**QUANTITATIVE DETERMINATION OF MICROALBUMIN (MALB)**

**PRINCIPLE OF THE METHOD**
Microalbumin-turbilatex is a quantitative turbidimetric test for the measurement of microalbumin (µALB) in human urine. Latex 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 in among otherwise apparently healthy people.

**REAGENTS**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
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<tbody>
<tr>
<td>Diluent (R1)</td>
<td>Glycine buffer 100 mmol/L, pH 10.0, Sodium azide 0.95 g/L.</td>
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<tr>
<td>Latex (R2)</td>
<td>Particles coated goat IgG with anti-human albumin, pH 8.2, Sodium azide 0.95 g/L.</td>
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<tr>
<td>µALB-CAL</td>
<td>Calibrator. Microalbumin concentration is stated on the vial label.</td>
</tr>
</tbody>
</table>

**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 Reference 1107072.

**PREPARATION**
Working reagent: Shake the latex vial gently before use. Prepare the necessary amount as follow:
1 mL Latex Reagent + 9 mL Diluent
Microalbumin Calibrator: Reconstitute → with 1.0 mL of distilled water. Mix gently and bring to room temperature for about 10 minutes before use.

**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.

**ADDITIONAL EQUIPMENT**
- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 540 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.

**PROCEDURE**
1. Bring the working reagent and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 540 nm (530-550)
   - Temperature: 37°C
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   - Working Reagent (mL)
   - Calibrator or sample (µL)

5. Mix and read the absorbance immediately (A1) and after 2 minutes (A2) of the sample addition.

**CALCULATIONS**

\[(A2-A1)_{sample} \times \text{Calibrator concentration} = \text{mg/L albumin}\]

**QUALITY CONTROL**
Control Sera are recommended to monitor the performance of manual and automated assay procedures.

**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 150 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:

<table>
<thead>
<tr>
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<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
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<tbody>
<tr>
<td>mean (mg/dl)</td>
<td>12.4 + 27.3</td>
<td>12.4 + 27.3</td>
</tr>
<tr>
<td>CAL</td>
<td>83.5</td>
<td>83.5</td>
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<tr>
<td>SD</td>
<td>0.28</td>
<td>0.28</td>
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<tr>
<td>CV</td>
<td>14.8</td>
<td>14.8</td>
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<tr>
<td></td>
<td>1.93</td>
<td>1.93</td>
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<tr>
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<td>2.28 Mean</td>
<td>2.28 Mean</td>
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<tr>
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<td>2.06</td>
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<tr>
<td></td>
<td>2.55</td>
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6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 95 samples ranging from 1 to 150 mg/L of microalbumin were assayed. The correlation coefficient (r) was 0.99 and the regression equation was y = 0.964x – 0.576. The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**
Glucose (2 g/L), hemoglobin (10 g/L) and creatinine (3 g/L), do not interfere. Urea (≥ 1 g/L) and bilirubin (≥ 10 mg/dL), interfere. Other substances may interfere.

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

**BIBLIOGRAPHY**