4 Metabolite profiles distinguish exposure to Zika and Dengue flaviviruses in human induced pluripotent stem cells (hiPSCs)

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Introduction

- Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) are required to comply with the donor eligibility requirements as per 21CFR 1271.
- The communicable diseases testing of donors includes testing for West Nile Virus (WNV) which belongs to the group of flaviviruses.
- Due to increase in global temperature, vector-borne flavivirus cases are increasing throughout the world. This can cause increased transmission of flaviviruses through donor cells and tissues.
- In this project, we seek to explore the potential of metabolomics as an innovative tool for detecting flaviviruses.. High-throughput metabolomics allows for the identification of small molecules and the underlying molecular mechanisms, paving the way for the rapid development of diagnostic biomarkers and the discovery of therapeutic targets.
- We studied Dengue (DENV) and Zika (ZIKV) viruses to investigate how metabolomic profiles can be leveraged for flavivirus detection. These viruses, predominantly transmitted by mosquitoes, have been reported in 71 countries and exhibit varying levels of virulence, with ZIKV being highly virulent and DENV comparatively less so.
- Among regulated cell therapy products, our research is focused on human induced pluripotent stem cells (hiPSCs) which represents an excellent resource for generating cell therapy products.

Objectives

1. Compare the sensitivity, and specificity of metabolomics to the Nucleic Acid Amplification Testing (NAT) and immunogenic detection assays.
2. Develop metabolite markers for detecting flaviviruses in hiPSCs.

Materials and Methods

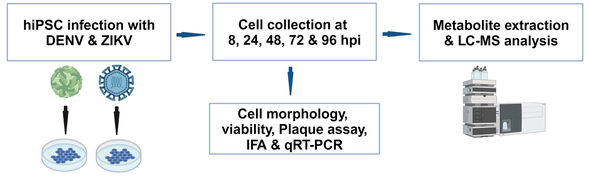


Figure 1. Study overview. hiPSCs were infected with DENV3 and ZIKV-MR766 strains at MOI 1. Samples were collected 8, 24, 48, 72 and 96 hours post infection (hpi). We assessed impact of viral infections on hiPSCs by examining cell viability, cytopathic effect (CPE), and immunofluorescence assay (IFA).

- To standardize metabolite extraction, cells in quantities of 0.2, 0.8, 2, and 8 million from both Mock and DENV-infected samples were analyzed using untargeted flow-injection mass spectrometry (FIA-MS), with ions annotated by matching inferred masses to the Human Metabolome Database.
- Additionally, LC-MS analysis was conducted at 0, 8, 24, 48, and 96 hpi to identify metabolites, with each condition (Mock, ZIKV, and DENV) including five replicates.

Results and Discussion

1. No significant changes observed in cell morphology and viability upon infection; however, hiPSC are more susceptible to ZIKV than DENV.

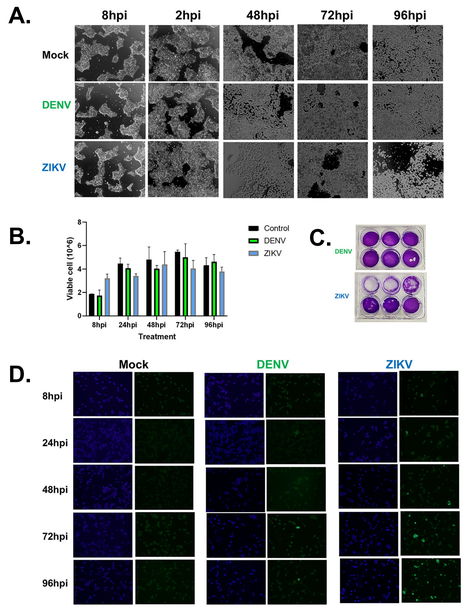
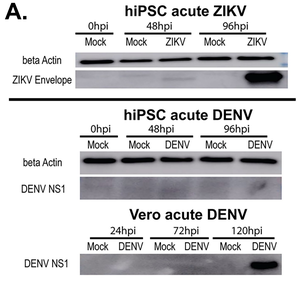


Figure 2. Characterization of hiPSC morphology and viability upon DENV and ZIKV infections. Morphology of hiPSCs (Mock, DENV and ZIKV) were captured using an inverted phase contrast microscope-100x (A). Number of viable cells were determined by trypan blue dye (B). Plaque assay to study infectious virions released from hiPSCs at 96 hpi (C). Immunofluorescence assay (IFA) with anti-4G2 antibody on different hours post infection (D).



B.

qRT-PCR

Actin	Mock	ZIKV	DENV	ZIKV	DENV
8hpi	21.1	21.4	20.8	31.6	00.0
24hpi	21.2	21.5	20.5	28.6	35.5
48hpi	22.2	21.7	21.0	21.8	26.9
72hpi	21.5	21.4	21.4	23.7	24.9
96hpi	21.0	21.4	21.7	19.3	22.2

Figure 3. Detection of DENV and ZIKA in infected hiPSCs. Western blot analysis with anti-ZIKV envelope, and anti-actin antibodies (A); with anti-DENV NS1, and anti-actin antibodies (B) in hiPSCs; and with anti-DENV NS1 antibody in Vero at different hpi. qRT-PCR (CT values) for Actin, ZIKV & DENV from mock and infected samples (D).

2. FIA-MS and LC-MS analyses detected changes in hiPSC metabolome after DENV and ZIKV infection.

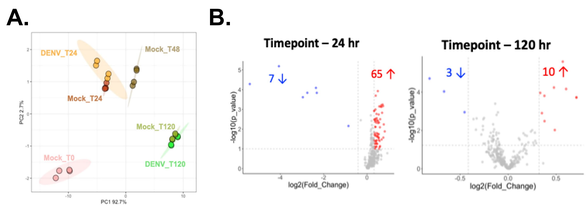


Figure 4. In FIA-MS analyses, early time point showed greater difference between mock and DENV. PCA representation of FIA-MS of 317 compounds shows separation of samples by time point (A) Volcano Plots depicting the number of significant features up (red) regulated and down (blue) with an FDR value <0.3 and fold change <0.75 or >1.25 (B).

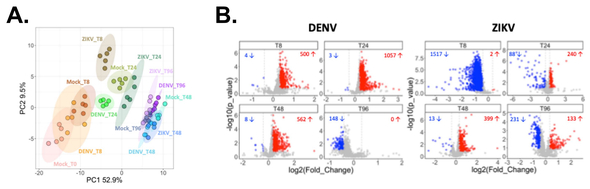
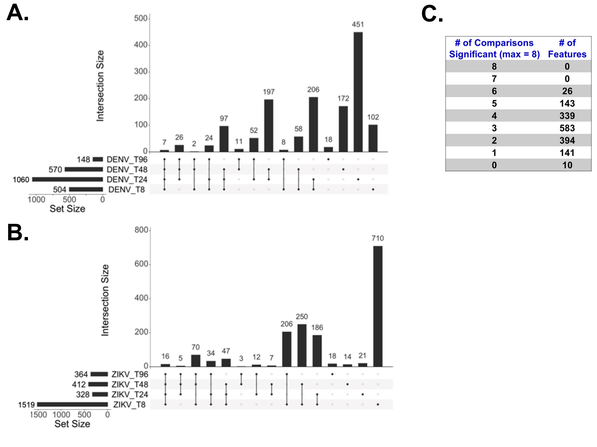


Figure 5. LC-MS/MS analyses identified differentially accumulated metabolites (DAMs) between mock vs DENV and mock vs ZIKV. PCA plot of 1636 features in positive ionization mode shows difference based on time and viral infection. Colors represent different groups (A). Volcano Plots depicting the number of significant features up (red) and down (blue) regulated with an FDR value <0.3 and fold change <0.75 or >1.25 (B).



C.

# of Comparisons Significant (max = 8)	# of Features
8	0
7	0
6	26
5	143
4	339
3	583
2	394
1	141
0	10

Figure 6. An UpSet plot representation shows overlap of significant features identified in the volcano plots in Figure 5. DENV (A) and ZIKV (B) across the four timepoints. Table count of significant features across all eight comparisons spanning both viruses (C).

3. Panel of candidate metabolites as biomarkers for flaviviruses detection.

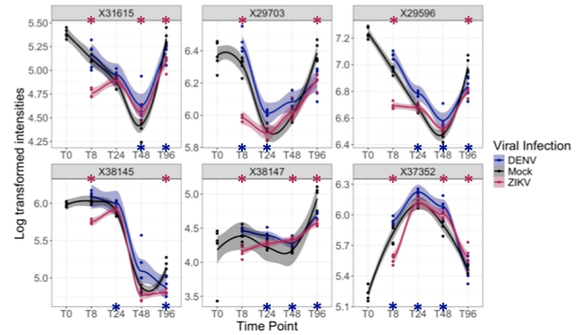


Figure 7. Differential regulation of potential markers of interest. LC-MS/MS analyses showed differential regulation of many features. Presented here are six metabolites of interest between mock vs DENV and mock vs ZIKV (statistical significance was calculated using t-test with and FDR correction).

Conclusions

- We demonstrated that 'Metabolomics' is a reproducible, and sensitive method for detecting flaviviruses in infected cells. It can differentiate infected from non-infected cells at an early time point (8hpi).
- In comparison to DENV, hiPSCs are more susceptible to ZIKV infection.
- LC-MS study identified overlapping but distinct metabolome profiles upon ZIKV and DENV infection.
- We discovered 6 potential metabolite markers in hiPSCs for flaviviruses detection.

Future directions:

1. Validate biomarkers in donor cells and tissues, including blood.
2. Study metabolomics in hiPSCs persistently infected with ZIKV and DENV.
3. Investigate whether these specific metabolites can also be utilized to detect other mosquito and tick-borne flaviviruses, such as WNV, PWV, etc.
4. Explore the potential of this technology for detecting challenging infectious diseases, such as herpes viruses.

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