Normalization of Organ-on-a-Chip Samples for Mass Spectrometry Based Proteomics and Metabolomics via Dansylation-based Assay

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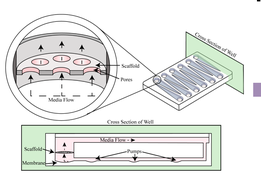
Abstract

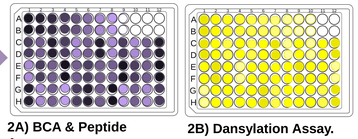
Lab-on-a-chip technology has rapidly expanded and has been made even more powerful with the use of mass spectrometry-based multi-omics analysis, which is well suited for the sample-limited nature of the lab-on-a-chip format. In our research, organ-on-a-chip technology allows us to achieve high-throughput experimentation without the use of model organisms, while simultaneously permitting us to focus on organ and sub-organ systems. However, there still remains the issue of chip-to-chip variability in analysis of the proteome and metabolome, which masks true biological changes that occur within the system. While this variability could be conquered via increasing *N*, doing so also increases cost and labor. We instead sought to improve our normalization methods to circumvent this issue. Here we utilize a CNBio liver chip to investigate mechanisms of action, toxicity, and host response following exposure to VX. To combat chip-to-chip variations, we developed a method for analysis and normalization of the CNBio liver chip, utilizing dansylation and BCA assays to achieve normalization of both the metabolome and proteome. This strategy resulted in reduced variation effects, allowing us to draw more statistically confident conclusions and observe trends that were a result of experimental conditions and not of systemic chip-to-chip variations. This method can easily be expanded to 'omics analysis of other organs-on-a-chip.

Methods



1) Cell Culture. Human hepatocytes were seeded on CNBio plates and exposed to 20 µM VX alongside un-exposed controls. Normalized sample sets went on to be assayed, while non-normalized sample sets underwent identical sample prep without the use of assay quantitation.

2) Pre-Acquisition Normalization.



2A) BCA & Peptide Assays. Proteins were precipitated out of the metabolomics fraction, assayed pre- and post-digestion, then normalized based on assay results.

2B) Dansylation Assay. Metabolomics fractions were labeled with dansyl chloride and measured against a standard curve, then normalized to the lowest-concentrated sample.

3) Mass Spectral Analysis. Normalized and non-normalized sample sets were analyzed on a Thermo Eclipse mass spectrometer with FAIMS interface in positive mode (for proteomics) or Thermo QExactive Plus Orbitrap mass spectrometer in reverse phase positive and negative ion modes and HILIC positive and negative ion modes (for metabolomics).

4) Statistical Analysis and Joint Pathway Analysis. Proteins and compounds from normalized and unnormalized datasets were filtered for adj. p-value of ≤ 0.05. Filtered data was input into Metaboanalyst's joint pathway analysis, utilizing Fisher's Exact Test set for enrichment analysis.

Results & Discussion

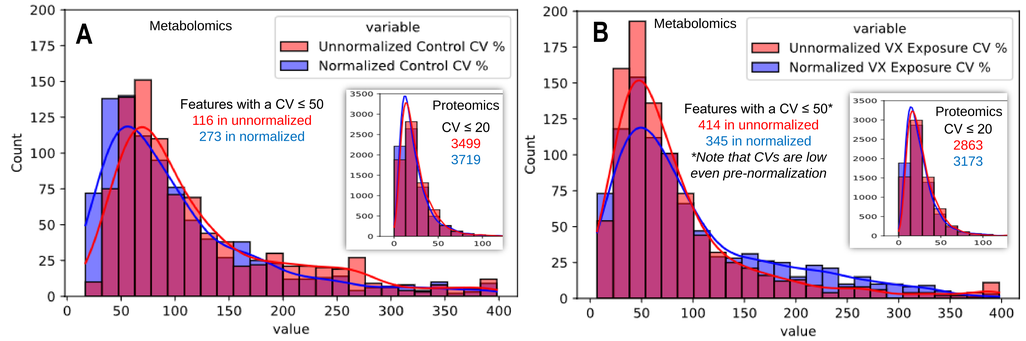


Figure 1. Coefficient of variation for reverse phase chromatography in positive ion mode mass spectrometry. A) Overlay of unnormalized (red) and normalized (blue) control samples and B) overlay of unnormalized (red) and normalized (blue) VX exposed samples. The curves in both panels are density lines.

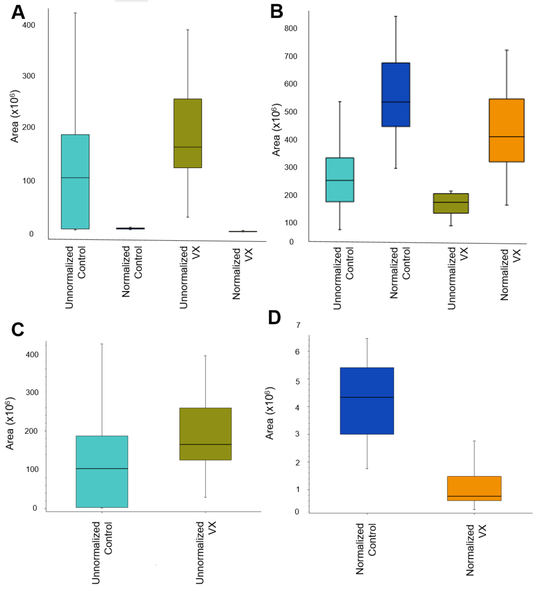


Figure 3. Box and whisker plots of selected metabolites with different results due to normalization in reverse phase positive ion mode. A) 1,3-Dimethylbutylamine in unnormalized and normalized samples, B) L-glutamic acid in unnormalized and normalized samples, C) 1,3-dimethylbutylamine in only unnormalized samples, and D) 1,3-dimethylbutylamine in only normalized samples.

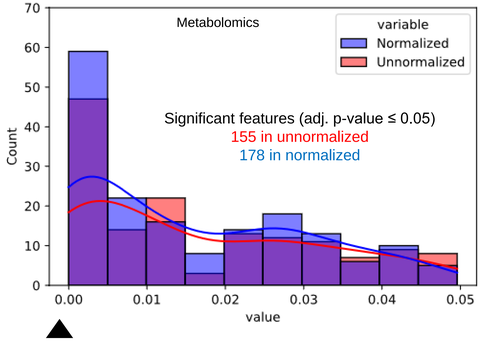


Figure 2. Overlay of reverse phase positive ion mode features determined to be significant (p-value ≤ 0.05). The curves are density lines.

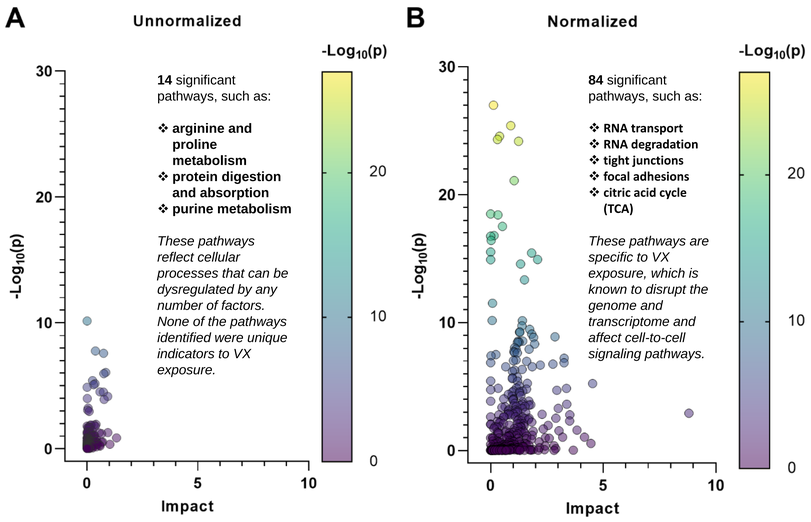


Figure 4. Joint pathway analysis of the proteomics and metabolomics data defined as significant (adjusted p.value ≤ 0.05) in the unnormalized (A) and the normalized (B) samples. The normalized sample results show many more pathways significantly enriched that are more biologically relevant to VX exposure.

Conclusion

Organ-on-a-chip systems allow for more precise understanding of the effects of toxins, environmental hazards, or potential drugs on cellular mechanics can be achieved in a more cost effective, higher throughput manner. However, in order to achieve more conclusive results from these samples, normalization strategies should be employed.

Normalizing metabolomic samples prior to data acquisition decreased CV values for the control samples by more than two-fold in the 0-50 range. This lead to identification of more significantly changing metabolites that could elucidate metabolic pathways affected by VX exposure. Similarly, the normalized proteomic samples identified proteins relevant to biochemical functions affected by VX. Finally, when normalized proteomic and metabolomic data are brought together, a more accurate picture of VX exposure emerges as demonstrated by the joint pathway enrichment. More than 80 pathways were identified as significant in the normalized data set that point to more specific effects of VX on the cell population, rather than the non-specific systems that were deemed significant in the unnormalized data.

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