Microalbúmina

Immunoturbidimetric method for quantitative determination of microalbuminuria

SUMMARY

Microalbuminuria is the urinary albumin excretion increase above normal levels but in the absence of visible clinical nephropathy. It is defined as the excretion from 30 to 300 mg albumin in 24 hours (20-200 ug/min) in 2 out of 3 urinary collections performed in a few weeks period.
Microalbumin determination (MAlb) is an important component in the screening of diabetic patients, since it allows the early detection of those individuals at risk of developing progressive renal disease and the implementation of adequate therapeutic measures.
Currently, microalbumin has been acknowledged as an independent risk factor for cardiovascular disease in patients with and without diabetes.

PRINCIPLE

The albumin reacts to the specific antibody forming insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the albumin concentration in the sample and may be spectrophotometrically measured.

PROVIDED REAGENTS

A. Reagent A: buffered saline solution, pH 7.6.
B. Reagent B: anti-human albumin monospecific antibodies.

NON-PROVIDED REAGENTS

- Saline solution.
- Wiener lab.'s **Microalbúmina Calibrador Turbitest AA**.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

The reagents are for “in vitro” diagnostic use.
All patient samples should be handled as though capable of transmitting infectious diseases.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not freeze.

SAMPLE

Urine
a) Collection: obtain the sample in the usual way. An early morning urine sample may be used as well as urines 3, 8, 12 or 24 hours after collection. The samples should not be collected after exercise, during urinary tract infections, acute stage diseases, after surgery or acute liquid accumulation.
b) Additives: not required.
c) Known interfering substances: no interferences are observed by creatinine up to 440 mg/dl, urea up to 4500 mg/dl, bilirubin up to 25 mg/dl (250 mg/l), ascorbic acid up to 500 mg/dl nor IgG up to 2300 mg/dl.
Urine samples containing hemoglobin and/or blood should not be used. Samples with visible turbidity should be centrifuged. Use only the supernatant to perform the assay.
See Young, S.D. in references for effect of drugs on the present method.
d) Stability and storage instructions: samples may be stored for 7 days refrigerated (2-10ºC) or for 2 months frozen (at -20ºC).

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Spectrophotometric square cuvettes
- Micropipettes and pipettes for measuring the stated volumes
- Kahn or hemolysis tubes
- Water bath at 37ºC
- Stopwatch

ASSAY CONDITIONS

- Wavelength: 340 nm
- Reaction temperature: 37ºC
- Reaction time: 10 minutes
- Sample volume: 70 ul
- Final reaction volume: 1.27 ml

Sample and reagent volumes may be proportionally changed without altering the calculation factors

PROCEDURE

CALIBRATION CURVE
In Kahn tubes, perform the following dilutions in saline solution of **Microalbúmina Calibrador Turbitest AA**: 1/1; 1/2; 1/4; 1/8 and 1/16, using saline solution as zero.

Diluted Microalbúmina Calibrador	70 ul
Reagent A	1000 ul

Homogenize and incubate for 5 minutes at 37ºC. Read each dilution absorbance at 340 nm (OD_1) taking the instrument to zero with distilled water. Then add:

Reagent B	200 ul

Homogenize and incubate for exactly 5 minutes at 37°C and immediately read the absorbance at 340 nm (OD_2) taking the instrument to zero with distilled water.
Calculate the difference in absorbance ($\Delta A = OD_2 - OD_1$) for each Calibrator dilution, including zero. Plot the difference in absorbance (ΔA) in graph paper against **Microalbúmina Calibrador** concentration in mg/l.

PROCEDURE FOR SAMPLES
In duly marked Kahn tubes, place:

Sample	70 ul
Reagent A	1000 ul

Homogenize and incubate for 5 minutes at 37°C. Read the absorbance at 340 nm (OD_1) taking the instrument to zero with distilled water. Then add:

Reagent B	200 ul

Homogenize and incubate for exactly 5 minutes at 37°C and immediately read the absorbance at 340 nm (OD_2) taking the instrument to zero with distilled water.

CALCULATIONS

1) Calculate the absorbance difference ($\Delta A = OD_2 - OD_1$) corresponding to each sample assayed. Interpolate this ΔA into the calibration curve to determine the MAlb concentration (mg/l) of the sample under study.
The samples with absorbances that are higher than the last calibration point should be diluted (1:2 ó 1:4) with saline solution and processed one more time. Multiply the obtained result by the dilution performed.

2) MAlb in urine (mg/24 hrs) = MAlb (mg/l) x V
where:
V = diuresis volume expressed in liters/24 hrs

3) To avoid the necessity to time urine collection the **MAlb/Creatinine Ratio** is used:

$$\text{MAlb/Creatinine (mg/g)} = 1000 \times \frac{\text{Microalbumin (mg/l)}}{\text{Creatinine (mg/l)}}$$

Being 1000 the Creatinine conversion factor from mg to g.

QUALITY CONTROL METHOD

Microalbúmina Control 2 niveles Turbitest AA.
The Controls are processed in the same way as the samples.

REFERENCE VALUES

Albumin urinary excretion

	mg/24 hrs	ug/min	mg/g Creatinine
Normal	< 30	< 20	< 30
Microalbuminuria	30-300	20-200	30-300
Clinical Albuminuria	> 300	> 200	> 300

It is recommended that each laboratory establish its own reference intervals, within its patient population. Microalbuminuria should always be reviewed with the patient's medical examinations, history and further laboratory results.

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
It is recommended to perform a complete recalibration when changing Reagent lot number or when suggested by Quality Control.
To avoid prozone problems in samples with antigen excess it is recommended that all samples be tested with test strips before the assay. The samples with protein levels higher than 250 mg/l should be diluted in saline solution so as to lie in the assay range. Avoid contamination to preserve the integrity of the reagents. Only use thoroughly clean and dry micropipettes for measurements.

PERFORMANCE

a) Reproducibility: evaluated by a modification of protocol EP5-A from CLSI. Thus, a control sample and samples with different microalbuminuria levels were tested. The intra-assay and total precision were calculated with the obtained data.

Intra-assay Precision

	Level	S.D.	C.V.
Normal	6.0 mg/l	± 0.17 mg/l	2.8 %
Pathological 1	30.8 mg/l	± 0.54 mg/l	1.8 %
Pathological 2	165.0 mg/l	± 0.70 mg/l	0.4 %
MAlb Control	50.2 mg/l	± 0.42 mg/l	0.8 %

Total Precision

	Level	S.D.	C.V.
Normal	6.0 mg/l	± 0.44 mg/l	7.4 %
Pathological 1	30.8 mg/l	± 1.00 mg/l	3.2 %
Pathological 2	165.0 mg/l	± 2.61 mg/l	1.6 %
MAlb Control	50.2 mg/l	± 0.82 mg/l	1.6 %

b) Detection limit: is the minimum analyte amount capable of being detected as a sample, different than zero, and corresponds to the concentration of 0.7 mg/l MAlb.
c) Assay range: corresponds to the exactly quantifiable interval of values and ranges from 4 mg/l to the last calibration point (250 mg/l approximately).
d) Prozone effect: not noted until 2700 mg/l MAlb.

The performance results were obtained using Konelab 60i autoanalyzer. Therefore, such values may differ whenever another autoanalyzer or manual technique is used.

PARAMETERS FOR AUTOANALYZERS

Refer to the applications that are specific for each autoanalyzer.

WIENER LAB PROVIDES

60 ml: 1 x 50 ml Reactivo A
1 x 10 ml Reactivo B
(Cód. 1513266)

60 ml: 1 x 50 ml Reactivo A
1 x 10 ml Reactivo B
(Cód. 1009338)

60 ml: 1 x 50 ml Reactivo A
1 x 10 ml Reactivo B
(Cód. 1009276)

60 ml: 1 x 50 ml Reactivo A
1 x 10 ml Reactivo B
(Cód. 1009656)

REFERENCES

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- Sacks et al. - Clin. Chem. 48: 436 (2002).
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- American Diabetes Association: Diabetic Nephropathy. Diabetes Care (Suppl 1) 26: S94 (2003).
- Tietz Textbook of Clinical Chemistry - Burtis, C.; Ashwood, E. (5º Edition) WB Saunders, 2001.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocol EP5-A, 1999.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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