.11	7.2
U 3	0.64	6.8

Between-run reproducibility (inter-assay):

3 serum samples and 2 urine samples were tested singly in 7 different runs on the same VIDAS instrument.

Sera	Mean concentration (mg/l)	CV (%)
S 1	3.84	6.4
S 2	1.73	5.8
S 3	1.63	6.9

Urine	Mean concentration (mg/l)	CV (%)
U 1	0.28	4.3
U 2	0.42	3.4

ACCURACY

Dilution test:

3 samples were diluted in a physiological saline solution and tested singly in 3 runs. The ratio of the mean concentration measured over the expected concentration is expressed as a mean recovery percentage.

Sample	Dilution factor	Expected concentration (mg/l)	Measured concentration (mg/l)	Recovery percentage
1	1/1	0.89		
	1/2	0.45	0.44	100
	1/4	0.22	0.19	85.6
	1/8	0.11	0.11	95.3
	1/16	0.06	0.05	89.9
	1/32	0.03	0.03	108
2	1/1	1.83		
	1/2	0.92	0.88	96.2
	1/4	0.46	0.41	89.7
	1/8	0.23	0.20	87.7
	1/16	0.11	0.09	78.9
	1/32	0.06	0.05	87.7
3	1/1	3.35		
	1/2	1.67	1.55	92.8
	1/4	0.84	0.79	94
	1/8	0.42	0.34	80.9
	1/16	0.21	0.16	76.2
	1/32	0.10	0.08	80

Correlation with another commercialized EIA reagent

Sera	n	Slope	Intersection at origin	Mean signal
Normal subjects	100	0.97	0.13	0.933
HIV + patients	87	0.9	0.44	0.941
Other patients	97	1.28	- 0.4	0.995

Urine	n	Slope	Intersection at origin	Mean signal
Normal subjects	49	1.04	- 0.007	0.989
Patients	98	1.03	0.09	0.986

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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5. ROMETTE et al. - β2 microglobuline: métabolisme, méthodes de dosage, variations pathologiques - Feuillets de biologie – 1992, vol. 33, n°189.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	07132J	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) WARNINGS AND PRECAUTIONS
2015/06	07132K	Technical	CONTENT OF THE KIT (30 TESTS) INSTRUCTIONS FOR USE

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