BioVendor Research and Diagnostic Products releases its new Uromodulin (UMOD, Tamm–Horsfall Protein, THP) ELISA. The Uromodulin ELISA kit has been optimized and validated for the quantitative determination of uromodulin in serum, urine and plasma samples.
Uromodulin (Tamm–Horsfall protein) is the most abundant protein excreted in the urine under physiological conditions. It is exclusively produced in the kidney and secreted into the urine via proteolytic cleavage. Its biological function is still not fully understood. Uromodulin has been linked to water/electrolyte balance and to kidney innate immunity. Also, studies in knockout mice demonstrated that it has a protective role against urinary tract infections and renal stone formation. Mutations in the gene encoding uromodulin lead to rare autosomal dominant diseases, collectively referred to as uromodulin-associated kidney diseases. These are characterized by progressive tubulointerstitial damage, impaired urinary concentrating ability, hyperuricemia, renal cysts, and progressive renal failure. Novel in vivo studies point at intracellular accumulation of mutant uromodulin as a key primary event in the disease pathogenesis. Recently, genome-wide association studies identified uromodulin as a risk factor for chronic kidney disease (CKD) and hypertension, and suggested that the level of uromodulin in the urine could represent a useful biomarker for the development of CKD.

Chronic kidney disease comprises a group of pathologies in which the renal excretory function is chronically compromised, mainly resulting from damage to renal structures. Most, but not all, forms of CKD are irreversible and progressive. CKD results from a variety of causes such as diabetes, hypertension, nephritis, inflammatory and infiltrative diseases, renal and systemic polycystic kidney disease, autoimmune diseases, renal hypoxia, trauma, nephrolithiasis and obstruction of the lower urinary ways, chemical toxicity and others.

There is no optimal diagnostic marker for renal function assessment in current nephrology. Cystatin C or creatinine quantifications allow for the glomerular filtration rate (GFR) estimation, and are limited to later forms of nephropathy.

The diagnostic efficiency of various urine protein determinations (protein/creatinine, albumin/creatinine, α-1-microglobulin, etc.) is often insufficient and the results can be confusing.

Novel urine markers such as NGAL and KIM-1 appeared recently but a serum screening marker—robust, sensitive and specific—is still urgently needed.

Therefore, a parameter allowing early nephropathy diagnosis and/or its etiology assessment by a single determination/measurement is sought extensively.
A Pilot Study Revealed Serum Uromodulin as a Unique Marker of the Presence of Nephropathy

Serum and urine specimen collection
Serum and urine samples collected from 112 nephropathy patients, and 15 age- and sex-matched healthy persons (66 males, 61 females) were obtained from the Nephrology Department. Prostejov Hospital, Prostejov, Czech Republic.

Diagnosis
Nephropathy (diabetic nephropathy, pyelonephritis, chronic renal insufficiency, interstitial nephropathy) was diagnosed by a nephrology specialist based on the incidence of at least three of the following: High serum creatinine, GF < 1 ml/s (MDRD or cystatin C determination), high serum cystatin C, high urine albumin/creatinine index, and high urine α-1-microglobulin.

Uromodulin determination
The uromodulin concentration in serum or urine samples was determined in the Prostejov Hospital laboratory using the BioVendor Human Uromodulin ELISA kit (RD191163200R) according to the manufacturer’s instructions.

Results

Urine Uromodulin
- Nephropathy patients had significantly lower values than healthy persons (4 860 vs. 11 165 ng/mL, p<0.01)
- BUT the values of nephropathy patients and healthy controls overlapped extensively

Serum Uromodulin
- Nephropathy patients had SIGNIFICANTLY LOWER VALUES than healthy persons (70.6 vs. 241 ng/mL, p<0.01)
- MINIMUM OVERLAP of healthy controls and nephropathy patients
Serum Uromodulin

The ROC analysis showed excellent characteristics of the serum UMOD determination for nephropathy diagnosis.

- **SENSITIVITY:** 92.1%
- **SPECIFICITY:** 100.0%
- **CUT-OFF:** 125.4 ng/mL
- **AUC:** 99% (nephropathy) for serum UMOD > 125.4 ng/mL

A Small Scale Pilot Study Confirming the Initial Discovery of the Serum Uromodulin Diagnostic Utility

**Serum and urine specimen collection**
Serum samples collected from 7 nephropathy patients and healthy sex- and age-matched controls were obtained from the Charles University, Prague, Czech Republic.

**Diagnosis**
Nephropathy (caused by ANCA-positive vasculitis) was identified by a nephrology specialist.

**Uromodulin determination**
The uromodulin concentrations in serum or urine samples were determined in the Charles University Laboratory using the BioVendor Human Uromodulin ELISA kit (RD191163200R) according to the manufacturer's instructions.

**Results**

**Serum Uromodulin**

- Nephropathy patients had **SIGNIFICANTLY LOWER VALUES** than healthy persons
- **NO OVERLAP** of the healthy person and pathology patient values
The BioVendor Human Uromodulin ELISA kit is suitable for the determination of uromodulin in serum, urine and plasma. Both urine and serum values are significantly lower in nephropathy patients compared with healthy persons. The serum uromodulin determination exhibited superior characteristics required for a nephropathy marker that might be used for screening purposes.

### Intended use
The RD191163200R Human Uromodulin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human uromodulin.

- The total assay time is less than 3.5 hours
- The kit measures uromodulin in serum, plasma (EDTA, citrate, heparin) and urine
- Assay format is 96 wells
- Standard is native protein based
- Quality Controls are human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized

### Clinical application
- Urolithiasis
- Urinary tract infections
- Nephropathies

### Test principle
In the Biovendor Human Uromodulin ELISA, standards, quality controls and samples are incubated in microtitation wells pre-coated with polyclonal anti-human uromodulin antibody. After a 60 minute incubation followed by washing, biotin labelled polyclonal anti-human uromodulin antibody is added and incubated with the captured uromodulin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of uromodulin. A standard curve is constructed by plotting absorbance values against uromodulin concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

### BioVendor Human Uromodulin ELISA (RD191163200R)

<table>
<thead>
<tr>
<th>HUMAN UROMODULIN ELISA</th>
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<tbody>
<tr>
<td>CAT. NO.: RD191163200R</td>
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<tr>
<td>Assay format</td>
</tr>
<tr>
<td>Samples</td>
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<tr>
<td>Controls</td>
</tr>
<tr>
<td>Standards</td>
</tr>
<tr>
<td>Limit of detection</td>
</tr>
<tr>
<td>Cross-reactivity</td>
</tr>
</tbody>
</table>

### Absorbance at 450 nm

![Graph showing absorbance at 450 nm vs. human uromodulin concentration](graph.png)
**Summary of protocol**

- Reconstitute Master Standard and prepare set of Standards
- Reconstitute QCs, dilute samples serum and plasma (50×), urine (2000×)
- Add 100 µl Standards, QCs and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Biotin Labelled antibody
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

---

**Precision**

Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>314.98</td>
<td>8.93</td>
<td>2.8</td>
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<tr>
<td>2</td>
<td>136.08</td>
<td>1.68</td>
<td>1.2</td>
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</table>

Inter-assay (Run-to-Run) (n=6)

<table>
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<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
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</thead>
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<tr>
<td>1</td>
<td>166.07</td>
<td>8.59</td>
<td>5.2</td>
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<tr>
<td>2</td>
<td>433.47</td>
<td>32.76</td>
<td>7.6</td>
</tr>
</tbody>
</table>

---

**Spiking recovery**

Serum samples were spiked with different amounts of human uromodulin and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>480.60</td>
<td>494.45</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>282.95</td>
<td>294.45</td>
<td>96.1</td>
</tr>
<tr>
<td></td>
<td>187.90</td>
<td>194.45</td>
<td>96.6</td>
</tr>
<tr>
<td>2</td>
<td>104.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>506.40</td>
<td>504.35</td>
<td>100.4</td>
</tr>
<tr>
<td></td>
<td>281.20</td>
<td>304.35</td>
<td>92.4</td>
</tr>
<tr>
<td></td>
<td>186.55</td>
<td>204.35</td>
<td>91.3</td>
</tr>
</tbody>
</table>

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**Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>437.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>215.25</td>
<td>218.78</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>110.70</td>
<td>109.39</td>
<td>101.2</td>
</tr>
<tr>
<td></td>
<td>54.60</td>
<td>54.69</td>
<td>99.8</td>
</tr>
<tr>
<td>2</td>
<td>423.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2×</td>
<td>212.05</td>
<td>211.50</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>108.10</td>
<td>105.75</td>
<td>102.2</td>
</tr>
<tr>
<td></td>
<td>51.65</td>
<td>52.88</td>
<td>97.7</td>
</tr>
</tbody>
</table>

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**Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.

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**Summary of protocol**

- Reconstitute Master Standard and prepare set of Standards
- Reconstitute QCs, dilute samples serum and plasma (50×), urine (2000×)
- Add 100 µl Standards, QCs and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Biotin Labelled antibody
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results
Preliminary Population and Clinical Data

The following results were obtained when serum samples from 105 unselected donors (58 men + 47 women) 22-65 years old were assayed with the Biovendor Human Uromodulin ELISA in our laboratory.

Age and Sex dependent distribution of uromodulin

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Mean Uromodulin (ng/ml)</th>
<th>SD Uromodulin (ng/ml)</th>
<th>Min. Uromodulin (ng/ml)</th>
<th>Max. Uromodulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>23-29</td>
<td>10</td>
<td>233.29</td>
<td>76.82</td>
<td>80.95</td>
<td>330.50</td>
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<tr>
<td></td>
<td>30-39</td>
<td>19</td>
<td>197.48</td>
<td>86.56</td>
<td>62.65</td>
<td>382.65</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>22</td>
<td>203.56</td>
<td>79.20</td>
<td>37.30</td>
<td>393.85</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>7</td>
<td>184.34</td>
<td>31.92</td>
<td>139.50</td>
<td>229.75</td>
</tr>
<tr>
<td>Women</td>
<td>22-29</td>
<td>9</td>
<td>215.11</td>
<td>77.84</td>
<td>123.00</td>
<td>406.70</td>
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<tr>
<td></td>
<td>30-39</td>
<td>14</td>
<td>269.14</td>
<td>110.61</td>
<td>92.90</td>
<td>501.15</td>
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<tr>
<td></td>
<td>40-49</td>
<td>17</td>
<td>220.60</td>
<td>56.53</td>
<td>107.70</td>
<td>312.05</td>
</tr>
<tr>
<td></td>
<td>50-61</td>
<td>5</td>
<td>206.24</td>
<td>73.34</td>
<td>88.20</td>
<td>282.25</td>
</tr>
</tbody>
</table>

Preliminary Population and Clinical Data

A SCREENING MARKER OF NEPHROPATHY

Related products

- RA05880R Human/Rat Angiotensin II ELISA
- RAG006R Human Angiotensin-Converting Enzyme 2 ELISA (ACE2)
- RSCPRH111R Rat beta2-Microglobulin ELISA
- RD194034200R Human Clusterin ELISA (ApoJ)
- RD491034200R Canine Clusterin ELISA (ApoJ)
- RD391034200CS Rat Clusterin ELISA (ApoJ)
- RD191009100 Human Cystatin C ELISA
- RD291009200R Mouse Cystatin C ELISA
- RD391009200R Rat Cystatin C ELISA
- RD191037100 Human Fetuin-A ELISA (AHSG)
- RHBA001R Human KIM-1 ELISA
- RRBA001R Rat KIM-1 ELISA
- RD191102200R Human Lipocalin 2/ NGAL ELISA
- RD181163100 Uromodulin Human, Rabbit Polyclonal Antibody
- RD184163100 Uromodulin Human, Sheep Polyclonal Antibody
- RMI001R Human Prorenin ELISA
- RIS004R Human Renin ELISA
- RD19113100R Human Prostaglandin D Synthase (Lipocalin-type) ELISA
- RD19116200R Human sRAGE ELISA
- RD191160200R Human Trefoil Factor 3 ELISA (TFF3)
- RD463163100 Canine Uromodulin (Canine urine isolated protein)
- RD663163050 Feline Uromodulin (Feline urine isolated protein)
- RD172163100 Human Uromodulin (Human urine isolated protein)
- RD563163100 Porcine Uromodulin (Porcine urine isolated protein)