3Rs Refinement - Use of hydrophobic sand in collection of analytical urine samples

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Introduction

Hydrophobic sand is a commercial product that is used in veterinary surgeries to collect cat urine samples for analysis. The sand has a hydrophobic coating and the urine forms as droplets on the top allowing it to be easily collected and stored. Currently, rodent urine collection for scientific purposes requires the use of metabolism cages.

The disadvantages of the metabolism cages are:
- Animal Welfare - mice are housed in metabolism cages for 16 hours
- Regulated procedure under the Animals (Scientific Procedures) Act
- Costs (buying the metabolic cages, maintenance of equipment and consumables)
- Takes a lot of technician time to set up, dismantle, wash and reassemble

Hydrophobic sand has the potential to reduce time, costs, animal stress, remove the regulation and improve animal welfare.

Method

A preliminary study was performed investigating the optimum time for urine collection. The times chosen to assess were 06:00, 12:00, 18:00 and 24:00hrs. A Latin-square study design was used to reduce the number of animals required. This also allowed us to determine whether habituation and/or the time of day had an effect on sample volume.
8 male and 8 female C57Bl6/J mice were used. They were held in a standard stock room with a 07:00 – 19:00hrs light cycle, 22ºC +/- 2 and 50% RH +/- 10.
The mice were housed in Tecniplast 1145 cages containing 200g of Kit4Cat™ hydrophobic sand for 1 hour, with food and water withheld. They were checked at 30 minute intervals and any urine present was collected with a 1mL syringe and decanted into plastic vials. These were stored at -80ºC. After 1 hour the mice were returned to their home cages and any remaining urine collected. This process was repeated weekly for 4 weeks until all mice had samples taken at each time point.

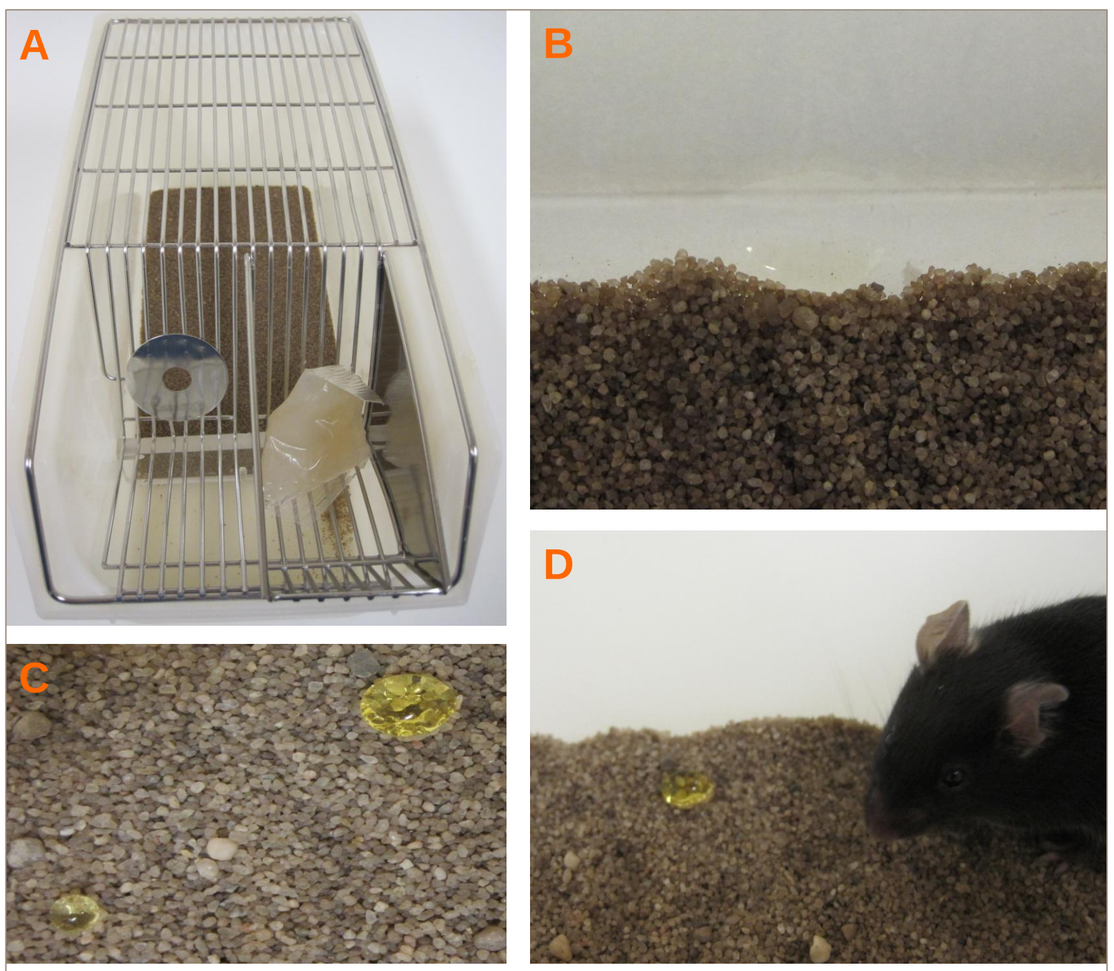
Using the results from this preliminary study, a comparison study was undertaken. A cross-over study design was used over 2 weeks comparing metabolism cages and hydrophobic sand. Environmental conditions were the same as the preliminary study but mice were sampled in North Kent Plastic Cages Ltd type 1 mouse cage with 150g Kit4Cat™ hydrophobic sand for 3 hours of collection starting at 18:00hrs. For this study 10 male and 10 female C57Bl6/J mice were used due to historical data from metabolism studies. Triple A Trading Solid Drink® was placed in the hopper as a substitute for food and water (see Figure 1 picture A). Urine samples were still taken at 30 minute intervals to reduce the risk of urine being lost to evaporation or contamination. Tecniplast rodent metabolism cages were used overnight on a 16 hour duration starting at 15:00hrs.

At the conclusion of the study the animals were euthanased using a Schedule 1 method followed by an appropriate confirmatory method, a necropsy was performed and the stomach contents were examined using a dissection microscope for evidence of the presence of hydrophobic sand.

Routine urine chemistry parameters were assayed using standard methodology on the Siemens Advia 1800 automated chemistry analyser, results were corrected for creatinine.

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Figure 1. Pictures of caging and urine droplets



A. Cage set up used for 3 hour urine collection including Solid Drink®. B. Example of uncollectible urine deposited on curved edge of the cage. C and D. Example droplets of urine that can be collected.

Results

For standard parameters in transgenic phenotyping we require a minimum sample volume of 0.2mL.
In the preliminary study we identified that the peak sample volume was obtained at 18.00hrs. At this time point samples meeting the 0.2mL requirement were obtained from only 69% of animals.
Using 3 hour collection 85% of the hydrophobic sand samples reached the 0.2mL minimum volume compared to 65% of the metabolism cage samples (see Table 1 for the combined results).

Post-mortem examination of stomach contents showed only 2 grains of hydrophobic sand in 1 of the 10 mice. The stomachs mainly contained Solid Drink®.

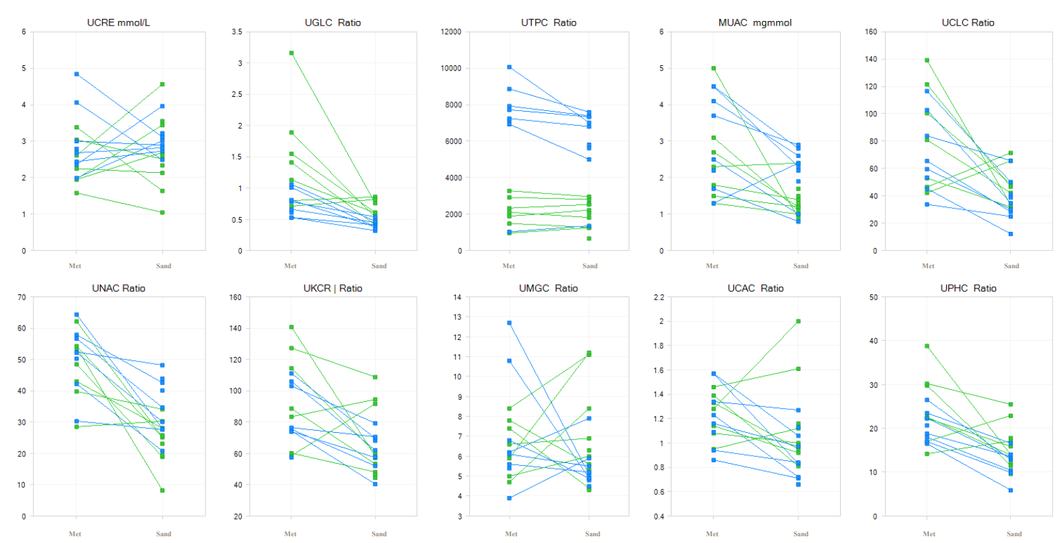
In the preliminary study it was observed that a proportion of the urine was deposited on the curved corners of the Tecniplast 1145 cage and therefore not touching the hydrophobic sand (see Figure 1 picture B). This prevented the urine from forming a droplet and made it hard to collect. In the second study switching to the North Kent Plastic cages enabled all the urine to be collected (see Figure 1 pictures C & D).

Table 1. Usable urine samples

	Hydrophobic sand		Met cages
	1 hour	3 hours	16 hours
	18 - 19:00hrs	18 - 21:00hrs	15 - 07:00hrs
> 0.2ml	69%	85%	65%
< 0.2ml	25%	10%	10%
no sample	6%	5%	25%

< 0.2ml could be measured with a 1 in 4 dilution

Figure 2. Measured urine parameters



Male / Female Measured urine parameters all corrected for creatinine.

Conclusions

Collection periods of 1 hour did not provide enough urine to give a robust sample for each mouse. In the second part of the study we increased the collection time to 3 hours with the addition of Solid Drink® to provide food and water. The 3 hour collection increased the samples that could be measured without dilution from 69% to 85%.

The habituation to the cages had no significant effect on urine volumes but there was an increasing trend towards greater volumes of urine after habituation (data not reported). It was also observed that repeating collections did not reduce the amount of urine the mice excreted. This opens up the possibility of increased frequency of urine collection without an impact on rodent welfare.

Comparison of 3 hour hydrophobic sand and 16 hour metabolism cage urine chemistry results (corrected for creatinine) did not identify any differences that would preclude the use of hydrophobic sand for standard urinalysis collection. Generally, the data points were closer together on the hydrophobic sand samples. This could be due to a reduction of circadian variation as collection was over a 3 hour period as opposed to 16 hours for the metabolism cage samples (see Figure 2).

Hydrophobic sand samples showed no evidence of faecal contamination. This may be due to the way faecal pellets can stick to the funnel under the metabolism cages which then contaminate the urine as it flows over the pellet. When collecting with hydrophobic sand there is little-to-no contact between urine and faeces.

Despite the apparent increase in technician time to collect the samples, overall the time taken to perform the full study using hydrophobic sand was significantly less compared to metabolism cages, when cleaning and set-up time was taking into account.

At post-mortem there was no significant ingestion of the hydrophobic sand. This removes the issue of any chemicals entering the rodent's body and possibly causing interference.

The use of hydrophobic sand has the potential to significantly reduce the amount of times metabolism cages are required, and so have a positive impact on animal welfare. As noted above, the studies were completed on C57BL6/J mice, other strains might respond differently requiring longer or shortened collection periods. We would advise any organisation planning to investigate the use of this product to perform their own pilot studies to determine the optimal approach for their facility.

Although we recognise that hydrophobic sand will never completely replace metabolism cages for urine collection (for example using radio labelled drugs when collection of the total urine output is required), we believe that this method of urine collection should be introduced widely.

Acknowledgements

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