



Quantitative determination of β_2 -microglobulin (β_2 -m) IDV

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

The β_2 -m Turbilatex is a quantitative turbidimetric test for the measurement of β_2 -microglobulin (β_2 -m) in human serum, plasma or urine. Latex particles coated with anti-human β_2 -m are agglutinated when mixed with samples containing β_2 -m. The agglutination causes an absorbance change, dependent upon the β_2 -m contents of the patient sample that can be quantified by comparison from a calibrator of known concentration.

CLINICAL SIGNIFICANCE

β_2 -m is a protein located on the surface of human lymphocytes and other nucleated cells. Free β_2 -m is filtered by the glomerulus and subsequently reabsorbed in the proximal tubular cells. Increased urinary excretion of β_2 -m is a sensitive indicator of renal insufficiency. Also, the β_2 -m level in serum is a useful marker of other diseases including carcinomas, lymphoid tumors, rheumatoid arthritis and AIDS.

REAGENTS

β_2-m Diluent (R1)	Tris buffer 20 mmol/L, pH 8.2. Preservative.
β_2-m Latex (R2)	Particles coated with goat IgG anti-human β_2 -m, pH 7.5. Preservative.
β_2-m CAL	Calibrator. β_2 -m concentration is stated on the vial label.
Optional:	β_2 -m Control.

PRECAUTIONS

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

CALIBRATION

Use β_2 - Microglobulin Calibrator.

The sensitivity of the assay and the target value of the calibrator have been standardized against the 1st International β_2 -m Standard from WHO.

The calibration is stable for 1 month.

Recalibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

PREPARATION

Working reagent: Shake the latex vial gently before use. Prepare the necessary amount as follows:

1 mL Latex Reagent + 4 mL Diluent

β_2 -m Calibrator:

Serum method: Reconstitute (→) with 1.0 mL of distilled water. Mix gently and bring to room temperature for about 10 minutes before use.

Urine method: Dilute reconstituted calibrator 1/6 with NaCl 9 g/L (50 μ L calibrator + 250 μ L NaCl 9 g/L).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during use. Do not use reagents over the expiration date.

Reagent deterioration: Presence of particles and turbidity.

Working reagent: Stable for 30 days at 2-8°C.

β_2 -m Calibrator: Stable for 1 month at 2-8°C or 3 months at -20°C.

Do not freeze; frozen Latex or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 540 nm filter.

SAMPLES

Fresh serum. Stable 7 days at 2-8°C or 3 months at -20°C.

Fresh urine. Adjust samples to pH 7-8 by the addition of K_2HPO_4 . Stable 2 days at 2-8°C or 2 months at -20°C.

The samples with particles or fibrin should be centrifuged before testing. Do not use hemolyzed or lipemic samples.

PROCEDURE

1. Bring the Reagents and the photometer (cuvette holder) to 37°C.

2. Assay conditions:

Wavelength: 540 nm (530-550).

Temperature: 37°C

Cuvette light path: 1 cm.

3. Adjust the instrument to zero with distilled water.

4. Pipette into a cuvette:

W. Reagent (mL)	1.0
Calibrator or sample (μ L)	10 (serum), 50 (urine)

5. Mix and read the absorbance immediately (A_1) and after 3 minutes (A_2) of the sample addition.

Gesam has instruction sheets available for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Serum:

$$\frac{(A_2 - A_1)_{\text{sample}}}{(A_2 - A_1)_{\text{Calibrator}}} \times \text{Calibrator concentration} = \text{mg/L } \beta_2\text{-m}$$

Urine:

$$\frac{(A_2 - A_1)_{\text{sample}}}{(A_2 - A_1)_{\text{Calibrator}}} \times \frac{\text{Calibrator concentration}}{6} = \text{mg/L } \beta_2\text{-m}$$

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used the GESAN β_2 -m Control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Serum: from 1.0 to 3.0 mg/L.

Urine: from 0.1 to 0.3 mg/L.

Each laboratory should establish its own reference range

PERFORMANCE CHARACTERISTICS

- Linearity limit:** Up to 18 mg/L (serum) and 3 mg/L (urine), under the described assay conditions. Samples higher results should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity depends on the sample-reagent ratio, as well as the analyzer used. It will be higher by decreasing sample volume, although the sensitivity of the test will be proportionally decreased.
- Detection limit:** Values less than 0.22 mg/L (serum) and 0.04 mg/L (urine) give non-reproducible results.
- Prozone effect:** No prozone effect was detected up to 100 mg/L (serum) and 20 mg/L (urine).
- Sensitivity:** Δ 0.048 A. mg/L (serum) and Δ 0.228 A. mg/L (urine).
- Precision:** The reagent has been tested for 20 days, using three different β_2 -m concentrations in an EP5-based study.

EP5	CV (%)		
	+/- 1 mg/L	+/- 3.2 mg/L	+/- 8.5 mg/L
Total	4.0%	3.4%	1.7%
Within Run	2.8%	2.0%	1.2%
Between Run	1.7%	1.5%	1.2%
Between Day	2.2%	2.4%	0.0%

- Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 36 samples of different concentrations of β_2 -m were assayed. The correlation coefficient (r) was 0.97 and the regression equation $y = 1.709x - 2.627$.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Serum method: bilirubin (20 mg/L), hemoglobin (10 g/L) and lipids (10 g/L), do not interfere. Rheumatoid factors (150 IU/mL), interfere.

Urine method: urea (urine) (50 g/L), uric ac. (20 g/L) and glucose (100 g/L), do not interfere.

Other substances may interfere⁷.

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