

# Urine Collection for Group-Housed Mice in Toxicology Studies Using the LabSand® Techniques – An Alternative Method



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## 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® (Coastline 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 hydrophobic, commercially available sand that keeps the urine afloat. 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.



Image 1: Urine collection using LabSand®

## 5 RESULTS CONTINUED

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

Mean urine volume placed in Petri dishes containing LabSand® (µL)	Mean urine volume recovered urine after a 5-hour storage period (µL)	Mean % Recovery
1550	112	6.69

Table 3: Comparison of Urine Chemistry Results Before and After Contact with LabSand® for 5 Hours in Capped Tubes.

		CREA (mg/dL)	NA (mmol/L)	K (mmol/L)	CL (mmol/L)	CA (mg/dL)	PHOS (mg/dL)
Before	Mean	42.69	31.17	92.71	33.17	11.24	55.08
	SD	58.46	10.59	62.54	8.44	9.95	83.06
	N	12	12	12	12	12	12
After	Mean	43.46	29.55	90.21	31.45	10.16	54.23
	SD	61.53	12.22	62.54	10.03	10.10	82.96
	N	11	11	11	11	12	12
% Difference		-2	5	3	5	11	2

**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).

## 3 MATERIALS AND METHODS

**Test System:** CD-1 mice (6 to 12 months old) from Charles River Canada Inc.

### Urine Collection:

Animals were food 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®10SG or Atlas® analyzer (Siemens, USA)  
Urine Chemistry: Modular P-800 analyzer (Roche, USA)

Table 4: Comparison of Urinalysis Results when Urine is Collected from Female Singly Housed Mice in Metabolic Cages (Single Collection) or Collected from Group-Housed Mice on LabSand® (Single or Multiple Collections).

Sample Number	Color	Clarity	Glucose	Bilirubin	Ketones	Blood	pH	Proteins	Specific Gravity
<b>Urinalysis Results from Singly-Housed Mice in Metabolic Cage at the End of a 5-Hour Collection Period.</b>									
1	straw	clear	neg	neg	neg	neg	7.5	3+	1.023
2	straw	cloudy	neg	neg	neg	neg	7.5	2+	1.018
3	straw	cloudy	neg	neg	neg	neg	7.5	2+	1.021
4	Dk yellow	cloudy	neg	neg	neg	neg	7.0	2+	1.027
5	straw	clear	neg	neg	neg	trace	7.5	2+	1.023
<b>Urinalysis Results from Group-Housed Mice on LabSand® at the End of the 5-Hour Collection Period.</b>									
6	straw	cloudy	neg	1+	neg	QNS	QNS	QNS	1.042
7	straw	cloudy	neg	neg	neg	neg	7.5	QNS	1.036
8	straw	cloudy	neg	neg	QNS	QNS	QNS	QNS	1.033
9	straw	cloudy	QNS	QNS	QNS	QNS	QNS	QNS	1.000
10	straw	cloudy	neg	neg	neg	1+	8.0	1+	1.030
11	straw	cloudy	neg	1+	neg	neg	8.5	1+	1.045
12	straw	cloudy	neg	neg	QNS	QNS	QNS	QNS	1.045
13	straw	cloudy	neg	QNS	QNS	QNS	QNS	QNS	1.045
14	straw	cloudy	QNS	QNS	QNS	QNS	QNS	QNS	1.033
15	straw	cloudy	neg	1+	1+	QNS	QNS	QNS	1.075
<b>Urinalysis Results from Group-Housed Mice on LabSand® with Multiple Collection (at Least Hourly) During a 5-Hour Period.</b>									
16	Light yellow	cloudy	neg	neg	neg	neg	6.0	neg	1.024
17	Light yellow	cloudy	neg	neg	1+	1+	7.0	neg	1.022
18	Light yellow	cloudy	neg	neg	neg	neg	7.0	neg	1.024
19	Light yellow	cloudy	neg	neg	1+	neg	7.0	neg	1.017
20	Light yellow	cloudy	neg	neg	neg	neg	7.5	neg	1.016
21	Light yellow	turbid	neg	neg	1+	neg	7.0	1+	1.025
22	Light yellow	turbid	neg	neg	1+	neg	7.0	1+	1.024
23	Light yellow	cloudy	neg	neg	1+	neg	7.0	1+	1.027

Legend: QNS: Insufficient quantity; neg: negative

## 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® (n=5), 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).

Table 1: Volume of Urine by Cage for Group-Housed Females with LabSand® Using different Collection Techniques.

	Single collection after 5 hours (AM)	Single collection after 5 hours (PM)	Multiple collections (at least hourly) in 5 hours (PM)
Mean Volume (µL)	123.3	183.9	800
Range (µL)	30-400	50-470	500-1200
SD	118.5	147.4	300
Number of Cages	9	9	8

### Urinalysis (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.

## 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).