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Rapid and sensitive direct detection and identification of poliovirus using nanopore sequencing

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Introduction

Global poliovirus surveillance involves the identification of the virus from both the stool samples of potential cases and from environmental samples (sewage) that can reveal transmission of the virus within a population. The current detection algorithm requires the culture of the virus within cell lines and can take 2-3 weeks to progress from sample receipt to a sequencing result, with confirmation of poliovirus being established through the genetic sequence of the VP1 capsid region. Rapid detection and identification of the virus is however essential in informing the correct response to a potential virus outbreak.

We have developed a rapid poliovirus amplification and sequencing method using the Oxford Nanopore MinION that has allowed us to provide viral identification in under three days. We can rapidly and accurately determine between the three poliovirus serotypes, identifying both wild-type and Sabin (vaccine) strains. We were also able to resolve samples containing mixtures of polioviruses, including vaccine-derived polioviruses (VDPVs) which can deviate from vaccine (Sabin) strains by as little as six nucleotides over the ~900 bp VP1 region.

Method

VP1 amplicons were generated using a nested PCR approach. Modifications to the inner primers that would facilitate library preparation were tested:

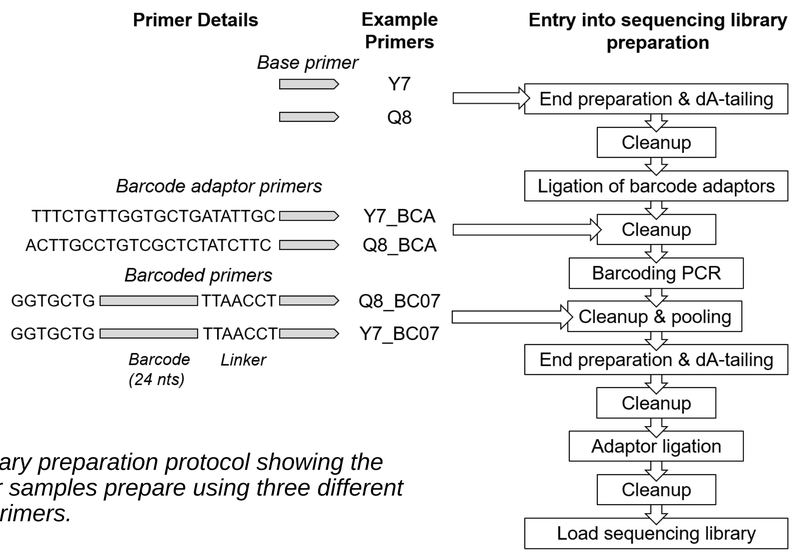


Figure 1 – Library preparation protocol showing the entry points for samples prepare using three different types of VP1 primers.

Primers with attached barcodes were found to be non-inferior, and greatly stream-lined library preparation. Prepared libraries were sequenced on the Oxford Nanopore MinION, with live basecalling and real-time analysis using RAMPART and a custom poliovirus module. Sequencing results were compared to those established by cell culture and Sanger sequencing, the current gold standard.

Results

Sensitive detection in stool

The protocol was tested on 155 stool samples at the WHO Regional Reference Laboratory in Pakistan. The samples contained a variety of wild-type poliovirus, Sabin strains and VDPVs. The following sensitivities and specificities were observed:

Serotypes	Sensitivity (%, CI)	Specificity (%, CI)
Wild-type 1	90.9 (75.7 – 98.1)	99.2 (95.5 – 100.0)
Sabin 2 and VDPV 2	92.5 (79.6 – 98.4)	98.7 (95.4 – 99.8)
Sabin 1 and 3 (alone or in mixtures)	88.3 (81.2 – 93.5)	93.2 (88.6 – 96.3)

Live Analysis

Sequencing runs were analysed in real-time via RAMPART (https://github.com/polionanopore/realtime-polio):

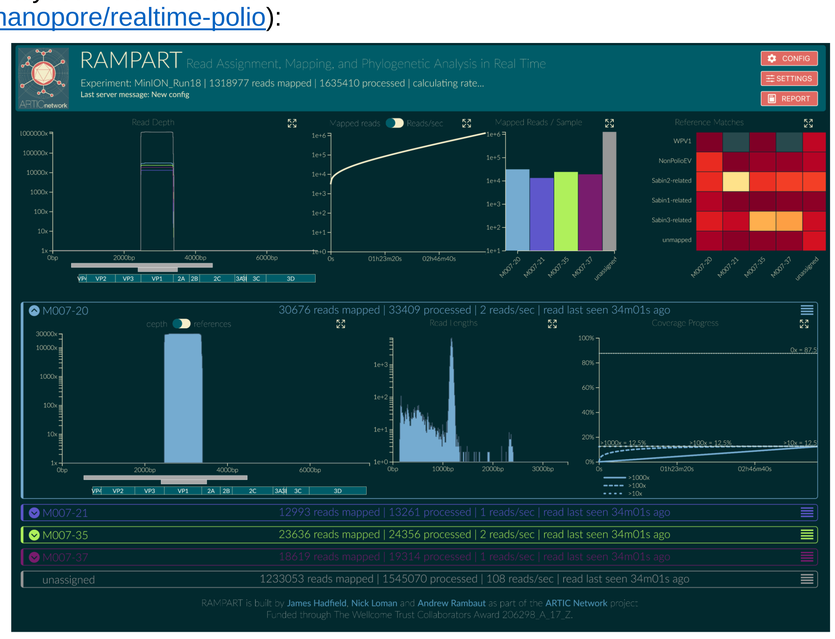


Figure 2 – Screenshot from RAMPART running the custom real-time poliovirus module. Samples are demultiplexed and reads mapped to a VP1 database containing polioviruses and non-polio enteroviruses.

Sensitive detection in sewage

36 environmental surveillance (ES) samples from Pakistan were tested using the protocol and the results compared to cell culture. Illumina sequencing was also performed in addition to Sanger sequencing of serotype-specific amplicons, allowing comparison across platforms:

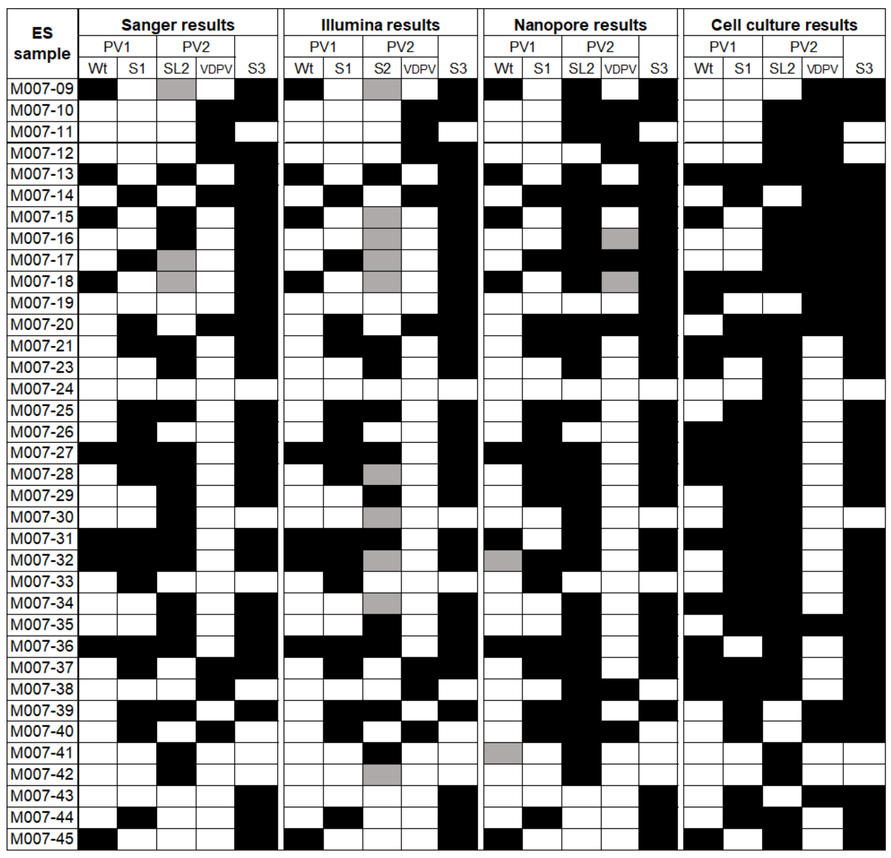
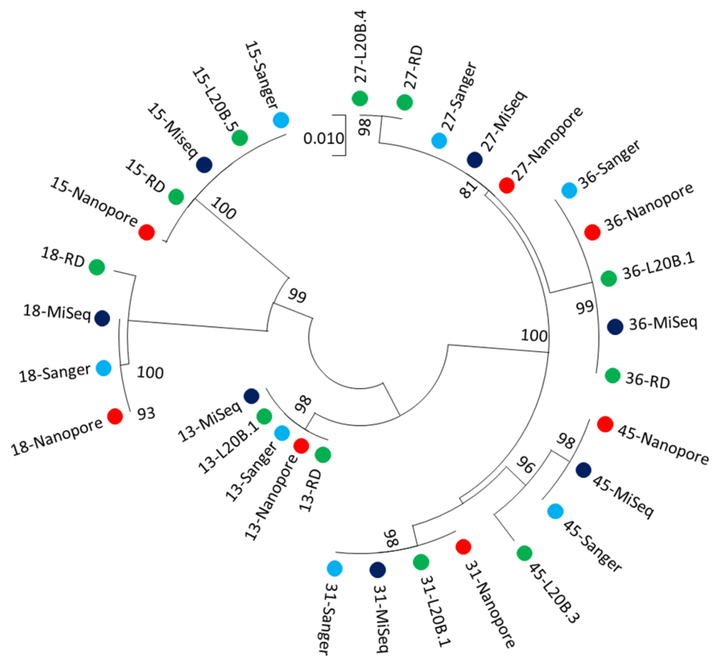


Figure 3- Polioviruses detected in Pakistan ES samples by cell culture or direct PCR followed by sequencing using three different methods. A black cell indicates detection of the virus. Sabin-related detections have been clarified as either Sabin (S) or VDPVs where possible. For Illumina and serotype-specific Sanger results, grey indicates the detection of Sabin poliovirus and the possibility of a VDPV based on multiple peaks and SNP analysis respectively. For nanopore results, grey indicates detection of the virus but at a low read threshold.

Accurate Consensus Identities

Where wild-type viruses were identified in ES samples using multiple techniques, consensus sequences were generated and clustered by relatedness:

Figure 4 – Relatedness of wild-type poliovirus 1 identified in ES samples in Pakistan using alternative methods. Consensus VP1 sequences derived by each sequencing platform (Sanger, MiSeq, nanopore) are shown in addition to the VP1 sequences of corresponding culture isolates (L20B, RD, labelled according to cell line used in isolation). Prefix numbers (15, 18,..) indicate the ES sample the sequence derives from.



Conclusion

We demonstrate a rapid and accurate method for poliovirus detection and typing via nested PCR with barcoded primers and nanopore sequencing. The method has been shown to be effective for both stool samples and sewage, and the consensus sequences generated closely match those determined by both Sanger and Illumina sequencing. Live analysis of the sequencing data is performed by a custom RAMPART module, with the overall process taking only 2-3 days from sample to final sequence. This method can be easily transferred to poliovirus laboratories and integrated into their existing infrastructure, facilitating more rapid responses as part of the global eradication effort.

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