Standardization of urine albumin measurement and reporting
Microalbumin Background:

- What is it?
- Why is it measured?
- How is it measured
  - Standardization: Why and How?
  - Current status of reporting microalbumin
  - Improving measurement of microalbumin
WHAT IS IT?

- Native albumin in serum:
  - Is a 585 AA protein
  - Has MW 66473 Da
  - Has 17 disulfide bond
  - Binds multiple ligands
  - There are C-terminal & N-terminal domain
  - Significant glycation (1-10%), higher in diabetes
  - Conformation changes influence filtration
  - Tubular uptake is receptor mediated
Proteinuria is the principal marker of kidney damage.

Albuminuria is the first evidence of renal dysfunction and an indication of diseases progression.
WHY IS IT MEASURED?

Readily available, accurate and specific
Reasonable cost
Minimally invasive
Familiar to all physicians and laboratorian
Have well defined reference ranges
Provide diagnostic and prognostic value
HOW IS IT MEASURED?

- Immunoassays
  - Primarily nephelometric and turbidimetric procedures
  - Influenced by:
    - Epitope(s) recognized by the antibodies
    - Ab reactivity with modified forms of albumin
  - Polyclonal assays are reactive with some modified albumin forms
HOW IS IT MEASURED?

- HPLC assays (size exclusion)
  - Does not resolve albumin from other co-eluting urine proteins causing overestimation
  - Hypothesis of “non-immunoreactive albumin” likely related to non-specificity of HPLC
### Review of assays to assess albuminuria

<table>
<thead>
<tr>
<th>Method</th>
<th>CV</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunonephelometry, Beckman</td>
<td>4.2% at 12.1 mg/L</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Immunoturbidimetry (Dade Behring)</td>
<td>5.3% at 45 mg/L</td>
<td>6 mg/L</td>
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<tr>
<td>Hemocue (point of care)</td>
<td>4.1% at 10.6 mg/L</td>
<td>6 mg/L</td>
</tr>
<tr>
<td>Radioimmunoassay</td>
<td>4.3% at 82 mg/L</td>
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</tbody>
</table>
ALBUMIN SPECIES IN URINE

- ALBUMIN IN URINE EXPOSED TO:
- Wider range of PH
- Ionic strength
- High concentration of urea, glucose and ascorbate
- Cleavage by peptidases
Albumin in urine is heterogeneous

- Large and small fragments exist in plasma and urine
- C- and N-terminal truncation occurs
- Tubular uptake is receptor mediated – influences enrichment of modified plasma forms in urine (e.g. glycated)
- Many ligands are concentrated in urine and bind to albumin
- Proteolytic degradation and chemical modifications may occur in tubules, bladder and urine after collection
Results reporting

- A variety of reporting systems:
  - Albumin concentration (e.g. mg/L)
  - Albumin excretion rate (AER, mg/24 h)
  - Albumin/creatinine ratio (ACR)
    - SI (molar) and non-SI units
    - mg/mmol
    - mg/g

- A variety of decision points with different numbers
SPECIMEN COLLECTION

- TIMED
- 24 - hour
- 12 – hour
- Spot urine
- Collection container
Specimen Handling

- Stable one week at 4 – 20c
- Storage at -20c causes fragmentation and 40% loss
- Samples should ideally be analysed fresh
- For prolonged storage, freeze at -80c
- Creatinine is also stable at 4 – 20c and -20 - -80c
- Inspect urine for clarity prior to analysis, centrifuge if cloudy
Recommendations:

- Albumin concentration (mg/L) is difficult to interpret and should not be reported alone.

- Albumin/Creatinine ratio should always be reported:
  - “mg/mmol” or “mg/g” should be used.
  - Problem for dipsticks.
Recommendations: urine albumin under development (1)

- Develop a reference method (LC-MS)
- Develop reference standard materials
- Clarify adsorption to containers
- Clarify biological variability
- Clarify molecular forms to measure
- Clarify current immunoassay performance
Recommendations(2)

• The term ‘urine albumin’ should be used rather than ‘micro-albumin’.
• Patients should be well at baseline. They should have no urinary tract infection, no acute febrile illness, no intense exercise within the previous 24 hours and not to be menstruating, no hematuria, nephrotoxic drugs.
• Recommended urine collection is a fresh, first morning void. A minimum of 5 ml should be collected.
• If a first morning void is not practicable, random spot samples are acceptable.
• Urine creatinine must also be measured.
• Samples not able to be delivered to the laboratory within 8 hours, should be refrigerated.
• Analysis should be performed on the day of receipt but samples can be stored for up to 7 days at 2-8 °C if necessary.
• Cloudy or particulate samples should be centrifuged prior to analysis.
• Positive ACR results must be confirmed, ideally on a fresh, first morning void, by repeat measurement on 1-2 occasions within 3 months
• Prolonged storage should be at -70 °C; samples should not be stored at -20 °C.
- Uncontrolled (i.e., malignant) hypertension
- Heart failure
- Pronounced hyperglycemia (>450 mg/dL), even if short-term

- Frozen urine specimens should be thawed only once prior to microalbumin testing (i.e., avoid repeated freeze-thaw cycles).
Microalbumin (MicroAlb) in the Diagnosis of Nephropathy of DM patients

Indications for Test

Semiquantitative and quantitative microalbumin determination in a timed (i.e., minimum of 12- up to 24-hour) urine collection is indicated in:

- All patients with DM initially upon achieving glycemic control (i.e., within 3 months of diagnosis) and capable of accurately and completely collecting a timed urine specimen
- The identification of patients with incipient DM-related chronic kidney disease (CKD) and differentially diagnosing those with microalbuminuria vs. those with macroalbuminuria
• Monitoring DM patients on a yearly basis, or more often (i.e., up to every 2 months), when adjusting therapy to reduce albuminuria of any degree
• DM patients with autonomic neuropathy suggested by symptoms such as tachycardia at rest, gustatory sweating, erectile dysfunction, or chronic diarrhea.
Procedure

1. Be sure that none of the following factors, known to give a false-positive test, is present:
   - Strenuous exercise within the previous 72 hours
   - Urinary tract infection
   - Acute febrile illness
   - Uncontrolled (i.e., malignant) hypertension
   - Heart failure
2. Instruct the patient to collect either random ("spot") or timed (minimum of 12-up to 24-hour) urine specimens. All urine samples must be refrigerated after collection or frozen, if testing will not be performed promptly.

3. Proceed with one or more of the following three methods for detecting microalbuminuria:
Method 1. Quantitative Microalbumin on a Random (“Spot”) Urine Sample

- Obtain a random (spot) urine specimen for quantitative microalbumin and creatinine determination.
- Request urine microalbumin and creatinine testing so the microalbumin (mcg)/ creatinine (mg) ratio can be calculated by laboratory personnel.
- If possible, confirm abnormal results for the microalbumin/creatinine ratio by Method 2.

Method 2. Quantitative Microalbumin on a “Timed” Urine Specimen

- Obtain a 12-to 24-hour urine collection for microalbumin and creatinine determination.
- The laboratory performing the microalbumin testing should report both the microalbumin concentration (mg/L) in the urine sample and microalbumin excretion rate (MAER) in mg/day, better report mg/gr creatinin.
For a 12-hour urine collection, be sure that the laboratory provides the MAER in mcg/min.

High-performance liquid chromatography (HPLC) measures total albumin, including immunoreactive and non-immunoreactive forms, and may allow better early detection of incipient diabetic nephropathy. Inform physican what detection method is performed,(overstimation of albumin).
**Method 3.** Semiquantitative Microalbumin on a Random (“Spot”) Urine

- Obtain a random urine specimen.
- Test the urine for microalbumin using a commercially available, semiquantitative dipstick method (e.g., Micral®).
- To ensure reliable results when using dipstick microalbumin methods, follow the manufacturer’s instructions precisely.
- Confirm abnormal results by Method 1 or, preferably, by Method 2.
**Interpretation**

1. Refer to “Albumin Concentration or Albumin Excretion Rate in the Assessment of Diabetic Nephropathy” (Table 1). Note that the ranges presented differ from and are lower than those identified by the American Diabetes Association (ADA, 2004).

2. Other causes of renal disease can occur in DM and should be suspected if:
   - Hematuria is present.
   - Azotemia occurs in the absence of proteinuria.
   - Nephrotic-range proteinuria is found in patients with relatively new-onset DM.
   - There is evidence of urinary tract obstruction and/or infection.
- Retinopathy is absent (as nephropathy of DM is usually seen in the context of concurrent retinopathy).
- The patient is taking drugs known to be nephrotoxic.
- The patient has malignant hypertension.

3. Persistently overt proteinuria or macroproteinuria (>500 mg/day) is irreversible and heralds the inevitable progression to end-stage renal disease (CKD Stage 5).

4. A cutoff value of 17 to 20 mg/L in a random urine specimen has a sensitivity of 100% and a specificity of 80% or better for the diagnosis of microalbuminuria when compared to a 24-hour timed urine collection as a reference standard.

5. Because of the known day-to-day variability in urinary albumin excretion, abnormal microalbumin tests by Methods 1 or 2 require confirmation by repeat testing.

6. An abnormal microalbumin test by Method 3 is not diagnostic and requires confirmation.
# TABLE 1

**Allbumin Concentration or Albumin Excretion Rate in the Assessment of Diabetic Nephropathy**

<table>
<thead>
<tr>
<th>Type of Urine Specimen</th>
<th>Normal</th>
<th>Incipient</th>
<th>overt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timed(24-hour)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;25 mg/day</td>
<td>25-250 mg/day</td>
<td>&gt;250 mg/day</td>
</tr>
<tr>
<td></td>
<td>&lt;20 mcg/minute</td>
<td>20-200 mcg/minute</td>
<td>&gt;200 mcg/minute</td>
</tr>
<tr>
<td>Random,semiquantitative (dipstick,such as Micral-Test&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>0 mg/L&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50-100 mg/L</td>
<td>≥300 mg/L</td>
</tr>
<tr>
<td>Random,quantitative</td>
<td>&lt;20 mcg/mg creatinine</td>
<td>20-250 mcg/mg creatinine</td>
<td>&gt;250 mcg/mg creatinine</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Reference gold standard.

<sup>b</sup> 10-20 mg/L is indeterminate.
Notes

1. Collecting a 12-hour overnight urine specimen is more convenient for most patients and has been shown to be about as accurate as a 24-hour collection.

2. Conventional immunochemical-based assays for microalbuminuria may not detect an unreactive fraction of albumin in urine resulting in an underestimate of protein excreted. High-performance liquid chromatography may allow early detection of incipient nephropathy by measuring all urinary albumins present.

3. Normal urinary microalbumin excretion is approximately 10 mg/day (range, 2.5 to 25 mg/d).

4. Microalbumin excretion must be ~ 500 mg/day or more before the qualitative urine dipstick test for microalbumin becomes positive.
5. CKD Stage 3 or incipient nephropathy (MAER= 25 to 250 mg/day) may antedate the onset of overt proteinuria (>250 mg/day) by 5 years and the onset of azotemia by 10 years.

6. The risk of microalbuminuria in patients with TIDM increases abruptly above a hemoglobin A1c value of 8.1%.

7. Detection of diabetic nephropathy at an earlier stage (i.e., incipient nephropathy) allows for earlier intervention utilizing strict glycemic control or blood pressure normalization (i.e., BP <120/<80 mmHg), often with modest dietary protein restriction.

8. Gustatory sweating was found to occur in 69% of patients with nephropathy of DM, 36% with neuropathy of DM, 5% in DM controls, and 5% in non-DM renal failure patients.
9. Proteinuria occurs in 15 to 40% of TIDM patients, with a peak incidence around 15 to 20 years of DM. In T2DM patients, the prevalence is highly variable, depending on population studied, ranging from 5% to 20%.

10. Diabetic nephropathy is more prevalent among African-Americans, Asians, and Native Americans than Caucasians.

11. In 140 TIDM patients with persistent microalbuminuria (20-200 mcg/min), 11% of patients on 1.25 mg ramipril, an angiotensin-converting enzyme (ACE) inhibitor, regressed to normoalbuminuria (<20 mcg/min), while 20% regressed on 5 mg ramipril, and 4% regressed on placebo (P=0.053) (O'Hare et al., 2000).

12. Combination therapy with medications from both the ACE inhibitor and angiotensin receptor blocker classes of agents work synergistically to reduce proteinuria.
Fractional Excretion of Sodium (FENa)

Indications for Test

Determination of the FENa is indicated:

- In DM patients with indeterminate or no known renal dysfunction whose intravascular volume status (high or low) is clinically difficult to ascertain, particularly in the context of hyperglycemia.

- To discriminate between the prerenal azotemia and oliguric phase of ATN in the context of hyperglycemic crisis.
Procedure
1. If possible, discontinue patient’s diuretic therapy for more than 24 hours.
2. Simultaneously obtain a random urine and blood sample.
3. Request glucose, sodium (Na), chloride (Cl), and creatinine testing on the blood sample and Na, Cl, and creatinine on the urine specimen.
4. Calculate FENa (%):
   \[
   \left( \frac{U_{Na}}{S_{Na}} \right) \times 100 \\
   \left( \frac{U_{Cr}}{S_{Cr}} \right)
   \]
   Where \( U_{Na}, S_{Na}, U_{Cr}, \) and \( S_{Cr} \) are the urine sodium, serum sodium, urine creatinine, and serum creatinine concentrations, respectively.
5. In extremely hyperglycemic, hyperosmolar states (e.g., serum glucose concentration > 600 mg/dL), most likely associated with prerenal azotemia, obtain serum (\( S_{Osm} \)) and urine (\( U_{Osm} \)) osmolality and a urine microscopic analysis for detection of casts in urinary sediment in lieu of FENa testing.
**TABLE 5.4**

Laboratory Tests Used to differentiate Prerenal Azotemia from Acute Tubular Necrosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Prerenal Azotemia</th>
<th>Acute Tubular Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNa (mEq/L)</td>
<td>&lt;20</td>
<td>&gt;40</td>
</tr>
<tr>
<td>UCr/SCr ratio</td>
<td>&gt;40:1</td>
<td>&lt;20:1</td>
</tr>
<tr>
<td>UOsm</td>
<td>100 mOsm &gt; SOsm</td>
<td>&lt; SOsm</td>
</tr>
<tr>
<td>Urinary sediment</td>
<td>Normal</td>
<td>Casts, cellular debris present</td>
</tr>
<tr>
<td>FENa</td>
<td>&lt;1%</td>
<td>&gt;2%</td>
</tr>
</tbody>
</table>

Note: UNa, urinary sodium (Na) concentration; UCr/SCr urine creatinine/serum creatinine ratio; UOsm, urine osmolality; SOsm, serum osmolality; FENa, fractional excretion of sodium = [(UNa/SNa)/(UCr/SCr)]x 100.
Interpretation
1. See “Laboratory Tests Used to Differentiate Prerenal Azotemia from Acute Tubular Necrosis” (Table 5.4).
2. FENa values:
   - 1-2% is typical in healthy individuals free of renal disease.
   - <1% indicates prerenal azotemia. Note that iron saturation (FeS) values < 1% can occur in acute renal failure from myoglobinuria or hemoglobinuria, iodinated radiocontrast nephropathy, hepatorenal syndrome, renal allograft rejection, burns, sepsis, urinary tract obstruction, acute glomerulonephritis, and drug-related alterations in renal hemodynamics. Occasionally, patients with oliguric and nonoliguric ATN will have an FeS<1%.
   - >2% indicates sodium leak associated with CKD Stages 3 to 5 and chronic or acute tubular damage (i.e., ATN).
3. Abnormal renal and adrenal function and the use of diuretics will interfere with assessments of intravascular volume made using urine Na and Cl concentrations.

4. Urine osmolality can be used to assess action of antidiuretic hormone (see Test 2.11.1) and to determine the etiology of polyuria or hypernatremia when diabetes insipidus is suspected.
Notes

1. The results of the FENa test in the differential diagnosis of acute renal failure must be interpreted in conjunction with the patient’s clinical course and the use of other urine and serum tests such as urine output and serum albumin and creatinine concentrations.

2. There are no “normal values” for urine electrolyte concentrations and osmolality, only “expected values” relative to specific clinical situations.