

Successful training of laboratories in Asia and Africa to use nanopore sequencing for poliovirus surveillance

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Background

Reducing the time taken to get from sample to sequence confirming the presence of a vaccine-derived poliovirus (VDPV) can in turn reduce the size of an outbreak by facilitating a faster response. Current gold standard methods for poliovirus detection rely on cell culture and shipping samples, sometimes internationally, to specialised sequencing laboratories, causing delays in identifying the virus. Using Oxford Nanopore sequencing technology can allow labs to undertake their own sequencing without the need to ship samples, and with low overhead cost and space requirement. We have developed a method for rapid direct detection of poliovirus using nanopore sequencing (DDNS) which can reduce the time taken from sample to sequence from weeks to days, and are in the process of training both national and international labs in its use for poliovirus surveillance. Once enough data is gathered, we can work towards having the method approved by the Global Polio Lab Network for use in all reference labs. These training workshops and feedback from the trainees has given us valuable insights into the expansion of nanopore sequencing into Asia and Africa, and here we discuss some of the challenges faced in the provision of training and in continued implementation, giving insight into how nanopore technology can be successfully deployed in these regions.

DDNS Method

Viral RNA is reverse transcribed and amplified in a nested PCR reaction with the second set of primers incorporating ONT PCR barcode sequences.

All samples are pooled equally by volume then concentrated and prepared for sequencing (SQK-LSK110, FLO-MIN106—soon shifting to kit14) on a MinION Mk1B, Mk1C, or GridION.

A complete pipeline for separating poliovirus types and creating consensus sequences, PIRANHA, has been packaged into a user friendly tool, Artifice, which can be run in Windows or Linux without the need for command line use.

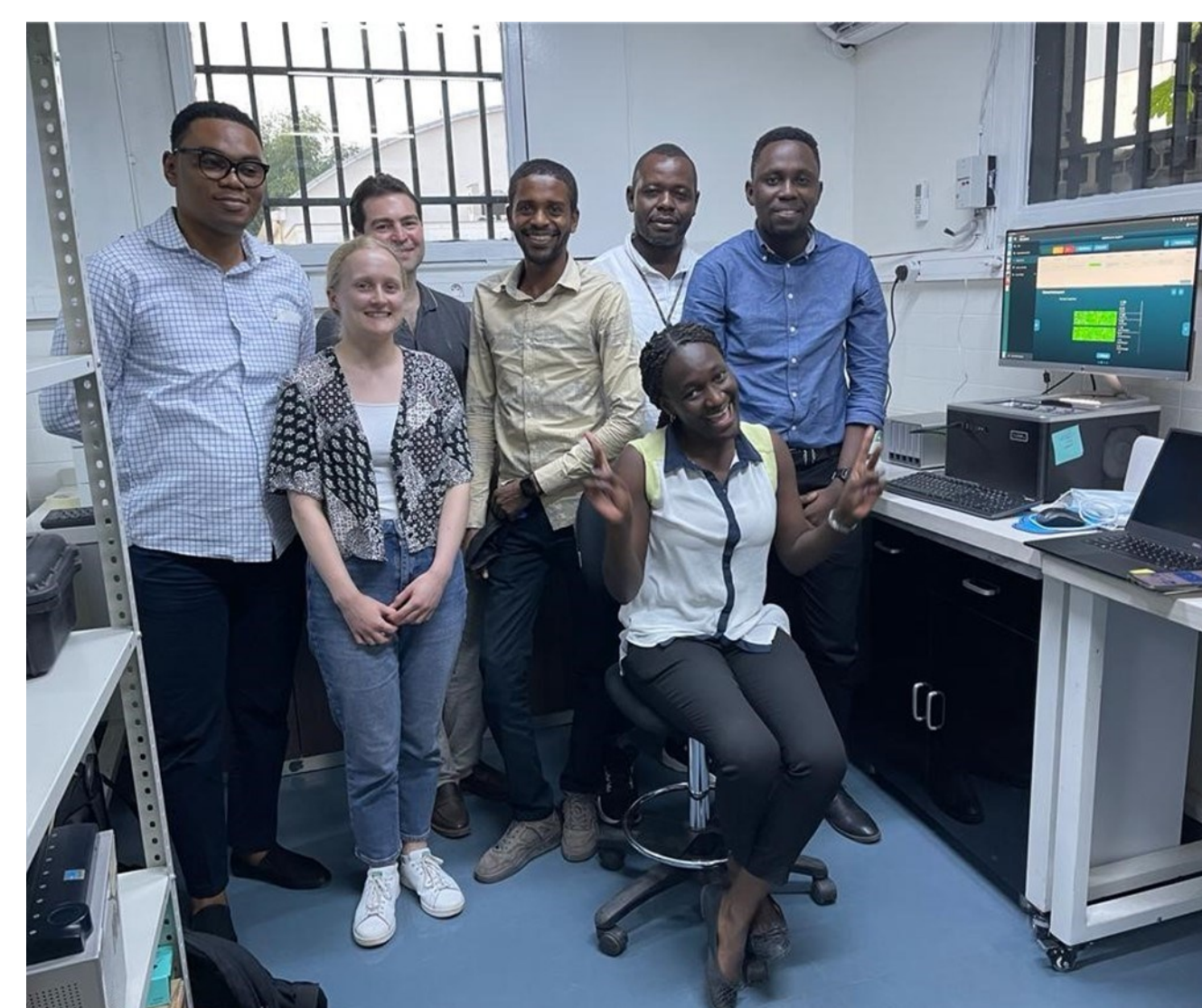
Training overview

- ◇ Two weeks training
- ◇ Combinations of presentations, discussions, and practical lab work
- ◇ Lab work can be performed first by the trainers before the trainees perform their own
- ◇ Sessions dedicated to sequence analysis and QA/QC of data
- ◇ Discussions on how to report the sequencing results
- ◇ Discussions on how to continue using DDNS
- ◇ Include extra sessions as requested e.g. Native barcoding for other amplicons of interest, phylogenetics



NIH, Pakistan, February 2020

In the lab, Pakistan, February 2020



INRB, the DRC, March 2022

In the lab, DRC, March 2022



CPHL, Oman, December 2022

Virtual training, October 2021



In the lab, Oman, December 2022

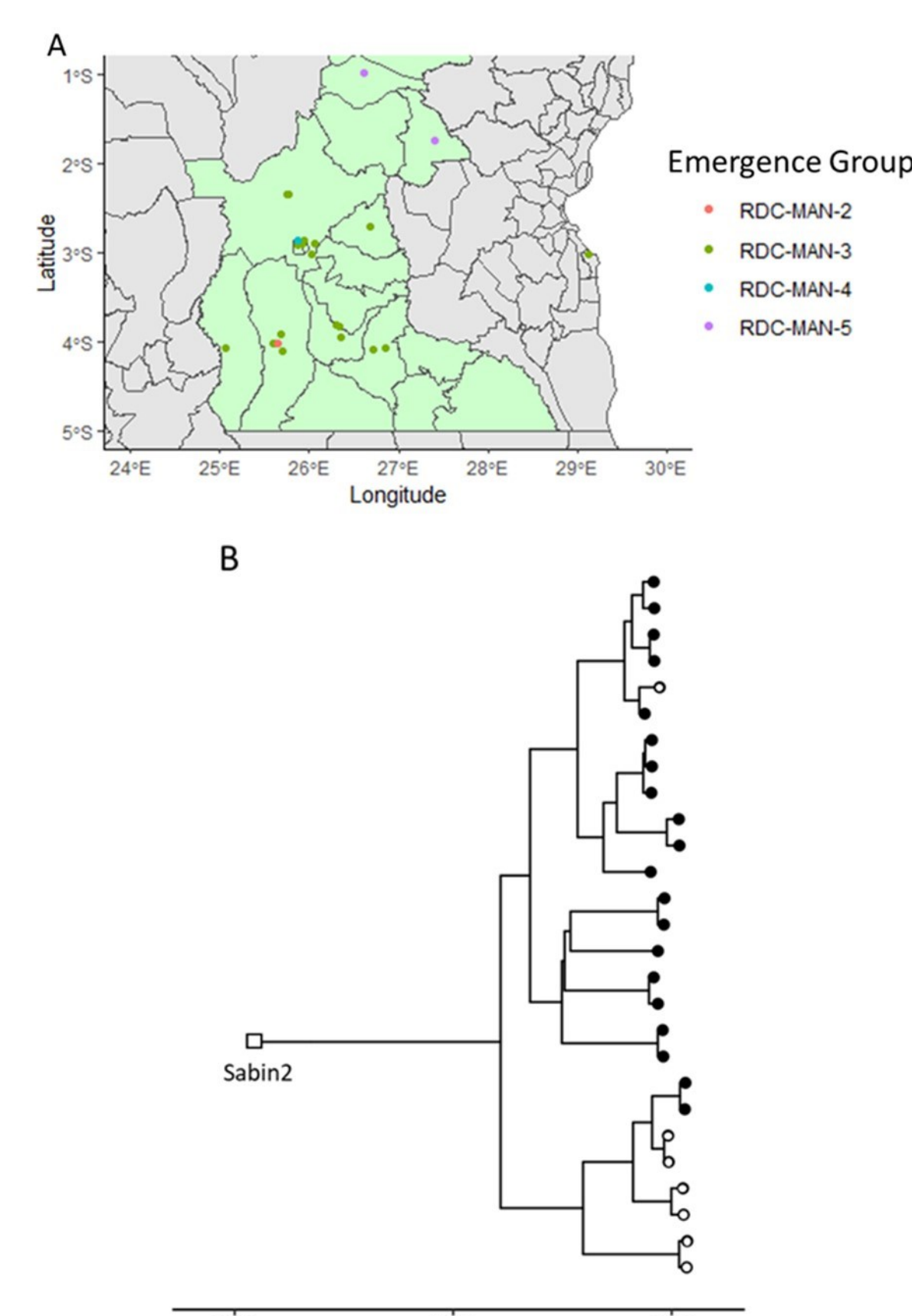
Top Tips:

- 1) Early contact to confirm dates and requirements gives plenty of time to organise travel and accommodation, visas, and reagents/equipment
- 2) Arrange follow up calls after training to provide support and ensure the lab has everything they need to continue sequencing
- 3) Have enough people so that there is always someone to work in the background, helping to organise the data and keep track of all experiments and samples

Impact of DDNS: a case study in the DRC

In August 2021, whilst continuing prospective testing of samples using the DDNS method, the lab in the DRC identified VDPV2 positive stool samples an average of 23 days before the same result was achieved by the current standard method. Over the course of the next 6 months the lab detected 36 VDPV2 positive stool samples associated with four separate VDPV2 outbreaks (A).

The consensus sequences obtained from DDNS had a mean sequence identity of 99.98% compared to the Sanger sequence obtained by the standard method. Figure B shows the relatedness of the RDC-Man-3 lineage, with solid nodes representing a DDNS sequence with matched result from the standard method.



Challenges faced

- ◇ Procurement
 - ⇒ Inflated costs from local suppliers and unreliable timing
- ◇ Power supply
 - ⇒ Labs do not always have a constant power supply, but often have back up generators
- ◇ Internet /Network
 - ⇒ Not all labs have strong internet connection and some have restrictions imposed on the network