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研究課題名（和文）Establishing a low-cost next-generation sequencing platform for on-site real-time surveillance of SARS-CoV-2 and other viruses in an unreached community from the Democratic Republic of Congo

研究課題名（英文）Establishing a low-cost next-generation sequencing platform for on-site real-time surveillance of SARS-CoV-2 and other viruses in an unreached community from the Democratic Republic of Congo

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研究成果の概要（和文）：このプロジェクトは、コンゴ民主共和国の遠隔地でも適用可能な迅速シーケンス用の携帯型ウイルス監視ツールキットを確立し、重要なマイルストーンを達成しました。協力協定、倫理クリアランス、ABS許可などの前提条件も満たされました。
しかし、試薬の調達や官僚的障害、COVID-19の制限により現地展開が大幅に遅れました。それにもかかわらず、DRCの農村部で1077人のサンプルが採取されましたが、日本への輸送でさらなる遅れが生じました。それでもこの研究は、携帯型ウイルスシーケンシングラボの実現可能性を証明し、将来の可能性を示しています。試薬は確保され、科学論文の発表に向けた取り組みが進行中です。

研究成果の学術的意義や社会的意義
The toolkit created in this project shows great potential for viral surveillance and investigations in remote areas of Africa. It promotes knowledge sharing, strengthens research capabilities, and empowers African communities, thus playing a crucial role in global health protection.

研究成果の概要（英文）：The project achieved significant milestones, establishing a portable viral surveillance toolkit for rapid sequencing that can potentially be applied in remote areas of the Democratic Republic of Congo (DRC). Administrative prerequisites were met, including collaboration agreements, ethical clearance, and obtaining the ABS permit.
However, field deployment faced substantial delays due to challenges in procuring reagents, bureaucratic hurdles, and COVID-19 restrictions. Despite these obstacles, surveys in rural DRC successfully sampled 1077 individuals. Yet, storing and transporting samples to Japan for analysis caused further delays. Nevertheless, this research proves the feasibility of a portable viral sequencing laboratory, showcasing its potential for future endeavors. Reagents have been secured for final analysis, and efforts are underway to evaluate the approach's validity for publication.

研究分野：Virology

キーワード：Viral genome　Nanopore sequecing　Congo　Hepatitis virus　Mpox virus

Establishing a low-cost next-generation sequencing platform for on-site real-time surveillance of SARSCoV-2 and other viruses in an unreached community from the Democratic Republic of Congo

1. Background (研究開始当初の背景)

The initial goal of this project was to create an affordable next-generation sequencing platform for immediate, real-time surveillance of SARSCoV-2 and other viruses in an underserved community in the Democratic Republic of Congo. As the SARS-CoV-2 pandemic waned, our focus transitioned to studying endemic viruses, particularly the hepatitis B virus (HBV).

HBV is a significant public health concern globally, particularly in sub-Saharan Africa, where it is endemic. HBV is an enveloped virus, with isolates being classified into ten phylogenetically distinct genotypes (A to J) determined based on full-genome sequence data or reverse hybridization-based diagnostic tests. HBV infection can lead to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, posing a substantial burden on healthcare systems and affecting the quality of life of millions of individuals. In highly endemic regions, such as parts of Africa, the prevalence of HBV can exceed 8%, with mother-to-child transmission and horizontal transmission during early childhood being the most common routes of infection.

Molecular surveillance of HBV involves the analysis of viral genetic sequences to monitor transmission patterns, track viral evolution, and identify the prevalence of different HBV genotypes and subgenotypes. This is crucial for understanding the epidemiology of the virus, implementing effective vaccination programs, and developing targeted antiviral therapies.

In Africa, the diversity of HBV strains is influenced by various factors, including human migration, cultural practices, and regional healthcare policies. Genotypes A, D, and E are predominantly found in this region, each associated with distinct clinical outcomes and responses to treatment. Molecular surveillance efforts in endemic areas are therefore essential for informing public health strategies and improving disease management.

Despite the high burden of HBV, molecular surveillance in many African countries remains limited due to resource constraints, lack of infrastructure, and insufficient access to advanced diagnostic tools. Enhancing molecular surveillance capabilities in these regions is critical for better understanding the virus's dynamics, optimizing vaccination

strategies, and ultimately reducing the incidence and impact of HBV-related diseases.

This study aims to establish a portable toolkit for rapid whole genome sequencing (WGS) of HBV using the MinION sequencer, including a workflow from sample to sequence and the associated bioinformatics pipeline. We also incorporate procedures for downstream analysis, interpretation, and management of genomic data in a surveillance context. Through testing and replication of methods across a community from Kasai Oriental in the Democratic Republic of Congo (DRC), we demonstrate the feasibility of real-time sequencing of HBV to rapidly inform policy decisions and disease management. We further highlight the potential for cost savings that could make routine genomic surveillance of HBV affordable in low-resource endemic settings. The developed toolkit shows potential for providing valuable insights into the molecular epidemiology of HBV and support the development of more effective control and prevention strategies tailored to the region's specific needs.

2. Research objectives (研究の目的)

This research aimed to set up an affordable next-generation sequencing platform for on-site detection and real-time surveillance of viral infections in rural communities from the DRC.

3. Research methods (研究の方法)

To facilitate HBV molecular surveillance in resource-limited settings, we developed a "lab-in-a-suitcase" platform—a portable toolkit designed to perform the entire process from blood sample collection to sequencing. This platform was equipped to operate under minimal laboratory infrastructure, with protocols specifically tailored to the conditions in the Democratic Republic of Congo (DRC).

3.1. Sample Collection and Nucleic Acid Extraction

Dried blood spot (DBS) samples were collected from individuals residing in Kasai Oriental, the DRC. The isolation of viral nucleic acids from these DBS samples was performed using the DNA QIAGEN kit, following the manufacturer's instructions. The extracted total nucleic acids were then resuspended in 50 µl of Tris-EDTA (TE) buffer.

3.2. PCR Amplification and sequencing of HBV Whole-Genome

HBV positive samples (tested with a rapid antigen test) were processed for whole genome amplification by using a polymerase chain reaction (PCR) protocol optimized for this

purpose. A total of 4 µl of the DNA template was used for the PCR reaction. Primers P1 (5'-CCGGAAAGCTTGAGCTCTTCTTTTTCACCTCTGCCTAATCA-3') and P2 (5'-CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG-3') were employed to target the HBV genome. The PCR amplification was optimized following this profile: denaturation at 94°C for 4 min followed by 11 cycles at 94°C for 40 s, 55°C for 1 min, and 72°C for 3 min; 11 cycles of 94°C for 40 s, 60°C for 1 min, and 72°C for 5 min; 11 cycles of 94°C for 40 s, 62°C for 1 min, and 72°C for 7 min; 11 cycles of 94°C for 40 s, 62°C for 1 min, and 72°C for 9 min; and a final extension step at 72°C for 10 min. The PCR reactions were carried out using the GoTaq polymerase mixture, as per the product recommendations, to ensure high-fidelity amplification of the HBV genome. This optimized PCR protocol allowed for the successful amplification of HBV whole-genome sequences, providing high-quality DNA suitable for subsequent molecular analyses.

The amplified sequences were then prepared for sequencing using the Oxford Nanopore Technologies (ONT) Rapid Barcoding Kit. Sequencing was conducted using the ONT MinION Mk1B device.

3.3. Bioinformatics

The bioinformatics analysis leveraged resources from the ARTIC network (https://artic.network/), which provides open-source tools for laboratory work, sequencing, bioinformatics, phylogenetics, and data analysis specifically aimed at viral outbreak responses. This comprehensive suite facilitated the processing and interpretation of sequencing data generated by our portable toolkit.

Additionally, specialized HBV bioinformatics tools were incorporated:

- The Genome Detective (https://www.genomedetective.com/): Used for viral genotyping to identify HBV genotypes present in the samples.
- The Geno2pheno (http://hbv.geno2pheno.org/): Utilized for profiling drug resistance, providing insights into potential antiviral treatment efficacy.

The combined use of these tools ensured robust, accurate, and context-appropriate molecular surveillance of HBV in the field. The protocol and toolkit were experimentally validated to confirm their effectiveness in the DRC's settings, demonstrating their utility for genomic surveillance in challenging environments.

4. Research results (研究成果)

4.1. Outcomes

Our project successfully established a "lab-in-a-suitcase" platform, which includes an

ONT MinION Mk1B sequencer (https://nanoporetech.com/ja/products/sequence/minion), a laptop, a Bento Lab (https://bento.bio/), a vortex device, a waist box, and a set of pipettes. During laboratory simulations, all equipment, along with necessary reagent boxes, could be packed into standard airline-sized checked luggage for easy transportation.

Experimental simulations were also successfully performed for whole genome amplification and sequencing of HBV from DBS samples collected from patients in the Kasai-Oriental province, DRC. The toolkit and protocol proved effective, successfully generating high-quality full-length HBV genomes. This allowed for detailed characterization of the genetic diversity and epidemiology of HBV in the region.

The successful implementation and utility of our portable laboratory in a resource-limited setting demonstrate its potential for field-based genomic surveillance. A scientific paper detailing these findings is currently being drafted for publication.

4.2. Challenges and Future Directions

A significant challenge encountered with our portable laboratory setup was the transportation of reagents from Japan to the DRC, a journey of approximately 24 hours by flight. The ONT reagents essential for this protocol require a cold chain transport, maintained at -20°C or -80°C. While transportation under dry ice was considered, it was not easily approved by the airlines. Additionally, establishing a direct supply chain to the DRC has proven difficult. As an interim solution, we conducted field surveys and decided to transport samples back to Japan for analysis. DBS used for developing the HBV toolkit are easy to transport and pose no infectious risk. However, they often yield inconsistent results due to the low viral DNA content, resulting in many PCR-negative outcomes from positive patient samples (as per rapid antigen tests).

To address this, we planned to adapt our analysis to use patient serum, which is expected to provide more reliable results. Serum samples have already been collected from Congolese residents, women in labor, and their newborns, and are stored at the National Institute of Biomedical Research (INRB), our collaborative institute located in Kinshasa, the DRC. Procedures are underway to transport these samples to Japan for analysis using our portable laboratory toolkit.

Looking ahead, we aim to further adapt our toolkit to analyze mpox viruses and other endemic viruses in the study population, enhancing its utility for broader viral surveillance efforts.

５．主な発表論文等

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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関