1 INTRODUCTION

There are many limitations associated with urine collection in mice including urine volume obtained, extended fasting periods and separation of group-housed males that increases the incidence of fighting when returned to co-housing. The LabSand® (Coastative Global, CA, USA) urine collection technique addresses these issues and offers a refined procedure in line with the 3Rs, providing valuable advantages to toxicology studies. LabSand® is a hydrophilic, commercially available sand that keeps the urine affixed. The LabSand® technique consists in replacing the cage bedding by the LabSand®. Animals are then left free in the cage and urine drops are collected using a pipette (Image 1).

2 OBJECTIVES

This study verified the impact of using the LabSand® technique, for collecting mouse urine, on urinalysis and urine chemistry parameters. Optimization of the collection procedures using LabSand® was also performed.

3 MATERIALS AND METHODS

Test System: CD-1 mice (5 to 12 months old) from Charles River Canada Inc.

Urine Collection:

Animals were fasted deprived with access to water for the duration of the urine collection (4 to 5 hours). The urine was collected following different regimens over a 4 to 5 hour period either in the AM or PM, to verify differences in volume obtained. Difference of the results obtained between a single sampling at the end of the collection period and multiple samplings (at least hourly) during the collection period was also verified.

LabSand® Techniques: Group-housed mice (3 per cage) placed in solid bottom cages with an adequate quantity of LabSand®, ~0.5 cm to cover the bottom of the cage. Urine drops collected with a pipette (single or multiple collections) and transferred in a polypropylene tubes with a cap.

Standard Metabolic Cages: Single-housed mice placed in metabolic cages with a jar placed at the bottom, where the urine is collected.

Analysis Techniques:

Standard Urinalysis Parameters: Multistick 810G or Atlas® analyzer (Siemens, USA)

Urine Chemistry: Modular P-800 analyzer (Roche, USA)

4 RESULTS

Urine Volume

A 5-hour collection period provided a higher volume of urine than a 4-hour period using the LabSand® technique (data not presented). Urine collected from group-housed mice on LabSand® after a 5-hour period in the afternoon (from 3 to 8 PM) provided a higher volume than when collected in the morning. Multiple collections (urine drops collected at least hourly) over a 5-hour period from group-housed mice on LabSand®, resulted in significantly higher urine volumes. When using these optimal conditions, the volume of urine obtained with LabSand® varied from 0.5 to 1.2 mL for female mice (Table 1) and allowed the analysis with the Atlas® analyzer. Similar trends were seen for males, with urine volume varying between 1.1 to 2.5 mL, when using optimal conditions (data not presented).

It was suspected that the low urine volume obtained when collected only once after 5 hours on LabSand® was due to evaporation. To verify this hypothesis, the same urine samples were evaluated before and after being placed on LabSand® in uncovered Petri-dishes for 5 hours. Following a 5-hour period on LabSand® (ms) the recovered urine volume ranged between 1.3% and 12.5% of initial urine volume and confirmed our hypothesis that urine samples were evaporating (Table 2).

Urinealyses (Table 4)

Samples collected from group-housed mice on Labsand® at the end of a 5-hour period were more concentrated: urine specific gravity was higher, color was stronger (straw versus light yellow). These differences were likely due to an evaporation process.

5 RESULTS CONTINUED

Table 2: Evidence of Evaporation of Urine Using LabSand® - Urine Volume Recovery After 5 Hours.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mean % Recovery (n=5)</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LabSand®</td>
<td>83.39 (83.24 - 83.50)</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Uncovered Petri-dish</td>
<td>66.33 (66.21 - 66.45)</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

Urine Chemistry (Table 3)

Results obtained before and after contact with LabSand® for 5 hours in capped tubes were found comparable for urine chemistry parameters: phosphorus (PHOS), sodium (Na), chloride (CL), creatinine (CREA), potassium (K) and urine calcium (CA).

6 CONCLUSION

The LabSand® technique is considered an acceptable alternative for urine collection on toxicology studies for urinalysis and urine chemistry. When using the LabSand® technique for urine collection in mice it is recommended to follow the optimal conditions to prevent any potential evaporation that could impact the urine chemistry results.

• Urine collection using a multiple sampling approach instead of collecting the urine only once at the end of the collection period.

• Urine transferred in a capped polypropylene tube.

• Collect the urine in the afternoon (3 to 8 PM).

Charles River, Montreal