**Introduction**

Exposure to indoor allergens is the most relevant environmental factor responsible for the development of allergic diseases. Rat (Rattus norvegicus) and domestic mouse (Mus musculus) allergens have been linked with asthma in several major urban centers, in some cases accounting for 95% of the indoor allergens present. Mouse allergens are responsible for the development of allergic diseases. Rat (Rattus norvegicus) and domestic mouse (Mus musculus) allergies are also common in the middle class and in laboratory animal workers. While both domestic mouse and rat urine samples are used in forensic environments, data on the clinical relevance of allergens derived from these rodents has not been studied thoroughly. The purpose of this study was to optimize an in-house ELISA to measure Mus m 1 and MUP in urine samples.

**Materials and Methods (I)**

**Preparation of In-House Reagents**

Urine Samples
- Collection of male domestic mouse, wild mouse, and rat urine samples.
- Dialysis of urine samples against distilled water for 48 hours at 4 - 8°C using a 3.5 K cutoff.

Protein Determination and Biotinylation of a Rabbit Polyclonal Antibody to MUP
- Immunoassay of two New Zealand rabbits with domestic mouse urine.
- Serum collection, pooling, and precipitation with 50% ammonium sulfate.
- Incubation of goat anti-rabbit IgG conjugated with AP (Chemicon, Temecula, CA).
- Electroblotting conditions: 150 mA for 3 hours.

**Comparison of the In-House and Commercial Antisera**

Western-blot of Domestic Mouse Urine: Immunoblotting for 3 hours.
- Membrane: PVDF, 0.2 µm pore size.
- Incubation of the in-house anti-MUg or commercial anti-MUg for 1 hour at 37°C.
- Incubation of goat (anti-rabbit) IgG conjugated with AP (Chemicon, Temecula, CA).

**Comparison of the following two assay conditions:**
- In-house Commercial

**Materials and Methods (II)**

**Assays Specificity**

Testing of various commercial and in-house mouse urine assay to measure MUP and commercial assay to measure Mus m 1.

**Testing of cross-reactivity:**
- Rat urinary extracts, mouse urinary extracts, and control sera.

**Results**

**Observed MUP levels in urine samples using the in-house and commercial ELISA:**
- Domestic Mouse: 1,843,000 ng/mL.
- Wild Mouse: 115,000 ng/mL.
- Rat: 168,000 ng/mL.

**Table 1: Total Protein, Mus m 1, and MUP Contents of the Urine Samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Protein (ng/mL)</th>
<th>Mus m 1 (ng/mL)</th>
<th>MUP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic Mouse</td>
<td>1,843,000</td>
<td>-</td>
<td>95,000</td>
</tr>
<tr>
<td>Wild Mouse</td>
<td>115,000</td>
<td>-</td>
<td>7,400</td>
</tr>
<tr>
<td>Rat</td>
<td>168,000</td>
<td>-</td>
<td>5,000</td>
</tr>
</tbody>
</table>

**Figure 1: Standard Curves Obtained Using the Assays to Measure MUP and Mus m 1.**
- Commercial ELISA: 1:50 to 1:102,400 (14.4 ng/mL to 0.014 ng/mL).
- In-house ELISA: 1:100,000 to 1:102,400,000 (18 ng/mL to 0.018 ng/mL).

**Table 2: Comparison of the following two assay conditions:**
- Western-blot assay of domestic mouse urine samples.
- Comparison of the in-House and Commercial Assays.

**Figure 2: Western-blot of Domestic Mouse Urine.**
- Both the MUP and MUC protein bands of approximately 28 kDa, which is the MUP described for Mus m 1 (Figure 1).