AUDIT

CE

µALB-TURBI

Microalbumin-immunoturbidimetry

turbidimetry

Quantitative determination of microalbumin (µALB)
IVD

Store 2 - 8ºC.

PRINCIPLE OF THE METHOD

Microalbumin - immunoturbidimetry is a quantitative turbidimetric test for the measurement of microalbumin (µALB) in human urine.
particles coated with specific antibodies anti - human albumin are agglutinated when mixed with samples containing µALB. The agglutination causes an absorbance change, dependent upon the µALB contents of the patient sample that can be quantified by comparison from a calibrator of known µALB concentration.

CLINICAL SIGNIFICANCE

Microalbuminuria is at present defined as an excretion rate for albumin between 20 and 200 mg/L, which is already above normal values but still below the values seen in patients with "conventional" proteinuria.
Microalbuminuria is a marker of an increased risk of diabetic nephropathy as well as cardiovascular disease in patients with insulin-dependent diabetes mellitus as well as with non-insulin-dependent diabetes mellitus. More recently, microalbuminuria has been found to be associated with cardiovascular disease also in the non-diabetic population. In fact, microalbuminuria may show to be a risk factor of cardiovascular disease among otherwise apparently healthy people.

REAGENTS

Diluent (R1)	Glycine buffer 100 mmol/L, pH 10.0. Preservative.
Reagent (R2)	Particles coated goat IgG with anti -human albumin, pH 8.2. Preservative.

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 Microalbumin Calibrator .
The sensitivity of the assay and the target value of the calibrator have been standardized against the International Reference Material CRM 470/RPPHS.
Recalibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

STORAGE AND STABILITY

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

Reagent deterioration: Presence of particles and turbidity.

Do not freeze; frozen Reagent 2 or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter.

SAMPLES

24 hours or random/ first morning urine specimen. It is recommended to adjust the pH at 7.0 with NaOH/HCL 1 mol/L.Stable 7 days at 2-8ºC when sodium azide 1 g/L is added to prevent contamination.
Urine should be centrifuged before testing.

PROCEDURE

1.Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength: 340
   Temperature: 37ºC
   Cuvette ligth path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

	Blank	Calibrator	Sample
R 1 (µL)	800	800	800
Calibrator (µL)	--	20	--
Sample (µL)	--	--	20

5. Mix and incubate for 5 min at 37ºC.
6. Read the absorbance (A_1) of the samples and calibrator.
7. Add:

	Blank	Calibrator	Sample
R 2 (µL)	200	200	200

8. Mix and incubate for 5 min. at 37ºC.
9. Read the absorbance (A_2) of the samples and calibrator, against the Blank.
10. Calculate the increase of the absorbance $\Delta A = A_2 - A_1$

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

CALCULATIONS

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

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used the Audit Diagnostics Microalbumin Control.

REFERENCE VALUES

Normal values up to 30 mg/24 hrs urine specimen and 20 mg/L in a first morning urine specimen.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Linearity limit:** Up to 290 mg/L, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. **Detection limit:** Values less than 2 mg/L give non-reproducible results.
3. **Prozone effect:** No prozone effect was detected up to 1000 mg/L.
4. **Sensitivity:** Δ 3.8 mA. mg/L.
5. **Precision:** The reagent has been tested for 20 days, using three different microalbumin concentrations in a EP5-based study.

EP5	CV (%)		
	+/ 10.36 mg/L	+/ 16.95 mg/L	+/ 57.33 mg/L
Total	4.5%	3.1%	2.5%
Within Run	1.9%	1.4%	1.1%
Between Run	4.1%	2.7%	2.3%
Between Day	0.0%	0.0%	0.0%

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 49 samples of different concentrations of microalbumin were assayed. The correlation coefficient (r) was 0.99 and the regression equation y = 0.424x +10.55

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Glucose (2 g/L), hemoglobine (10 g/L) and creatinine (3 g/L), do not interfere. Urea (≥ 1 g/L) and bilirrubin (≥ 10 mg/dL), interfere. Other substances may interfere[6]

NOTES

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

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