MICROALBUMIN (Albumin in urine)

In vitro diagnostic reagents for the quantitative determination of albumin in urine (MAU) by means of particle-enhanced turbidimetric immunoassay.

Clinical Significance

Increased albumin excretion detectable only by sensitive immunoassay (microalbuminuria) has been used for some years as a predictor of incipient nephropathy and cardiovascular disease in diabetic patients. Microalburninuria has also been associated with hypertension and increased risk of cardiovascular disease in non-diabetic patients. Microalbuininuria occurs in response to acute inflammatory conditions such as ischaemia, trauma,and termal injury, surgery, pancreatitis, and inflammatory bowel disease. In many of these conditions albumin excretion increases within minutes or hours of the initiating stimulus and only last for 24 to 72 h. The degree of microalburninuria is proportional to the severity of the inflammatory insult, is predictive of outcome, and is not associated with any other features of renal impairment. Interest in measuring subclinical elevations in the albumin excretion rate has focused on individuals with an already established diagnosis of diabetes or essential hypertension. Providing proper care is taken to minimise the influence of exercise and poor metabolic control of the albumin excretion rate, the urinary albumin level has proved to be an excellent predictor of the progression to overt nephropaty in both insulin-dependent and non-insulin dependent diabetes.

Principle

This MAU test is based upon the reactions between albumin and latex-covalently bound antibodies against human albumin. MAU values are determined photometrically.

Reagents

A - Buffer - 45 mL of phosphate buffer, pH: 8.5, < 0,1 % sodium azide as preservative.

B - Latex reagent – 5 mL of a suspension of latex microparticules covalently bound anti-albumin antibodies in a neutral aqueous solution, and < 0,1 % sodium azide as preservative.

C.- Calibrator – 1 mL. Human - based reference fluid. Preservative: sodium azide, 0.075 %. All raw materials of human origin used in the manufacture of this product showed no reactivity when tested for HBsAg, anti-HIV-1/2 and HCV with commercially available test methods. However, this product should be handled as though capable of transmitting infectious diseases

Reagent Preparation

Working Reagent is prepared with 1 part of Latex Reagent and 9 parts of Buffer Reagent. Prepare a fresh WR based on its workload. Shake gently the reagents before pipetting.

Calibration Curve and Controls

Analytical Range up to 250 mg/L.

Calibrator 1	100 µl of MAU Calibrator*
Calibrator 2	100 µl of Calibrator 1 + 100 µl of Saline Solution
Calibrator 3	100 µl of Calibrator 2 + 100 µl of Saline Solution
Calibrator 4	100 µl of Calibrator 3 + 100 µl of Saline Solution
Calibrator 5	100 µl of Saline Solution

(*) See values on the label or on the insert. Multiply by the appropriate factor.

For quality control use a suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Stability

Reagents in the original vial is stable to the expiration date on the vial label when capped and stored at +2 - +8ºC. Immediately following the completion of an assay run, the reagent vial should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2 - +8ºC after use. Do not freeze reagents. The MAU latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarted. The MAU buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarted.
WR is stable for up to two weeks at 4ºC. **It is recommended that each Laboratory prepares a fresh Working Reagent based on its workload.**

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines. As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required but not provided

Spectrophotometric analyser. Controls.

Specimens

Use 12 or 24 hour collection. centrifuge urine specimens. Screen these specimens using an albumin test strip. If the result is negative (approx. below 300 mg/L), analyse the specimens undiluted. If the result is positive, dilute the specimen with specific protein sample diluent to obtain a concentration below 250 mg/L .

Procedure

Wavelength		600 nm	
Temperature		37ºC	
Cuvette		1cm light path	
Measurement against distilled water blank.			
Bring the reagents at 37ºC and pipette:			
	Calibrator	Sample	Blank
Calibrator	3 µl	---	---
Sample	---	3 µl	---
Distilled Water	---	---	3 µl
Work. Reagent	500 µl	500 µl	500 µl
Mix and measure absorbance immediately (A1) incubate 4 min (37ºC), after incubation read absorbance (A2).			

Calculation

Plot the calibration curve and the sample concentration is obtained by interpolation the sample absorbance (A2-A1) in the calibration curve.
If is an one point calibration:

$$\frac{(A2-A1)_{sample} - (A2-A1)_{blank}}{(A2-A1)_{calibrator} - (A2-A1)_{blank}} \; x Calibrator Concentration$$

Linearity

The range interval for the multipoint calibration method is from 0 to 250 mg/L. With this method you can use the one point calibration procedure using a calibrator without dilutions, because it is linear at least up to 170 mg/L. When values exceed the range the samples should be diluted with saline solution and the result should be multiplied by the appropriated factor. In an one point calibration, when values exceed 125 mg/L, the samples should be diluted with saline solution and the result should be multiplied by the appropriated factor.

Reference Values

For timed overnight urine collections an albumin excretion rate greater than 20 µg/min is considered to abnormal. These data are to be interpreted as a guide. Each laboratory should establish its own reference intervals.

Sensitivity

Calculating the mean plus 3SD of twenty replicates of zero standard resulted in a lower limit of detection less than 5 mg/L.

Prozone Effect

The system did not show prozone phenomenon at least up to 400 mg/L.

Assay Precision

Intra-assay coefficients of variation (CV) for three samples (MAU values ranging from 30 to 150 mg/L) were between 1.9 and 3.7 %. Inter-assay CVs were between 2.4 and 4.5 %.

Method comparison

70 samples were correlated with a commercial procedure. When comparing the results by lineal regression the result was: y = 0,96 x + 4,1 and r = 0,981

Analytical characteristics have been obtained in a single experiment in a conventional spectrophotometer. Therefore, the data expressed in the present document should be interpreted as a guide example.

Literature

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Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symbols figurant sur les etiquettes. Spiegazione dei simboli utilizzati sull'eticheta. Significado dos símbolos indicados nas etiquetas. Erläuterung der symbole auf den etiketten.

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