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Preparation, storage and use of the positive and negative controls for DDNS V.2

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We use this protocol and it's working

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Abstract

This protocol 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).

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

Positive control (Coxsackievirus A20) – Supplied by MHRA, contact Dr. Erika Bujaki at **erika.bujaki@mhra.gov.uk**. and Dr. Thomas Wilton at **thomas.wilton@mhra.gov.uk**. and copy Dr. Javier Martin at **javier.martin@mhra.gov.uk**).

Before start

Positive control:

- Content: CVA20 known to amplify in the first round PCR (semi-nest) and second round PCR (nested VP1).
- 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 first round PCR (semi-nest) and second round PCR (nested VP1).
- Purpose:
  - Check for non-specific signal and false-positive results.

CVA20 positive control reconstitution

1 Reconstitution of the positive control

Note

The CVA20 positive control must be reconstituted before use. No attempt should be made to weigh out the freeze dried material

Safety information

Infectious material – Handle virus in a Class II Microbiological Safety Cabinet (MSCII)

1.1 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

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

Reconstituted CVA20 positive control in 1mL of NFW

1.3 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

2 Prepare the CVA20 positive extraction control for RNA extraction as follows:

Note

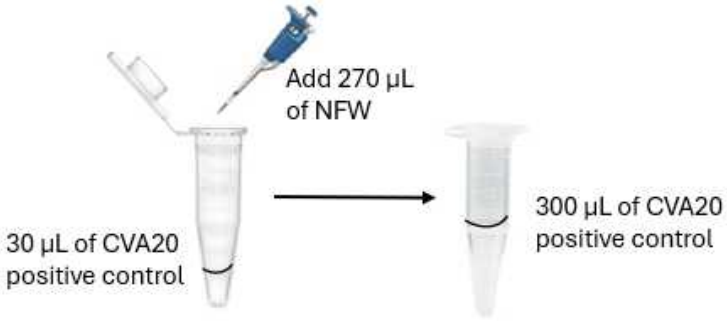
Include a positive extraction control and a negative extraction control for every RNA extraction
batch with new preparations of solutions.

2.1 Retrieve a 30 µl aliquot of the CVA20 from the -20°C freezer.

2.2 Allow to thaw at room temperature whilst in the MSCII cabinet

2.3 Briefly centrifuge for 5 secs.

2.4 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.

3 Prepare the negative extraction control as follows:

3.1 Aliquot 300 µl of nuclease free water (NFW) into a sterile 1.5ml Eppendorf tube (DNase/RNase free) labelled ExNTC (extraction negative control)

4 Perform RNA extraction of the samples in parallel with the positive and negative controls.

5 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 is not for immediate use, store at - 80°C