UTILIZING PROTEIN SEQUENCING FOR THE DETECTION OF VIRAL HEMORRHAGIC FEVER BIOMARKERS

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INTRODUCTION

Viral hemorrhagic fever (VHF) infections are caused by RNA viruses such as dengue and hantaviruses.[1] These infections can result in severe and often life-threatening symptoms. VHF infections pose a significant risk to Department of War (DoW) personnel in many areas of responsibility due to the high prevalence of mosquitoes, ticks, and other potential VHF vectors. In 2024, over 1,000 cases of dengue virus were reported in Florida and over 6,000 cases in Puerto Rico.[2] For this reason, a better rapid diagnostic tools are needed for the detection of VHF infections, such as dengue, both in domestic and foreign areas of operational activity

Human serum albumin (HSA) is a potential and valid biomarker for VHF infections in human urine samples. For example, a 2020 study showed a >10-fold increase in urinary albumin in 30% of patients and a >100-fold increase in 57% of patients with a VHF infection.[3] HSA is a 66.5 kDa, monomeric, globular protein composed of 585 amino acids[4]. It is composed of three repeated homologous subunits and is used as diagnostic biomarker for diseases such as chronic kidney disease[4]. A clinical urine sample is considered normal when albumin levels are under 30 µg/mL and abnormal when the concentration is higher than 30 µg/mL, also known as microalbuminuria.[5] Changes in albumin levels are non-specific physiological responses and cannot be used as a standalone diagnostic tool for VHF infections.

Next-generation protein sequencing (NGPS) has become an important tool for identifying proteins, protein variants, and post-translational modifications. The Quantum-Si NGPS technology represents a groundbreaking advancement in protein sequencing, enabling the detection of biomarkers for VHF viruses and other pathogens. The Quantum-Si platform uses Endoproteinase LysC to cleave protein sequences after the lysine (K) amino acid residue, generating distinct peptides (Figure 1). These peptides are then activated, conjugated to K-linker, and sequenced on the Quantum-Si Platinum® Pro instrument.[6] This study aims to present the sequencing of HSA using the Quantum-Si Platinum Pro instrument as a method to detect VHF biomarkers in urine samples.

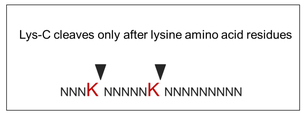

Figure 1. Lys-C cleavage of protein at lysine amino acid.

MATERIALS AND METHODS

- A total of 8 urines were collected onsite at the Naval Health Research Center and tested on the Fisherbrand Urine Analyzer for microalbumin concentration and A:C (albumin to creatinine) ratio. All samples were confirmed normal with an A:C ratio of less than 30 mg/g.
- Normal urine samples were digested with LysC at a concentration of 9.4 nM HSA. Samples were prepared and sequenced following the Quantum-Si Library Preparation and Sequencing Kits, which recognize 15 amino acids (Table 1).
- For the protein quantification experiments, 7 barcoded peptides (Table 2) were synthesized by the commercial vendor InnoPep, Inc. (San Diego, CA).
- Barcoded peptide experiments were accomplished by mixing 3 different concentrations (0.5, 1.0, and 2.0 µM) of purified HSA protein with a 1 µM mixture of the seven barcoded peptides before sequencing on the Quantum-Si Platinum Pro instrument.
- Four positive and four negative clinical dengue urine samples were prepared and sequenced using the Quantum-Si kits and Platinum Pro instrument.
- The peptide alignment and protein inference data were generated in the Quantum-Si Platinum Pro instrument (Figure 2). Sequenced data were extracted from the Quantum-Si Platinum Pro instrument and analyzed on the DAVID Bioinformatics tool[7] and jvenn[8] websites.

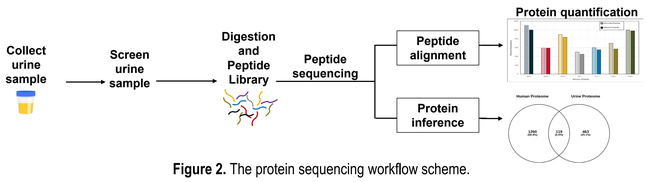

Figure 2. The protein sequencing workflow scheme.

RESULTS

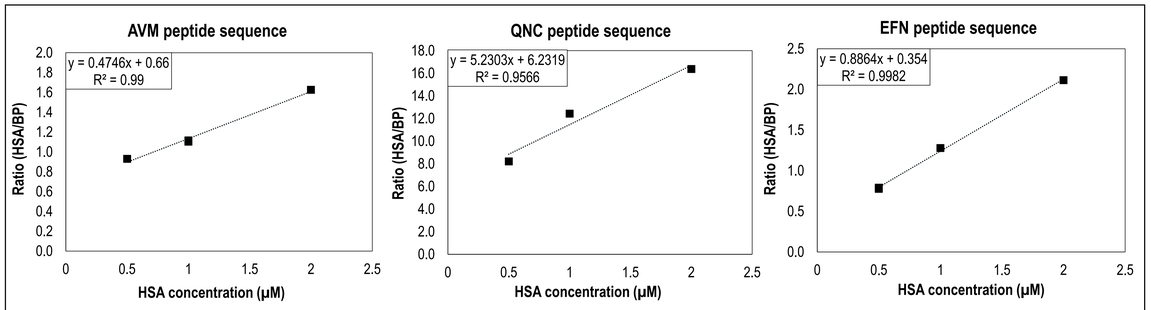

Figure 3. Standard concentration curves for HSA peptides AVM, QNC, and EFN used for protein quantification.

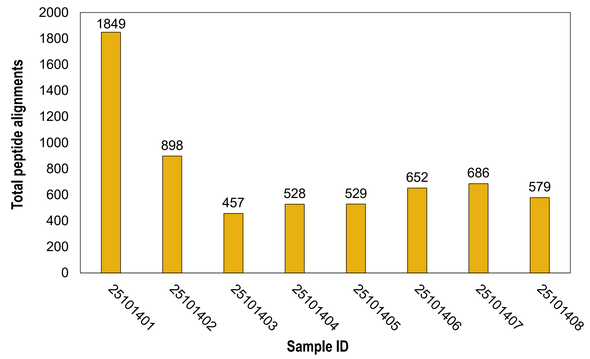

Figure 4. The total HSA peptide alignments for all urine samples.

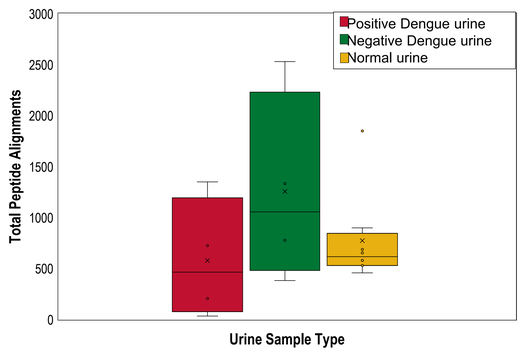

Figure 5. The total HSA peptide alignment comparison between urine sample types.

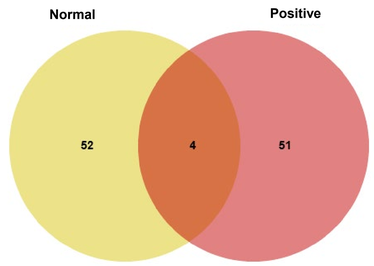

Figure 6. Venn diagram comparing proteins found in normal and positive Dengue urine samples.

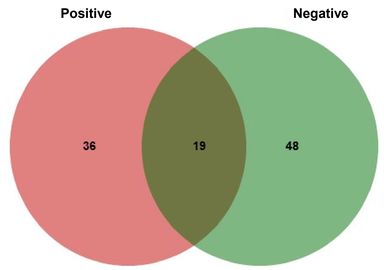

Figure 7. Venn diagram comparing proteins found in positive and negative Dengue urine samples.

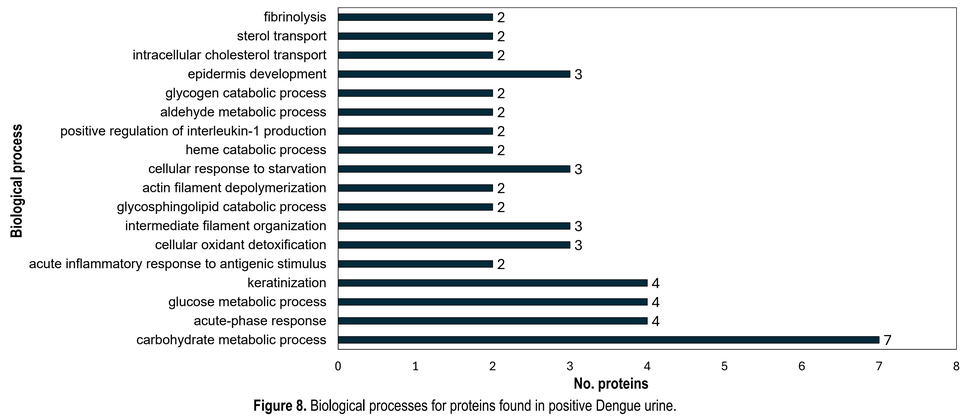

Figure 8. Biological processes for proteins found in positive Dengue urine.

TABLES

Table 1. Quantum-Si Sequencing Recognizers and Binding Amino Acids

Recognizer	Binding amino acid residue
FYW	Phenylalanine, tyrosine, tryptophan
LIV	Leucine, isoleucine, valine
R	Arginine
DE	Aspartic acid, glutamic acid
GAS	Glycine, alanine, serine
NQM	Asparagine, glutamine, methionine

Table 2. Barcoded HSA Peptides used for Protein Quantification Experiments

Peptide name	Sequence
BC_AVM	H-**NDYRL**AVMDDFAAFVEK-**NH_2**
BC_QNC	H-**QRESFL**QNCELFEQLGEYK-**NH_2**
BC_EFN	H-**FQRLA**EFNAETFTFHADICTLSEK-**NH_2**
BC_ALV	H-**ELFNR**ALVLIAFAQYLQQCPFEDHVK-**NH_2**
BC_YLY	H-**FALRQD**YLYEIARRHPYFYAPELLFFAK-**NH_2**
BC_FQN	H-**RQAEL**FQNALLVRYTK-**NH_2**
BC_AWA	H-**RFEQI**AWAVLARSQRFPK-**NH_2**

DISCUSSION

The standard concentration curves generated using barcoded peptides demonstrated high linearity, with coefficient of determination (R^2) values exceeding 0.95 for each peptide. This robust calibration model enabled the highly accurate calculation of HSA concentrations across experimental samples (Figure 3).

The technical workflow, encompassing enzymatic digestion, library preparation, and single-molecule sequencing using the Quantum-Si Library Preparation Kit, Sequencing Kit, and Platinum Pro instrument, was successfully executed for all normal urine samples. At a known baseline HSA concentration of 9.4 nM, all analyzed urine samples consistently yielded over 450 total peptide alignments, with individual sample yields ranging from 457 to 1,849 alignments (Figure 4). This workflow achieved a significant increase in peptide identification, successfully mapping at least 23 out of 60 potential HSA peptides per sample. Consequently, the platform demonstrated a sequence coverage rate exceeding 40% for the target protein.

Quantitative differences in peptide recovery were observed when comparing patient cohorts (Figure 5). Positive dengue urine samples (n=4) generated an average of 578 total HSA peptide alignments. In contrast, normal control urines yielded an average of 772 alignments, while negative dengue urines exhibited the highest density with an average of 1,256 total HSA peptide alignments.

Comparative protein inference analysis revealed distinct proteomic signatures across the cohorts (Figures 6 & 7). A core set of four proteins was mutually identified in both normal and positive dengue urine samples. Overlap analysis between the dengue cohorts revealed 19 shared proteins. However, strong cohort-specific differences were observed: 36 proteins were uniquely detected in positive dengue urine samples, whereas 48 proteins were isolated exclusively in negative dengue specimens.

CONCLUSION

High diagnostic utility: Urinary proteomics successfully distinguished between normal, positive, and negative dengue cohorts, offering a non-invasive screening tool.
Unique dengue biomarkers: Positive cohorts exhibited 36 unique urinary proteins involved in interleukin-1 production, heme, and carbohydrate metabolism pathways (Figure 8).

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ACKNOWLEDGMENTS

This work was supported by the Joint Science and Technology Office for Chemical and Biological Defense/Defense Threat Reduction Agency (DTRA), Research and Development Directorate, Chemical and Biological Technologies Department, Medical Science and Technology Division under project number CB11492.