

## **Amplicon-based sequencing**

Alt-text Figure 5 - Flowchart of a sequencing protocol from viral RNA isolation to sequencing analyses

A flow diagram showing the processes following RNA extraction and how many days these take. The first flow is ordered as follows: RNA extraction, RT-PCR, PCR Amplification, Library Preparation, Illumina Sequencing, and Sequence Analysis. These processes take three days. The second flow is RNA extraction, qPCR detection, and Report. These processes take two days.

Alt-text Figure 6 - The ARTIC sequencing protocol

The ARTIC sequencing protocol. The protocol is as follows: A single strand of RNA is completed with cDNA synthesis. This undergoes multiplex PCR with untailed primers. Pools are combined and quality controlled. Barcodes are added or NGS libraries prepared. These form a sequencing library. This library is normalised, quality controlled and sequenced in either Oxford Nanopore or Illumina machinery.

Alt-text Figure 7 - The Midnight protocol

This is a diagram of the Midnight protocol. It shows the steps and the time taken for each. The process begins with RT-PCR (265 minutes), and then pooling (5 minutes). A 1200bp amplicon is rapidly barcoded (15 minutes), and then these are subjected to sample pooling, SPRI clean, quantification, and rapid adapter addition (all taking 35 minutes). Finally, these are loaded into the sequencer (10 minutes).