analytical chemistry

Technical Note

Cite This: *Anal. Chem.* 2019, 91, 4958−4963

pubs.acs.org/ac

Controlling Preanalytical Process in High-Coverage Quantitative Metabolomics: Spot-Sample Collection for Mouse Urine and Fecal Metabolome Profiling

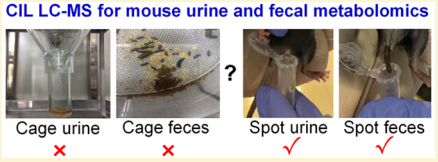
Deying Chen,[†] Jiong Yu,[†] Zhehua Zhang,[†] Xiaoling Su,[†] Liang Li,*[,†,‡] and Lanjuan Li*[,†]

[†]State Key Laboratory and Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China

[‡]Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

Supporting Information

ABSTRACT: Compared to conventional MS and NMR techniques, high-performance chemical isotope labeling (CIL) LC-MS provides accurate relative quantification of many more metabolites in biological samples. However, to apply this technique for urine and fecal metabolomics studies of animal models, the entire workflow, including the preanalytical process, needs to be strictly controlled to avoid or minimize quantitative errors. In this study, we report our investigation of the effects of mouse urine and fecal sample collection methods on CIL LC-MS metabolome analysis. Metabolic-cage collection and spot-sample collection of urine and feces were compared in a mouse model of CCl_4-induced liver disease. ^{13}C-/^{12}C-dansylation LC-MS was used for quantitative profiling of the amine-/phenol-submetabolome changes. A total of 5026, 4963, 4238, and 4600 peak pairs or metabolites were detected from spot urine, spot feces, cage-collected urine, and cage-collected feces, respectively. It was found that samples collected using metabolic cages, widely used in low coverage metabolomics, could be contaminated with food as well as cross-specimen (urine in feces or feces in urine) to the extent that metabolomic comparison of different groups of mice could be seriously compromised in high-coverage metabolomics. In contrast, spot urine and spot feces could be collected without contamination and should be used in CIL LC-MS metabolomics.



Mice are widely used in animal models for biological, medical, and clinical research. Among various biospecimens useful for mouse metabolomics, urine and feces are two common samples that can be noninvasively collected.[1] Analytical methods, including NMR, GC-MS, and LC-MS, have been used to analyze the metabolomes of mouse urine and feces.[2,3] Due to limited sensitivity and/or quantitative accuracy of these methods, only a small number of relatively high abundance metabolites are quantified in untargeted or targeted metabolomics. However, with recent advances in chemical isotope labeling (CIL) LC-MS, it is now possible to carry out accurate relative quantification of chemical-group-based submetabolomes using proper labeling reagents with high coverage.[4−7] Thousands of metabolites can be profiled in one submetabolome analysis alone (e.g., all amine-containing metabolites).[5] On the other hand, this technical advance demands a much more stringent control of the preanalytical process. This is because the overall accuracy of metabolome analysis can be strongly affected by any error that may be introduced in the process leading to the actual analysis. Errors introduced in preanalytical steps may become the dominant source of the overall error when the analysis error is comparatively small.

In mouse urine and fecal metabolomics, sample collection is a crucial step in the preanalytical process. Introduction of a small amount of contaminants during urine or feces collection may not be a problem if an analytical method is not sufficiently sensitive to detect the contaminant or not very accurate to tell the difference of a metabolite concentration with and without contaminant interference. However, this will not be the case when a highly sensitive and highly accurate method is used for metabolome profiling. The concentrations of urinary or fecal metabolites can be changed by the contaminant metabolites that are detectable and in common with those of urine or feces. Thus, a commonly used protocol for low-coverage metabolomics may not be applicable for high-coverage metabolomics. The objective of this study is to determine a suitable sample collection method that is compatible to high-coverage quantitative metabolome analysis of urine and feces in mouse model studies.

By far, the most common method of collecting urine and feces from a mouse is to use a metabolic cage.[8,9] It is convenient to carry out. It also allows urine and feces to accumulate over a period of time (e.g., 24 h) inside a cage to represent an average period-sample. However, cage collection is prone to urine/feces cross-contamination as well as contamination from food. An alternative sample collection method is spot collection where urine or feces is collected afresh from a mouse.[8,10] Because of actual or presumed

Received: January 17, 2019
Accepted: March 22, 2019
Published: March 22, 2019

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958−4963

inconvenience in spot-sample collection as well as small sample sizes collectable (e.g., 20−100 μL urine), there were only a few reports of using this method to collect mouse urine[1,11−14] and feces[1,15] for metabolomics. One study indicated that spot urine collection was not recommended as low volumes of samples collected in some mice were not sufficient for NMR analysis.[12]

In this report, we show that the commonly used metabolite-cage collection is not suitable for CIL LC-MS based urine and fecal metabolomics. We describe an optimized spot-collection method to collect urine and feces quickly and conveniently with no contamination. The spot-collection method is incorporated into a mouse urine and fecal metabolome profiling workflow for high-coverage quantitative metabolomics.

EXPERIMENTAL SECTION

Mouse Model. All animal experiments were conducted according to a protocol that was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University, Hangzhou, China. A total of 42 healthy male C57BL/6 mice (Shanghai SLAC laboratory animal Co. Ltd., Shanghai, China), weighing 18−20 g and at 6 weeks of age, were housed in groups under standard conditions, fed a standard chow, and allowed access to sterile water for 2 weeks prior to building the chemical exposure model with the injection of either olive oil (control mice) or CCl_4 dissolved in olive oil (v/v = 1:1; liver injury or disease mice) at dose of 2 mL/kg body weight intraperitoneally. Supporting Information, Figure S1A summarizes the samples collected from five groups of mice, two methods of sample collection, and six time points of collection (day 0 to day 9 after treatment). Supporting Information, Note S1-1 describes tissue histopathology and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level measurement.

Sample Collection. In the spot feces collection method, fresh feces were collected by lifting the mouse from the cage using one hand to grab the end of tail. Mouse defecated quickly once held properly, likely due to fear. The feces could be collected into a 2.0 mL Eppendorf tube. Only 2 out of 6 mice in day 3 and 1 out of 6 mice in day 5 from the CCl_4-treated group did not defecate, likely due to their sickness with injured liver. Mice that failed to defecate were placed individually into an empty cage for 30 min, followed by performing the spot collection procedure again. In the second attempt, collection of feces was successful in all three cases. This method of spot feces collection was also found to be successful in our other mouse fecal metabolomics studies (to be reported).

For spot urine collection, fresh samples were collected into a 2.0 mL Eppendorf tube by application of gentle trans-abdominal pressure over the bladder to induce urination. One micturition from a mouse could produce approximately 20 to 100 μL of urine. This volume of sample may not be adequate for less sensitive metabolome profiling method; however, it is sufficient for the highly sensitive CIL LC-MS method. The tubes containing the freshly collected urine were placed on ice and then transferred to a −80 °C freezer.

In the cage-sample collection method, mouse metabolism cage (Type Y-3101, Yuyan Instruments Co. Ltd., Shanghai, China) was used to separate liquid from solid (Supporting Information, Figure S1B). Collection of the accumulated feces and urine over a 24 h period was done daily between 9:00 and 10:00 AM. Feces were collected with a small forceps directly from the cage floor, pooled per cage and frozen on ice. Urine was pipetted from the collecting vial at the bottom of the cage into a 5 mL Eppendorf tube which was then placed on ice. The cage-collected 24 h urine was centrifuged at 4000 rpm for 10 min. The supernatant was filtered through 0.22 μM filter (Agela Technologies, China). The filtered urine was aliquoted into 2 mL vials and stored at −80 °C.

Sample Processing and Analysis. After the stored samples were thawed, metabolites were extracted and labeled for CIL LC-MS analysis. For fecal metabolite extraction, feces were subjected to sequential solvent extraction by water and acetonitrile as descried previously.[16] Supporting Information, Note S1-2 provides the details of the CIL LC-MS workflow, the experimental protocol for metabolite extraction,[16] dansylation labeling,[5] LC-UV for sample normalization,[17] and equal-mole ^{12}C-reagent-labeled sample mixing with ^{13}C-reagent-labeled pool. The ^{12}C-/^{13}C mixtures were analyzed using Agilent 1290 UPLC linked to Agilent electrospray ionization time-of-flight mass spectrometry, as detailed in Supporting Information, Note S1-3. The resultant LC-MS data were subjected to IsoMS data processing,[18−20] multivariate and univariate statistical analysis, Dns-standard library,[21] and MyCompoundID[22] metabolite identification, as described in Supporting Information, Note S1-4.

RESULTS AND DISCUSSION

Liver Injury Model and Time-Course Metabolome Profiling. In this study, we used a mouse model of chemical exposure to examine how sample collection methods can significantly affect the metabolomics results. CCl_4 can cause liver damage and mice exposed to this chemical have been used to study liver disease and functions.[23] In our study, ALT and AST measurements were performed to assess the regeneration of liver function and hepatic fibrosis (Figure 1A). One day after CCl_4 exposure (i.e., day 1), ALT and AST activities were significantly increased. By day 3, the activities were reduced to less than half of the day 1 activities and approached to almost normal levels on day 5, 7, and 9. Histological examination of the acute injury tissue of liver was also performed (Supporting Information, Figure S2). The tissue injury and recovery results corroborated well with the ALT and AST measurements. Thus, the time-course events included the acute liver injury after CCl_4 exposure, injury repair and injury recovery over a period of 9 days.

We used this model of time-course profiling, instead of a simple binary comparison at one time point, to examine the effects of sample collection methods on quantitative results of the metabolomes (Figure 1B,C and discussion below). This offers several advantages, as described in Supporting Information, Note S2-1.

Metabolome Analysis. In this work, we focused on the use of dansylation LC-MS to profile the amine/phenol submetabolome. Since urine and feces have very different metabolite compositions, we compared the urine and fecal metabolomes separately, using a ^{13}C-dansyl-labeled pooled sample as the internal standard. Within each sample type, we compared two collection methods, namely, cage and spot collections. A total of 576 ^{12}C-/^{13}C-labeled mixtures were produced and analyzed by LC-MS from experimental duplicates of 72 spot fecal samples (i.e., 6 daily samples × 2 collection methods × 6 mice/method), 72 cage fecal samples, 72 spot urine samples, and 72 cage urine samples.

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958−4963

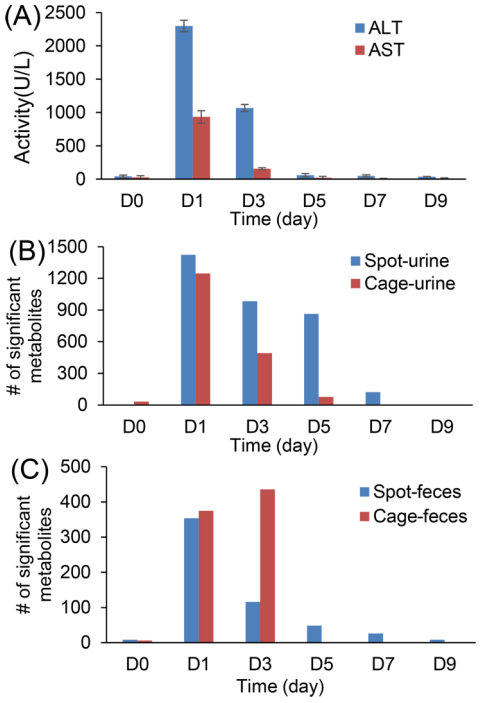


Figure 1. (A) Measured activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood. Inflammation or injury to liver cells, a condition known as hepatocellular liver injury, leaks AST and ALT into the bloodstream. Error bar indicates ±1 SD (standard deviation) of three measurements (i.e., three mice). Plots of the number of metabolites with significant changes in binary comparison of disease mice and control mice in different days using the metabolome data of spot urine and cage urine (B) and spot feces and cage feces (C). The number of significantly changed metabolites was determined by comparing all peak pairs of disease mice vs control mice at an individual day using a volcano plot with student *t* test (see Supporting Information, Figures S9–S12).

There were a total of 5026, 4963, 4238, and 4600 peak pairs or metabolites detected from spot urine, spot feces, cage urine and cage feces, respectively. We identified 72, 82, 76, and 72 metabolites using the dansyl library (Supporting Information, Tables S1, S2, S3, and S4), matched 1428, 979, 1324, and 952 peak pairs using accurate mass search to the HMDB library (Supporting Information, Tables S5, S6, S7, and S8) and 4210, 3805, 3713, and 3481 peak pairs to the one-reaction EML library (Supporting Information, Tables S5, S6, S7, and S8), respectively. Thus, 85.2%, 78.3%, 89.4%, and 77.2% of the detected peak pairs could be either positively identified or mass-matched to metabolite structures in metabolome databases in the four different types of samples. This high level of coverage for the amine/phenol submetabolome offered us a unique opportunity to examine how sample collection method can influence the metabolome results.

Multivariate Statistical Analysis. Figure 2 shows the principal component analysis (PCA) plots of the metabolome data generated from experimental duplicate analyses of 72 spot urine, 72 cage urine, 72 spot feces, and 72 cage feces collected from the CH_4-exposed or disease mice in day 0, 1, 3, 5, 7, and 9. The data from the QC samples (i.e., 1:1 mixture of ^{12}C-/^{13}C-dansyl-labeled pool) are also shown; they cluster to each other, indicating excellent reproducibility in LC-MS data acquisition. In all cases, separation of urine or fecal samples collected from different days is visible. However, data clustering within a group and the extent of separation between groups are different. Intragroup clustering is tighter for most of the groups from spot urine samples (Figure 2A) and spot feces samples (Figure 2C), compared to the cage urine (Figure 2B) and cage feces (Figure 2D). Day-to-day group separation and intragroup clustering can be more readily seen in the orthogonal projections to latent structures discriminant analysis (OPLS-DA) plots shown in Supporting Information, Figure S3. Comparison of the model parameters (R^2X, R^2Y, and Q^2) indicates that spot urine metabolome data provide better separation than cage urine and spot feces data also give better separation than cage feces.

Similar observations were found in the PCA and OPLS-DA plots of urine and feces collected from the control mice (Supporting Information, Figures S4 and S5). Overall, for most of the disease or control mice with samples collected from individual days, the intragroup metabolomic data cluster to each other more tightly when spot urine or spot feces were analyzed, compared to cage samples. This finding seems to be counterintuitive, as one would expect the cage samples should cluster together more tightly, considering that the cage sample is a daily average sample, while spot urine is from one time point. However, this notion assumes that both cage collection and spot collection are free from sample contamination interference. As it is shown below, this is not the case for cage-collected samples.

Cage Collection versus Spot Collection. In cage collection, cross-contamination among urine, feces and food cannot be avoided. Supporting Information, Figure S6 shows pictures of representative cases where some feces were touched with food, while others were partially dissolved or soaked with urine. There were food and feces in the urine collection funnel and tube and the urine itself. We would pick the feces with no obvious presence of food and urine to reduce the risk of collecting contaminated feces. We would pipet out the liquid from the urine collection tube to avoid solid feces or food at the bottom. This way of collecting and using cage urine and cage feces may be fine for the analysis of metabolites that are present in much higher concentrations in urine than feces and food or in feces than urine and food. However, in high coverage metabolomics where many low abundance metabolites can be detected and quantified, a small amount of cross-contamination can present a huge problem. We have examined the extent of cross-contamination among urine, feces and food by comparing the peak pairs detected from these three different types of samples.

To study food contamination to the urine or fecal metabolome, the peak pairs detected from food or mouse feed itself (i.e., the food metabolome) were determined by three repeat injections of experimental triplicates of ^{12}C-/^{13}C-dansyl-labeled food mixtures (n = 9). These food peak pairs were then compared to those detected in urine and feces. Figure 3A shows the number of common peak pairs found in food and spot urine collected from control mice in different days, while Figure 3B shows the number of common pairs found in food and cage urine. The common pair number detected in food and cage urine is much higher than that in food and spot urine [e.g., there is an average of 1100 ± 45 (n = 72) common pairs in six control mice for cage urine, compared to 652 ± 52 for spot urine]. Spot urine should be free of food contamination and the common pairs found in spot urine and food represent the common metabolites in these two types of samples. A significant increase in common pairs in cage urine

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958–4963

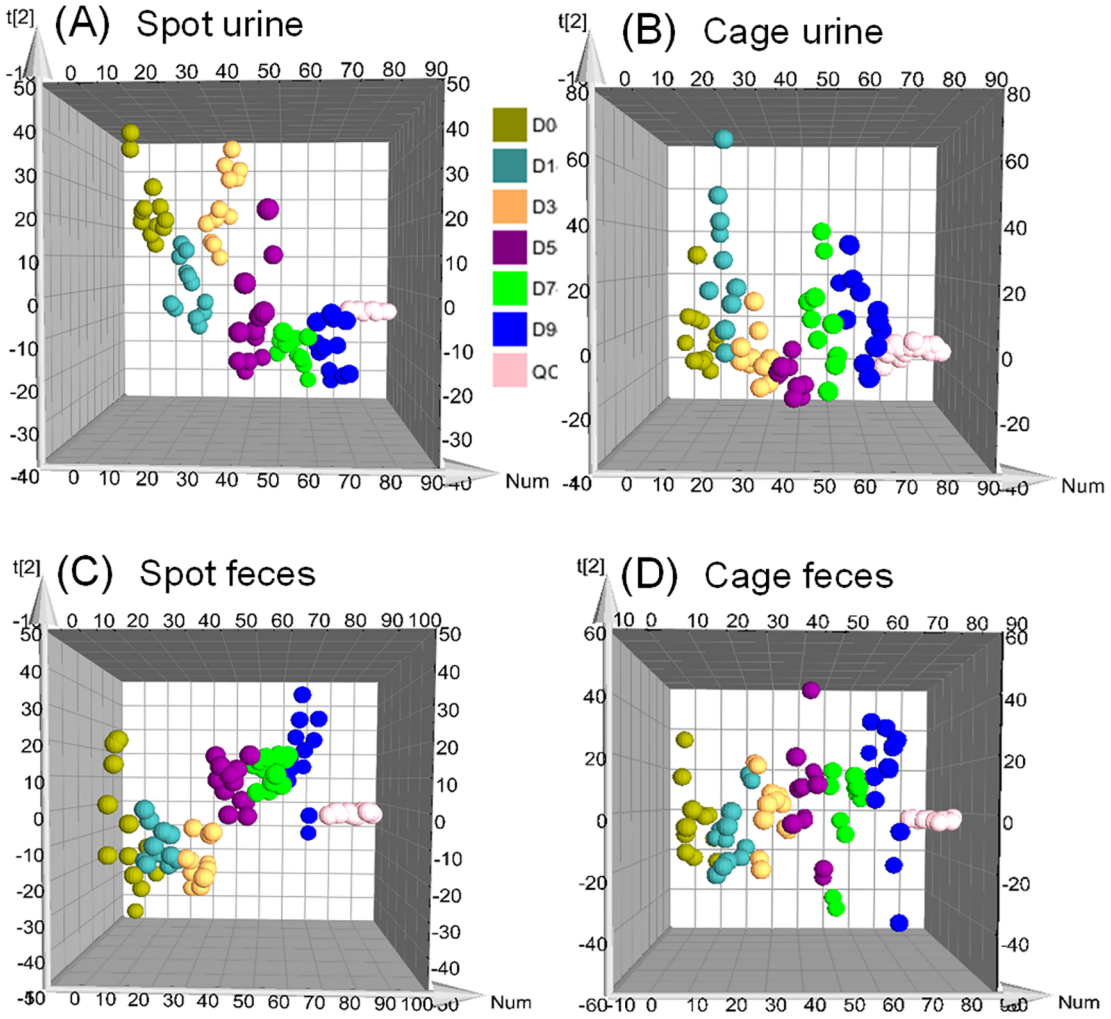


Figure 2. PCA plots of disease mice metabolome data from (A) spot urine, (B) cage urine, (C) spot feces, and (D) cage feces.

indicates severe food contamination in the cage urine samples. We note that there was a report of food contamination in mouse urine collected in a cage even using a less sensitive method, NMR,[24] although cage urine is still widely used in mouse metabolomics.

On fecal samples, Figure 3C shows the common pair number found in food and spot feces and Figure 3D shows those found in food and cage feces. The common pair number found in food and cage feces only increases slightly, compared to those of food and spot feces. Statistical analysis of peak pair number comparison between Figure 3A,B has a p-value of 5.4×10^{-9}, compared to 1.3×10^{-5} between Figure 3C,D, using student t test. Thus, compared to cage urine, the extent of food contamination is much less in cage feces. This is most likely due to the great care that was taken to pick only the feces with no visible food attached for analysis. Similar observations were found for the disease mice where cage urine samples were more severely contaminated with food than cage feces (Supporting Information, Figure S7).

Besides food contamination, cross-specimen contamination is another major problem. This is shown in Figure 4 in the comparisons of the common peak pair numbers detected in spot urine, spot feces and cage feces collected from six control mice in different days. In this case, all peak pairs found in spot urine were combined to represent the clean urine metabolome and then compared to the peak pairs detected in individual spot or cage fecal samples to determine the common pairs (Figure 4A,B). There are more common pairs detected in cage feces (747 ± 39, $n = 72$) than spot feces (603 ± 48), indicating the presence of urine contamination in cage feces. Similarly, all peak pairs found in spot feces representing the clean fecal metabolome are compared to those found in individual spot urine samples (Figure 4C) and cage urine samples (Figure 4D) to determine the number of common pairs. The average number of common pairs detected in cage urine (725 ± 74, $n = 72$) is not statistically different from that found in spot urine (699 ± 34), indicating that cage urine is, on average, not significantly contaminated with feces. The p-value of peak pair number comparison between Figure 4A and B is 0.0072, compared to 0.044 between Figure 4C and D, from student t test. However, as shown in Figure 4D, in some individual cage urine samples, the number of common pairs is significantly higher than those found in Figure 4C (e.g., the first sample of day 0, 1, 3, and 9 with near 900 common peak pairs found). These individual cage urine samples were likely contaminated with feces (e.g., the urine-dissolved feces might have leaked into the urine collection tube). Similar observations were found for the disease mice (Supporting Information, Figure S8).

Taken together, it is clear that the cage collection method can introduce food and cross-specimen contamination to an extent that peak pair or metabolite detection can be

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958−4963

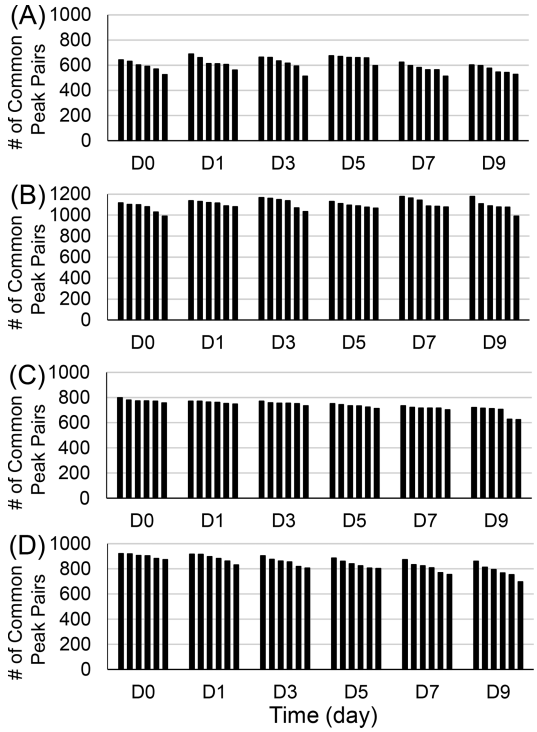


Figure 3. Number of common peak pairs found from control mice in food and individual samples collected in different days from (A) spot urine, (B) cage urine, (C) cage feces, and (D) cage feces. Each bar represents the average peak pair number from duplicate analysis of one sample.

significantly altered. The major contamination in cage urine comes from the food, while cage feces can be contaminated with both food and urine. The question is whether this contamination would affect the metabolome profiling results in high coverage quantitative metabolomics. We used our time-course mouse model to address this important question.

Supporting Information, Figures S9–S12 show the volcano plots from which we determined the significant metabolites (≥1.5-fold change and false-discovery rate $q \leq 0.05$) separating the disease and control groups in each time-point. The number of significant metabolites is plotted in Figure 1B using the spot urine and cage urine metabolome data and in Figure 1C using the spot feces and cage feces metabolome data. For spot samples, the change in the number of significant metabolites follows a pattern of significant increase in day 1 and then gradual decrease from days 3 to 9. This pattern of change is consistent with the ALT and AST level changes (Figure 1A), indicating the metabolome data can follow the liver injury and recovery. However, for cage samples, the patterns of changes are very different and not consistent. Supporting Information, Notes S2-2 and -3, provides detailed analysis of the metabolome data for discovering biomarkers of liver injury and recovery.

CONCLUSIONS

We have shown the importance of using a proper sample collection method (i.e., spot-sample collection) for urine and fecal metabolomics of mouse models. The traditional method of using metabolic cages for sample collection is prone to food and cross-specimen contamination that can cause errors in urine or fecal metabolome analysis when a high-coverage quantitative technique such as CIL LC-MS is used.

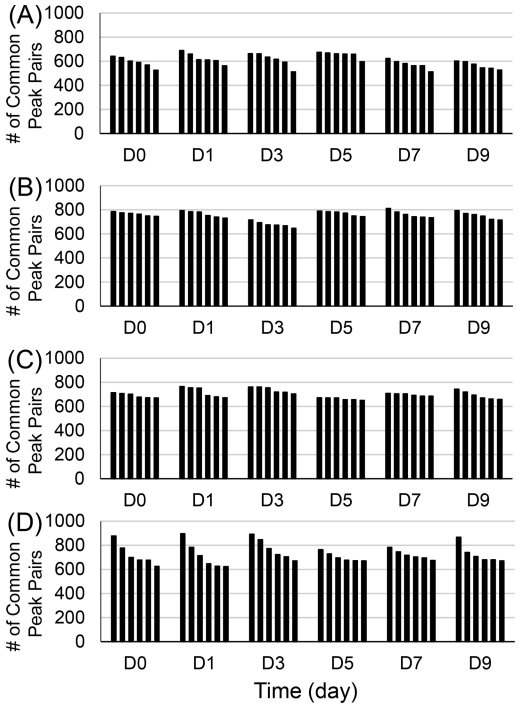


Figure 4. Number of common peak pairs found from control mice in (A) the clean urine metabolome (all spot urine peak pairs) and individual spot fecal samples collected in different days, (B) the clean urine metabolome and individual cage fecal samples, (C) the clean fecal metabolome (all spot feces peak pairs) and individual spot urine samples, and (D) the clean fecal metabolome and individual cage urine samples. Each bar represents the average peak pair number from duplicate analysis of one sample.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b00310.

Supplemental Note S1 for experimental, Note S2 for biomarker discovery, Figures S1–S12 for study design and analysis (PDF)
Table S1 for metabolite identification results (XLSX)
Table S2 for metabolite identification results (XLSX)
Table S3 for metabolite identification results (XLSX)
Table S4 for metabolite identification results (XLSX)
Table S5 for metabolite identification results (XLSX)
Table S6 for metabolite identification results (XLSX)
Table S7 for metabolite identification results (XLSX)
Table S8 for metabolite identification results (XLSX)
Table S9 cited in Note S2 (XLSX)
Table S10 cited in Note S2 (XLSX)
Table S11 cited in Note S2 (XLSX)
Table S12 cited in Note S2 (XLSX)
Table S13 cited in Note S2 (XLSX)

AUTHOR INFORMATION

Corresponding Authors

*E-mail: liang.li@ualberta.ca.
*E-mail: ljli@zju.edu.cn.

ORCID

Jiong Yu: 0000-0001-5737-9419
Liang Li: 0000-0002-9347-2108

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958–4963

Lanjuan Li: 0000-0001-6945-0593

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 21804118), Independent Project Fund of the State Key Laboratory for Diagnosis and Treatment of Infectious Disease (SKL DTID), General Medical Science and Technology Plan Project of Zhejiang Province (No. 2018KY366), and Public Welfare Technology Application Research Plan Project of Zhejiang Province in China (No. 2018C37059). This work was also supported by a visiting professorship to L.L. by Zhejiang University K. P. Chao's Hi-Tech Foundation for Scholars and Scientists. Additional support was provided by the Natural Sciences and Engineering Research Council of Canada, Canada Research Chairs, Canada Foundation for Innovation, Genome Canada and Alberta Innovates.

■ REFERENCES

(1) Yap, I. K. S.; Li, J. V.; Saric, J.; Martin, F. P.; Davies, H.; Wang, Y. L.; Wilson, I. D.; Nicholson, J. K.; Utzinger, J.; Marchesi, J. R.; Holmes, E. *J. Proteome Res.* **2008**, *7*, 3718−3728.
(2) Deda, O.; Gika, H. G.; Wilson, I. D.; Theodoridis, G. A. *J. Pharm. Biomed. Anal.* **2015**, *113*, 137−150.
(3) Khamis, M. M.; Adamko, D. J.; El-Aneed, A. *Mass Spectrom. Rev.* **2017**, *36*, 115−134.
(4) Higashi, T.; Ogawa, S. *J. Pharm. Biomed. Anal.* **2016**, *130*, 181−193.
(5) Guo, K.; Li, L. *Anal. Chem.* **2009**, *81*, 3919−3932.
(6) Luo, X.; An, M. R.; Cuneo, K. C.; Lubman, D. M.; Li, L. *Anal. Chem.* **2018**, *90*, 8314−8319.
(7) Yuan, B. F.; Zhu, Q. F.; Guo, N.; Zheng, S. J.; Wang, Y. L.; Wang, J.; Xu, J.; Liu, S. J.; He, K.; Hu, T.; Zheng, Y. W.; Xu, F. Q.; Feng, Y. Q. *Anal. Chem.* **2018**, *90*, 3512−3520.
(8) Kurien, B. T.; Everds, N. E.; Scofield, R. H. *Lab. Anim.* **2004**, *38*, 333−361.
(9) Wettersten, H. I.; Ganti, S.; Weiss, R. H., Metabolomic Profiling of Tumor-Bearing Mice. In *Cell-Wide Metabolic Alterations Associated with Malignancy*; Galluzzi, L.; Kroemer, G., Eds.; Elsevier Academic Press Inc.: San Diego, 2014; Vol. *543*, pp 275−296.
(10) Chew, J. L.; Chua, K. Y. *Lab Anim.* **2003**, *32*, 48−50.
(11) Peng, J.; Guo, K.; Xia, J. G.; Zhou, J. J.; Yang, J.; Westaway, D.; Wishart, D. S.; Li, L. *J. Proteome Res.* **2014**, *13*, 4457−4469.
(12) Pelantova, H.; Buganova, M.; Anyz, J.; Zelezna, B.; Maletinska, L.; Novak, D.; Haluzik, M.; Kuzma, M. *J. Pharm. Biomed. Anal.* **2015**, *115*, 225−235.
(13) Montoliu, I.; Cominetti, O.; Boulange, C. L.; Berger, B.; Siddharth, J.; Nicholson, J.; Martin, F. P. J. *Anal. Chem.* **2016**, *88*, 7617−7626.
(14) Laiakis, E. C.; Trani, D.; Moon, B. H.; Strawn, S. J.; Fornace, A. J. *Radiat. Res.* **2015**, *183*, 382−390.
(15) Hamalainen, A.; Heistermann, M.; Fenosoa, Z. S. E.; Kraus, C. *Gen. Comp. Endocrinol.* **2014**, *195*, 68−79.
(16) Xu, W.; Chen, D. Y.; Wang, N.; Zhang, T.; Zhou, R. K.; Huan, T.; Lu, Y. F.; Su, X. L.; Xie, Q.; Li, L.; Li, L. J. *Anal. Chem.* **2015**, *87*, 829−836.
(17) Wu, Y. M.; Li, L. *Anal. Chem.* **2012**, *84*, 10723−10731.
(18) Zhou, R.; Tseng, C. L.; Huan, T.; Li, L. *Anal. Chem.* **2014**, *86*, 4675−4679.
(19) Huan, T.; Li, L. *Anal. Chem.* **2015**, *87*, 1306−1313.
(20) Huan, T.; Li, L. *Anal. Chem.* **2015**, *87*, 7011−7016.
(21) Huan, T.; Wu, Y. M.; Tang, C. Q.; Lin, G. H.; Li, L. *Anal. Chem.* **2015**, *87*, 9838−9845.
(22) Li, L.; Li, R. H.; Zhou, J. J.; Zuniga, A.; Stanislaus, A. E.; Wu, Y. M.; Huan, T.; Zheng, J. M.; Shi, Y.; Wishart, D. S.; Lin, G. H. *Anal. Chem.* **2013**, *85*, 3401−3408.
(23) Hillebrandt, S.; Goos, C.; Matern, S.; Lammert, F. *Gastroenterology* **2002**, *123*, 2041−2051.
(24) Gu, H.; Pan, Z. Z.; Duda, C.; Mann, D.; Kissinger, C.; Rohde, C.; Raftery, D. *J. Pharm. Biomed. Anal.* **2007**, *45*, 134−140.

DOI: 10.1021/acs.analchem.9b00310
Anal. Chem. 2019, 91, 4958−4963