O0560 Metagenomic MinION whole-genome sequencing of emerging/re-emerging RNA viruses directly from clinical samples

Liana Kafetzopoulou*[1][2][4], Sophie Duraffour[2][3], Anke Thielebein[3], Lisa Oestereich[3], Roger Hewson[1][2], Julian Hiscox[2][4], Richard Vipond[5][6], Stephan Günther[7], Miles Carroll[5][6], Steven Pullan[5][6]

[1]*Public Health England, Research, Porton Down, United Kingdom*, [2]*NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, United Kingdom*, [3]*Bernhard Nocht Institute for Tropical Medicine, Virology Department, Hamburg, Germany*, [4]*Institute of Infection and Global Health, Department of Infection Biology, Liverpool, United Kingdom*, [5]*NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom*, [6]*Public Health England, National Infection Service, Porton Down, United Kingdom*, [7]*Bernhard Nocht Institute for Tropical Medicine, Department of Virology, Hamburg, Germany*

Background: Emerging and re-emerging viruses cause a significant global disease burden, ranging from mild febrile illness to haemorrhagic fevers. Rapid and unbiased identification methods, such as metagenomic sequencing, are vital for the identification and characterisation of an emerging pathogen for which little prior knowledge is available. Portable methodologies for field use are required during such outbreaks, especially when they occur away from established diagnostic laboratories. The aim of this work is to assess the feasibility and sensitivity of direct metagenomics on clinical samples both for detection and genome sequencing using the MinION.

Materials/methods: Archive real-time RT-PCR positive patient samples (serum, plasma, urine) of viral pathogens of interest (chikungunya, dengue, Lassa, West Nile) were selected to represent a range of viral titres seen in clinical samples, along with previously undiagnosed samples collected from febrile patients. RNA from each sample was reverse-transcribed and randomly amplified by Sequence Independent Single Primer Amplification and sequenced on the Oxford nanopore MinION.

Results: Sequencing of low to mid-range Ct value samples yielded high read depth of near complete genome coverage. Higher Ct value sample sequencing provided sufficient information for virus identification and further investigation. Taxonomic classification of reads using Kraken lead to the identification of a dengue/chikungunya co-infection and identified viruses present in a subset of the previously undiagnosed samples. The effectiveness of taxonomic classification and *de novo* assembly varied across the pathogens investigated.

Conclusions: We have demonstrated that direct metagenomic sequencing of clinical serum, plasma or urine samples, without the need for viral enrichment beyond DNase treatment, can reliably produce complete viral RNA genome sequences. Real-time genomic surveillance utilising the MinION for metagenomic sequencing provides the potential for both prompt viral identification and genomic sequencing, which is useful for viral surveillance and epidemiology.