May 18, 2023

Quality guidance on the use of run controls for direct detection of poliovirus by nanopore sequencing

DOI
dx.doi.org/10.17504/protocols.io.14egn2jdmg5d/v1

Joyce Akello[1], Manasi Majumdar[2], Alex Shaw[1], Catherine Troman[1], Erika Bujaki[2], Javier Martin[2], Nick Grassly[1]

[1]Imperial College London; [2]National Institute for Biological Standards and Control

Joyce Akello
Imperial College London

DOI: dx.doi.org/10.17504/protocols.io.14egn2jdmg5d/v1

Document Citation: Joyce Akello, Manasi Majumdar, Alex Shaw, Catherine Troman, Erika Bujaki, Javier Martin, Nick Grassly 2023. Quality guidance on the use of run controls for direct detection of poliovirus by nanopore sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2jdmg5d/v1

Created: February 27, 2023

Last Modified: April 19, 2024

Document Integer ID: 77699

Keywords: Poliovirus, Direct detection, Nanopore sequencing, Extraction run controls

Funders Acknowledgements:
Bill and Melinda Gates Foundation

Abstract

This document describes the laboratory process for the use of run controls (positive and negative controls) for performing direct detection of poliovirus by nanopore sequencing (DDNS assay).

Guidance on the use of run controls for direct detection of poliovirus by nanopore sequencing

Purpose

To outline the key steps of how and when to use the run controls for DDNS assay including preparation, storage, and outcome interpretation. The run controls are used to demonstrate that the entire DDNS workflow starting from RNA extraction to obtaining a sequence is successfully performed.

Associated forms & documents

- DDNS protocol v2.0 (**DDNS protocol v (protocols.io)**)
- DDNS metadata and QC
- Poliovirus direct detection and nanopore seqeuncing (DDNS) FAQs (**Poliovirus direct detection and nanopore sequencing (DDNS) FAQs (protocols.io)**)

Reagents

- Coxsackie virus A20 –(Supplied by NIBSC, contact Dr. Manasi Majumdar at **Manasi.Majumdar@nibsc.org** and copy Dr. Javier Martin at **Javier.Martin@nibsc.org**)
- Nuclease free water (NFW)
- RNA extraction reagents (Recommend using the MagMAX Viral RNA Isolation kit performed manual or automated [ThermoFisher], the QIAamp Viral RNA Kit [Qiagen], or Roche High Pure Viral RNA Kit with proteinase K [Roche])

Equipments

- Microbiological Safety Cabinet, Class II (MSCII)
- PPE (Gloves, Lab coat)
- Vortex mixer
- Benchtop centrifuge
- Sterile 1.5ml Eppendorf tubes DNase/RNase free
- Calibrated pipettes single channel (0.2µl to1000µl)
- Sterile pipette tips with filters (10-1000µl)
- Trend monitored freezer, -20^{0}C and refrigerator, 4 ^{0}C
- Disposal Dispo-safe “sweetie” jar or bio-bin

Procedure

The positive and negative controls will both undergo all steps from sample RNA extraction, PCR amplification to nanopore sequencingrun in parallel with the samples. This is to ensurethat the entire DDNS workflow is controlled for cross contamination, failed RNA extraction, PCR amplification, and sequencing.

Positive control:
- Content: CVA20 known to amplify in the PanEV RT-PCR and nested VP1 PCR
- Purpose:
  - Demonstrate that the DDNS method is successfully performed and giving the expected level of sensitivity and specificity as characterised during technical optimisation.
  - Confirms that negative results are accurate.

Negative control:
- Content: Nuclease free water (NFW) known not to amplify in the PanEV RT-PCR and nested VP1 PCR
- Purpose:
  - Check for non-specific signal and false-positive results.

Run controls preparation, storage, and use

CVA20 positive control reconstitution

Note: The CVA20 positive control must be reconstituted before use.
No attempt should be made to weigh out the freeze dried material
Infectious material – Handle virus in a Class II Microbiological Safety Cabinet (MSCII)

1. Working in the MSCII, reconstitute the vial containing the lyophilised CVA20 (freeze dried material) by adding 1mL of nuclease free water (NFW)

Lyophilised CVA20 positive control

2. Vortex briefly to ensure that the material completely dissolves in water giving a colourless liquid

Reconstituted CVA20 positive control in 1mL of NFW

3. Aliquot the solution into single-use volumes of 30 µl in 1.5mL sterile eppendorf tubes (DNase/RNase free) and store all aliquots at -20^{0}C for future use.

Note: The operator must label the tubes with the date the positive control was reconstituted.

Aliquots of the resuspended CVA20 can be store at -20°C for up to 5 weeks. Aliquoting will reduce the chance of source contamination as well as help to preserve the stability of the CVA20 solution by reducing the number of freeze-thaw cycles of the entire solution.

Use of the positive extraction control (CVA20) and negative extraction control during RNA extraction.

Note: Include a positive extraction control and a negative extraction control for every RNA extraction batch with new preparations of solutions.

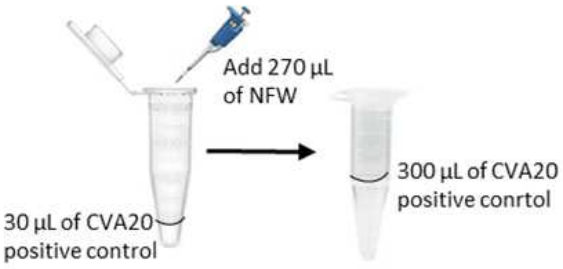
4. Prepare the CVA20 positive extraction control for RNA extraction as follows:

a. Retrieve a 30 µl aliquot of the CVA20 from the -20^{0}C freezer

b. Allow to thaw at room temperature whilst in the MSCII cabinet

c. Briefly centrifuge for 5 secs.

d. Add 270 µl of NFW and pipette up and down to mix the solution. The solution is now ready for immediate RNA extraction



Note: It is the responsibility of the operator to check that the batch positive control is added to the process.

5. Prepare the negative extraction control for RNA extraction as follows:

a. Aliquot 300 µl of nuclease free water into a sterile 1.5mlEppendorf tube (DNase/RNase free)labelled ExNTC (extraction negative control)

6. Perform RNA extraction of the samples in parallel with the positive and negative control ensuring to include run extraction controls on the first and last RNA extraction batch run of the day

7.The elutes / purified RNA from the controls and samples following RNA extraction can now be processed according to the DDNS protocol.

Note: The elutes /purified RNA should be kept on ice after extraction and while working with it. If the eluted RNA if not for immediate use, store at - 80°C

Run controls validation

The PCR products of the run controls can be checked prior to sequencingto ensure that the RNA extraction and PCR amplification steps worked efficiently. Checking of the run control PCR products can be performed using the Agilent TapeStation system or gel electrophoresis. For the sample RNA extraction and the PCR amplification assay run to be accepted as valid, the PanEV RT-PCR and nested VP1 PCR results for the positive and negative

extraction controls as well as the PCR no template control must be of the excepted results as resolved and visualised by the TapeStation or gel electrophoresis (see figure 1 below)

For the controls to meet QC requirements and validate each DDNS assay run, refer to the DDNS metadata and QC document for guidelines.

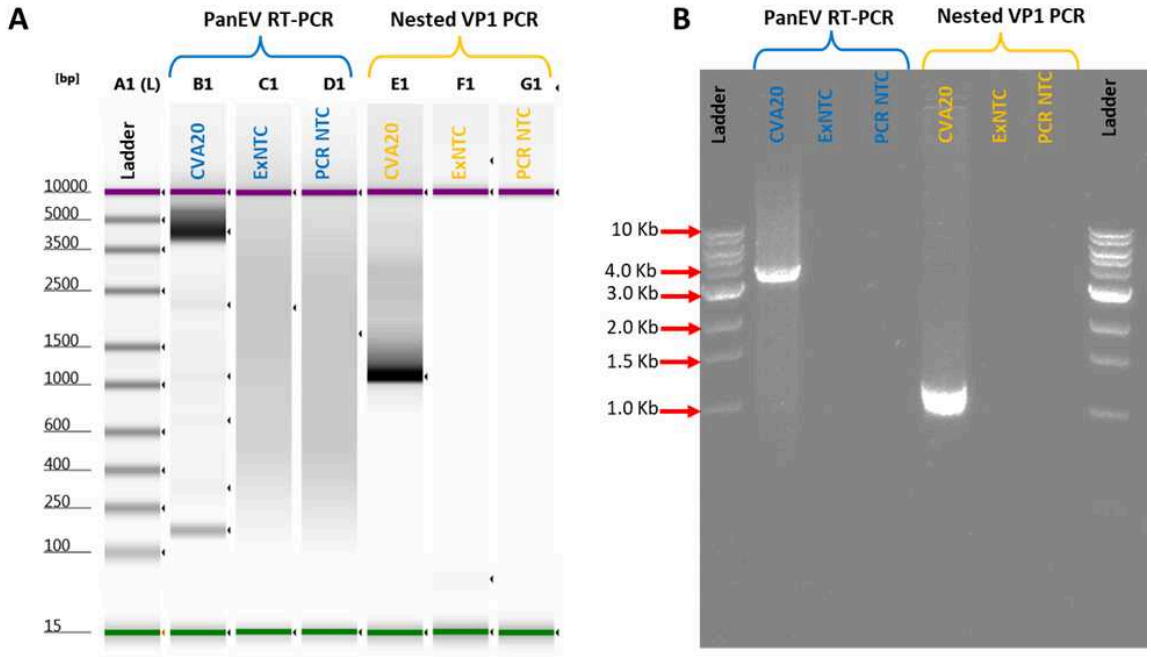


Figure 1. The Tapestation results for the DDNS assay controls from PanEV RT-PCR and nested VP1 PCR products (Panel A) and the gel electrophoresis results for the DDNS assay controls from PanEV RT-PCR and nested VP1 PCR products (Panel B). The positive extraction control labelled as CVA20 with a PanEV RT-PCR band at approximately 4.2kb and nested VP1 PCR band at approximately 1.2kb. The negative extraction control as ExNTC with no band in both the PanEV RT-PCR and nested VP1 PCR. The PCR no template control as PCR NTC with no band in both the PanEV RT-PCR and nested VP1 PCR.