MICROALBUMIN

Turbidimetric Immunoassay

Reagent for quantitative determination of the excretion of albumin (MAL) in the human urine

REF 23010
R1 1 x 50 mL
R2 1 x 5 mL
R3 1 x 1 mL

REF 23011
R1 2 x 50 mL
R2 1 x 10 mL
R3 1 x 1 mL

TECHNICAL SUPPORT AND ORDERS
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Fax : (33) 03 23 25 256

CLINICAL SIGNIFICANCE (1) (2)
Diabetic nephropathy, which is accompanied by irreversible damage and persistent proteinuria, is a major cause of death in persons with insulin-dependent diabetes mellitus. An early sign of diabetic nephropathy are small albumin secretions in urine, i.e. Microalbumin. Therefore, detection of kidney (glomerular) damage that is minimal and reversible is important.

PRINCIPLE (1)
Photometric measurement of turbidity, corresponding to antigen-antibody reaction, by the end-point method at 340 nm.

REAGENTS

Vial R1
BUFFER (Concentration in the Test)
Saline Accelerator
Sodium Azide 0.95 g/L

Vial R2
ANTI-MAL (Concentration in the Test)
Phosphate buffered Saline
Polyclonal Anti-Human Albumin (Goat) (variable)
Sodium Azide 0.95 g/L

Vial R3
MAL Standard Super High (REF 23012)
Pooled liquid stabilized human plasma (Sodium azide 0.95 g/L).

The concentration of this standard is batch-specific (see Assigned value on the label of the vial)

REAGENTS PREPARATION
Liquid Reagents, ready for use.

SAFETY CAUTIONS
B I O L A B O reagents are designated for professional, in vitro diagnostic use.
• Use adequate protections (overall, gloves, glasses).
• Do not pipette by mouth.
• In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
• Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
• Material Safety Data Sheet is available upon request.
• Waste disposal: Respect legislation in force in the country.

All specimens, standards and controls should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

STABILITY AND STORAGE
Store at 2-8°C, away from light (Do not freeze).
• Unopened reagents are stable until expiry date stated on the label.
• Once opened, when free from contamination, reagents R1 and R2 are stable at least for 3 months at 2-8°C, 24 h at room temperature and 30 days on the board of analyser.
• Once opened, when free from contamination:
  Well recapped and stored in the original vial, standard (vial R3) is stable at least for 6 weeks.

SPECIMEN COLLECTION AND PREPARATION (1) (3)
24h Urines or 3 random urine samples collected over the course of 1 week (minimizes intraindividual variation).
If the test cannot be carried out on the same day, the urine may be stored at room temperature for 2 days and at 2-8°C for maximum 14 days.

It is recommended to use centrifuged urines.

INTERFERENCES (7)
Results of tests measured on a clinical chemistry analyser COBAS MIRA:

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration (If applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>Do not interfere up to 4300 mg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Do not interfere up to 560 mg/dL</td>
</tr>
<tr>
<td>Calcium</td>
<td>Do not interfere up to 30 mmol/L</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Do not interfere up to 31.8 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Do not interfere up to 375 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Do not interfere up to 32.6 mg/dL</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Do not interfere up to 0.280 abs</td>
</tr>
<tr>
<td>Glucose</td>
<td>Do not interfere up to 1000 mg/dL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Do not interfere up to 25 mg/dL</td>
</tr>
<tr>
<td>Chlorides</td>
<td>Do not interfere up to 1400 mg/dL</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Do not interfere up to 98 mg/dL</td>
</tr>
</tbody>
</table>

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED
1. Saline (0.9 %)
2. Standards and Controls.

CALIBRATION
• Vial R3 enclosed in the kit or Standard REF 23012
• Or Standard Set REF 23013 traceable to Reference Material (RPPHS/CRM470) from IFCC

Use as indicated in the insert (§ MANUAL PROCEDURE) to generate a reference curve.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. If control values are out of range, even after using a new vial of serum.
QUALITY CONTROL

- BIOLABO Control REF 23014
- Assayed control referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, use a new vial of control and repeat the test.
3. If control is still out of range, use a new vial of calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (4) (5) (6)

2nd morning urines (4):
- Adults: < 20 mg Albumin/g Creatinine or < 2,26 g (34,35 µmol) Albumin/mol Creatinine
- Childs (3 to 5 years old) (5):
  - < 20 mg/L (0,304 µmol/L) Albumin or < 37 mg Albumin/g Creatinine

24 h Urines (6):
- < 20 mg/L (0,304 µmol/L)
- < 30 mg/24h (0,456 µmol/24h)

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

The performance studies for MAL reagents were realised on a clinical chemistry analyzer (COBAS MIRA).

<table>
<thead>
<tr>
<th></th>
<th>Low level</th>
<th>Normal level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within run</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean mg/L</td>
<td>22.3</td>
<td>48.6</td>
<td>98.1</td>
</tr>
<tr>
<td>S.D. mg/L</td>
<td>0.76</td>
<td>1.49</td>
<td>2.47</td>
</tr>
<tr>
<td>C.V. %</td>
<td>3.4</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Expected C.V. %</td>
<td>&lt; 6%</td>
<td>&lt; 4.5%</td>
<td>&lt; 4.5%</td>
</tr>
</tbody>
</table>

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<tr>
<td><strong>Between run</strong></td>
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</tr>
<tr>
<td>N = 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean mg/L</td>
<td>23.5</td>
<td>48.5</td>
<td>100.3</td>
</tr>
<tr>
<td>S.D. mg/L</td>
<td>1.30</td>
<td>2.72</td>
<td>3.78</td>
</tr>
<tr>
<td>C.V. %</td>
<td>5.5</td>
<td>5.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Expected C.V. %</td>
<td>&lt; 8%</td>
<td>&lt; 6%</td>
<td>&lt; 6%</td>
</tr>
</tbody>
</table>

Detection limit: approx. 2.2 mg/L
Sensitivity: approx. 0.420 abs for 200 mg/L
Specificity: Monospecific
Prozone effect: tested up to 6000 mg/L, no effect found within the measuring range (approx. to 0.728 ΔA)
Comparison with a commercially available reagent (same method): using 100 specimens between 2.2 and 400 mg/L
y = 0.9961 x + 1.5992
r = 0.9974

LINEARITY

The assay is linear between 2.2 and 400 mg/L.
Above 400 mg/L, dilute the specimen with saline and re-assay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Standard curve:
Generate a Standard curve by successive 1:2 dilutions of Standard enclosed in the kit (vial R3) or Standard High REF 23012 in saline (5 different levels are recommended), or use the ready for use Standard Set REF 23013.
Use saline as sample to determine zero point

Test:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standards</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (Vial R1)</td>
<td>900 µL</td>
<td>900 µL</td>
<td>900 µL</td>
</tr>
<tr>
<td>Saline</td>
<td>60 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standards</td>
<td>60 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>60 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mx well. Read absorbances (Abs A1) of standards, controls and assays at 340 nm against Blank.

Mx well. Incubate for exactly 5 minutes at room temperature. Read absorbances (Abs A2) of standards, controls and assays at 340 nm against Blank.

Note:
Application procedures on clinical chemistry analyzers are available upon request.

CALCULATION

Calculate the result as follows:

Calculate ΔAbs (Abs A2 – Abs A1) for standards, controls and assays.
Plot a Standard Curve “Concentration = f(ΔAbs)”.
Read the concentration of controls and samples on the graph.

REFERENCES

(5) Hubbuch A. Results of a multicenter study of provisional reference ranges for albumin in urine of children and adults. Publication de Roche