TARGET ENRICHED NGS REVEALS WIDE BREADTH OF VIRUSES CAUSING ACUTE UNDIFFERENTIATED FEVER IN THAILAND

Presentation # 548

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Abstract

Background: Despite extensive laboratory testing, infectious agents were not detected in approximately 50% of patients hospitalized for acute undifferentiated febrile illnesses (AUFI) at Siriraj Hospital, Bangkok, Thailand. Unbiased Metagenomic Next Generation Sequencing (mNGS) enables detection of any microbe present in patient samples. Target enrichment for viruses represents a highly sensitive and cost-effective approach for overcoming host background in clinical specimens. Plasma collected from 811 patients with undiagnosed AUFI between 2014- 2021 were analyzed by mNGS coupled to target enrichment to identify known and novel viruses.

Methods: Plasma was pre-treated with benzonase before extraction on an Abbott *m*2000 instrument. mNGS libraries were prepared from double-stranded cDNA with Illumina Nextera XT reagents on an epMotion then combined in pools of 24 and hybridized to Comprehensive Viral Research Panel (CVRP; Twist Biosciences) probes covering >15,000 strains of vertebrate viruses. Captured viral sequences were amplified, quantified, and sequenced together on a MiSeq. Reads were taxonomically classified by the SURPI pipeline and aligned in CLC Bio Genomics Workbench software.

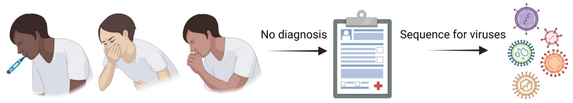
Results: CVRP method optimization enabled sequencing of 24-48 libraries per MiSeq run, for which >50% genome coverage was obtained with model viruses spiked into clinical specimens at 1000 cp/ml. This approach revealed an array of >24 different viruses found in 30% of samples. Dengue was the most prevalent at 4.9%, with all four genotypes detected. Other common causes of AUFI such as Chikungunya, HIV-1, HAV, HBV, HCV, and CMV were present in 1.5-2.5% of cases. Less prevalent (<1%) infections included HEV, HSV-2, EBV, HHV-6, Enterovirus B&D and Parvovirus B19. We also encountered sporadic cases of Measles, Cardiovirus, West Nile, Rotavirus, Picobirnavirus, Polyomavirus 4&5, Kubovirus, and Rabies lyssavirus.

Conclusions: Target capture successfully demonstrated that viruses were important etiologies for unresolved cases of AUFI in Thailand. This data has led to a better understanding of the epidemiology of this clinical syndrome and has implications for proper management of AUFI, including lower rates of unnecessary testing and antimicrobial use.

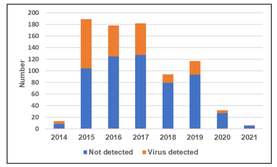
Recruitment of Patients with AUFI

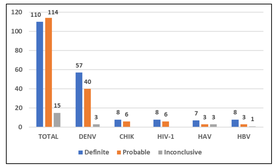
Acute undifferentiated febrile illness (AUFI) is defined as patients with febrile illness without an obvious cause of infection in this study. Most patients presented with acute fever of <14 days and flu like symptoms. Their plasma or sera were tested by indirect immunofluorescent assays (IFA) for the diagnosis of leptospirosis, scrub typhus, and murine typhus at the Division of Infectious Diseases and Tropical Medicine Laboratory, Department of Medicine, Siriraj Hospital. Left over plasma, serum and blood samples were deidentified and archived in -70^{0}C freezer.

The clinical information retrieved from their medical records included demographic data, initial clinical presentation such as duration of fever and results of laboratory investigations such as complete blood counds, blood chemistries, and NS1 rapid test, as appropriate. This study protocol was approved by The Ethical Committee at the Faculty Medicine Siriraj Hospital, Mahidol University (COA no: Si391/2021).



Summary of the Study

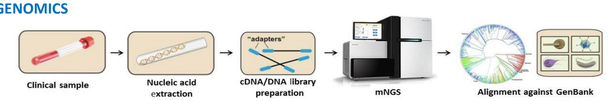




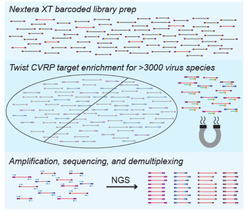
(Left) Overall, 243 (30%) out of 811 AUFI patient samples had evidence of a viral infection. The majority of them were positive for dengue (n=100, 11%), with DENV4 being the most prevalent genotype. (Right) A high degree of agreement was observed between virus target enrichment NGS data and clinical presentation.

Virus Target Enrichment Workflow

METAGENOMICS



CVRP TARGET ENRICHMENT



BIOINFORMATICS

Figure 2. Benzonased plasma from patients with AUFI were extracted and nucleic acid was converted to cDNA. Nextera XT was used to fragment and barcode NGS libraries. Sample libraries were pooled and hybridized to biotin-labeled CVRP probes tiling >3000 virus species. Viral sequences captured by streptavidin beads were amplified by KAPA PCR and then sequenced on a MiSeq. SURPI was used to taxonomically identify microbial reads.

CVRP Sensitivity and Multiplexing

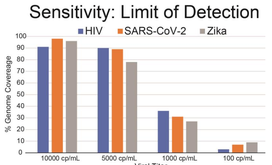
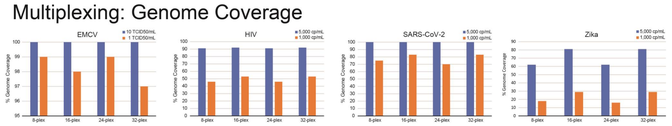
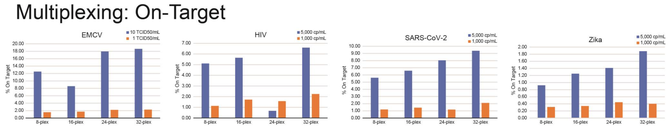


Figure 3. (top) Viruses of known titer (HIV, SARS-CoV-2, Zika) were serially diluted in patient plasma and 24 NGS libraries were pooled and enriched by CVRP. Percent coverage shows >30% of a viral genome is detected above 1000 cp/ml.

Viruses of known titer (EMCV, HIV, SARS-CoV-2, Zika) were serially diluted in patient plasma and 8, 16, 24, or 32 NGS libraries were pooled and enriched by CVRP. **(middle)** Multiplexing does not affect genome coverage. **(bottom)** Increased multiplexing actually improves percent on-target rates at the higher 5000 cp/ml titer.





References

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3. Briese, T., A. Kapoor, N. Mishra, K. Jain, A. Kumar, O. J. Jabado, and W. I. Lipkin. 2015. 'Virome Capture Sequencing Enables Sensitive Viral Diagnosis and Comprehensive Virome Analysis', *MBio*, 6: e01491-15.

Viruses Detected and Their Agreement with Clinical Data

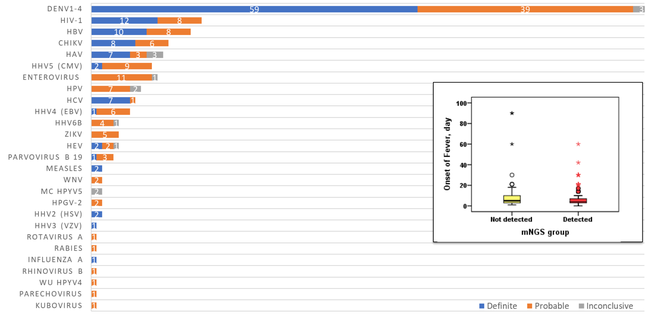

Figure 4. CVRP virus determinations were compared to clinical presentations as documented in patient records. A 'definite' case had an alternative confirmation (e.g. PCR). A 'probable' case had consistent symptoms, but without an alternative confirmation. An 'inconclusive' case indicates the NGS result was not consistent with symptoms or alternative diagnosis was made. (*inset*) The median duration from onset of symptoms was similar between the viral-detected group and the not detected group [4 days IQR 2,7 days and 5 days IQR 2,10 days respectively].

Viral Diversity: Dengue, Chikungunya, HIV, HBV

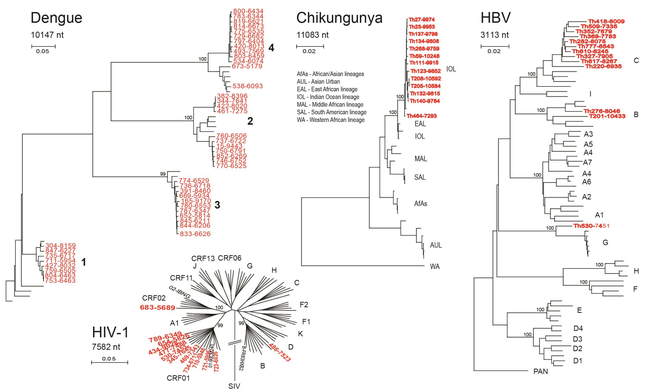

Figure 5. Complete genome sequences were aligned and analyzed by neighbor-joining phylogenetic trees. Newly obtained AUFI sequences are in red, reference strains are in unlabeled. All 4 genotypes of Dengue were observed in Thailand. Most strains of HIV were CRF01, with one subtype B and one CRF02. Chikungunya strains were all from the Indian Ocean lineage. HBV sequences were primarily genotype C, with 2 B and an outlier G sequence.

Discussion

Overall, we identified viruses in 30% of AUFI patient specimens, including relatively prevalent viruses such as Dengue, HIV-1, HBV, Chikungunya and HAV, and infrequently detected viruses such as VZV, Rotavirus A, Parechovirus, Kubovirus, West Nile virus, Enterovirus D68, Rabies lyssavirus virus and Measles. The clinical presentations of these patients (mainly acute fever and flu like symptoms or AUFI) were similar in both the relatively prevalent and the infrequently detected viral infections. Although AUFI could be the clinical manifestation of any viral infection, further studies to confirm the significance and impact of these viruses and to identify other non-viral agents as the causes of AUFI in Thailand are needed.