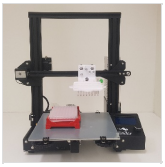

Sep 09, 2020

High-throughput SARS-CoV-2 RNA extraction from nasopharyngeal swabs using repurposed 3D printers v2.

Forked from High-throughput SARS-CoV-2 RNA extraction from nasopharyngeal swabs using repurposed 3D printers.

Koen Vandelannoote[1]

[1]University of Melbourne

1 *Works for me* This protocol is published without a DOI.

Koen Vandelannoote

Koen Vandelannoote

ABSTRACT

Here, we describe a semi-automated workflow for paramagnetic bead-based extraction of SARS-CoV-2 RNA from human upper respiratory samples that uses low-cost repurposed *Creality3D* Ender-3 3D-printers and readily accessible reagents. The protocol described here is for the use of four instruments in parallel, each processing 24 samples, 96 samples per batch.

ATTACHMENTS

20200908_Ender_VX-500_extraction_template.xlsx RNA_ex_v10.gcode

PROTOCOL CITATION

Koen Vandelannoote 2020. High-throughput SARS-CoV-2 RNA extraction from nasopharyngeal swabs using repurposed 3D printers v2.. **protocols.io** https://protocols.io/view/high-throughput-sars-cov-2-rna-extraction-from-nas-bk6qkzdw

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KEYWORDS

Ender VX-500, RNA extraction, 3D printer, SARS-CoV-2, nasopharyngeal swabs, COVID-19

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41904

MATERIALS TEXT

REAGENTS:

- Lysis buffer ([M]**6 Molarity (M) Guanidinium thiocyanate** , [M]**150 Milimolar (mM) Tris-Cl** pH**8** , [M]**30 Milimolar (mM) EDTA** pH**8** , [M]**90 Milimolar (mM) DTT** , 3% (v/v) Triton X-100).

- SPRI bead mix prepared for RNA binding as described in https://openwetware.org/wiki/SPRI_bead_mix

This SPRI bead mix is a drop-in substitute for RNAClean XP beads (Beckman Coulter) at about 1/100 of the cost.

- 100% Ethanol
- 80% Ethanol solution
- Nuclease free water (NFW)

PLASTIC CONSUMABLES:

- 96-well plates with V-shaped wells (Greiner – Ref. 651201)

It is highly recommended to work with the specified 96-well plates. The use of alternative plates requires careful modifications to the Ender VX-500 "print protocol".

- 8-tube PCR strips (SSIbio - Ref. 3247-00)

It is highly recommended to work with the specified strip tubes. The use of alternative strips requires careful modifications to the Ender VX-500 "print protocol".

- 96 deep-well plates **(**Axygen product number P-DW-11-C-S**)**

SAFETY WARNINGS

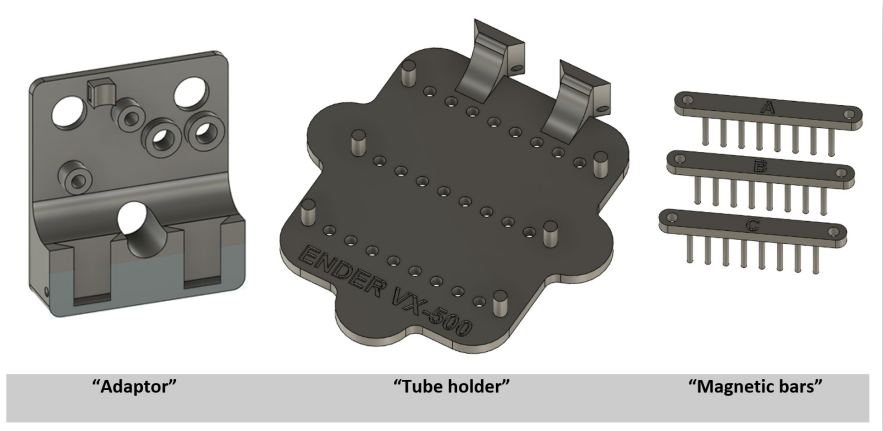
Nasopharyngeal swab samples that may contain respiratory viruses should be handled in a Class II BSC. Standard PC2 laboratory personal protective equipment is required. This includes a long sleeved laboratory gown, gloves and safety glasses. Additionally, a face mask should be worn.

The lysis buffer contains guanidine thiocyanate which may produce hazardous gases (including hydrogen cyanide and chlorine) when combined with bleach (sodium hypochlorite) and/or strong acids.

Ethanol is a highly flammable liquid.

BEFORE STARTING

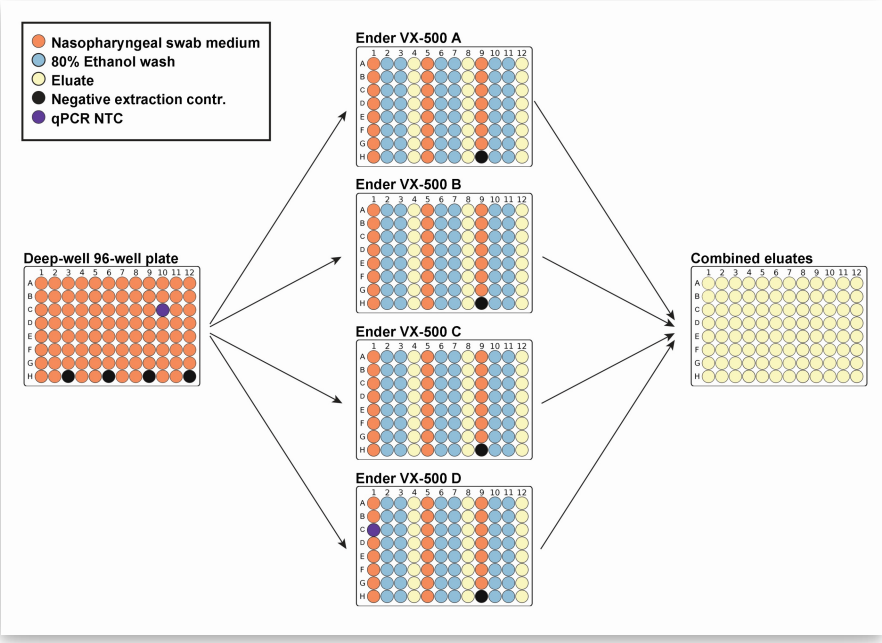
This protocol provides directions for the use of a 3D Printer RNA extraction apparatus we named the "Ender VX-500". The platform essentially consists of a *Creality3D Ender-3* printer repurposed by installing three different custom designed interlocking attachments on the printer's extruder mounting mechanism.



These models can be obtained from https://www.thingiverse.com/koen_vdl/designs

A 3D print “job” can be executed on the printers which will emulate the movements required to make the magnetic combs on the mounted attachments run a paramagnetic bead-based RNA extraction protocol.

Here we describe how we can efficiently process 91 nasopharyngeal swab samples in parallel on 4 Ender VX-500 machines.



Graphical overview of the extraction procedure

Pre-process sample information

1

Scan the barcodes of 91 nasopharyngeal swabs using a barcode scanner and the "20200908_Ender_VX-500_extraction_template.xlsx" spreadsheet.

- Populate the “SampleID” column of the “Sample log” sheet
- Randomly include a negative extraction control for every set of 24 swabs in the sheet.
- Randomly include a PCR no-template-control (NTC) control in the sheet.

The spreadsheet will automatically parse 5 plate dispensing charts that can be printed for convenience:

- 4 charts represent the plate layouts of the 4 Ender VX-500 extraction runs (of 24 samples each).
- 1 chart represents the plate layout of the 96-well plate on which the eluates of the 4 extraction runs will be

combined.

The "20200908_Ender_VX-500_extraction_template.xlsx" spreadsheet can be found as an attachment under the abstract tab.

Pre-dispense the samples into a deep-well 96 well plate

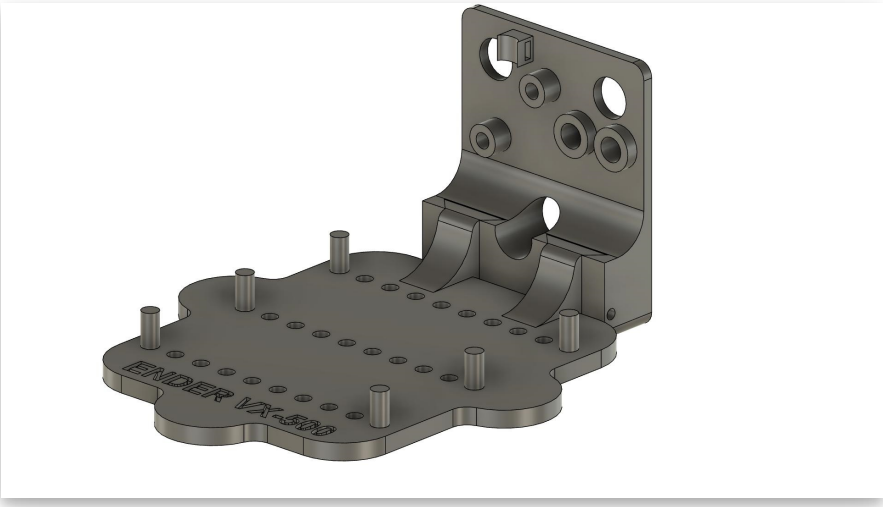
2 Transfer **100 µl** of swab transport medium into a deep-well 96 well plate in a Class II BSC using the plate layout charts as a guide.

Prepare four Ender VX-500 machines for extraction

3 Attach three 8-tube PCR strips onto the “Tube holder”.

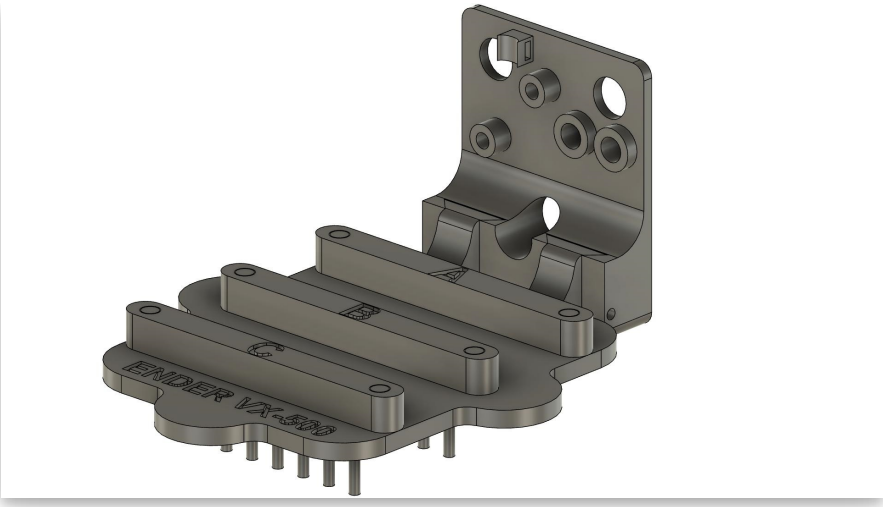
- The caps of the strip fit tightly into the openings on the “Tube holder”.
- Only hold the strips by the caps and make sure not to touch the outside of the tubes.

4 Seat the “Tube holder” into the “Adaptor”.



5 Lock the “Tube holder” in place by inserting the provided Allan hex wrench. This will ensure the “Tube holder” is always inserted in the same position.

6 Place three “Magnetic bars” in the openings of the “Tube holder”.



Prepare four 96-well plates for extraction

7 Add **18.5 µl** Lysis buffer to columns 1, 5 and 9 of a 96-well plates with V-shaped wells using a multichannel pipette.

Resuspend any salted out guanidine thiocyanate crystals before dispensing the buffer.
Incubate **65 °C** for **00:10:00** .

8 Add **80 µl** NFW to columns 4, 8 and 12 using a multichannel pipette.

9 Add **150 µl** **80 % (v/v) Ethanol** to columns 2, 3, 6, 7, 10 and 11 using a multichannel pipette.

- The ethanol concentration will quickly lower in the air flow of the BSC.
- Always use a freshly made up Ethanol solution.

10 Transfer / pipet-mix **37 µl** of nasopharyngeal swab transport medium from the deep-well plate into columns 1, 5 and 9 using using a multichannel pipette and the relevant plate dispensing chart.

11 Incubate the plate **Room temperature** for **00:01:00** .

12 Transfer / pipet-mix **30 µl** SPRI bead mix into columns 1, 5 and 9 using a multichannel pipette.

Shake the SPRI bead mix before use (the beads settle quickly).

13 Transfer / pipet-mix **85.5 µl** **100 % (v/v) Ethanol** to columns 1, 5 and 9 using a multichannel pipette.

14 Incubate the plate **Room temperature** for **00:05:00** .

The prepared plates need to be extracted on the Ender VX-500 machines immediately after this incubation step.

Extract 4 plates on 4 Ender VX-500 machines

15 Insert the four 96-well plates onto four Ender VX-500 machines prepared for extraction

Ensure the microplates are correctly oriented by checking the position of the A1 marker.

16 Power on the Ender VX-500 machines.

17 Use the knob on the Ender VX-500 to start the extraction by selecting “Print from TF” and selecting “RNA_ex_v10.gcode”.

The "RNA_ex_v10.gcode" print job which emulates the RNA extraction can be found as an attachment under the abstract tab.

18 The Ender VX-500 will:

- Collect bead-RNA complex from the lysate (**00:05:00**)
- Wash 1: vortex (**00:01:00**) & collect bead-RNA complex (**00:01:30**)
- Wash 2: vortex (**00:01:00**) & collect bead-RNA complex (**00:01:30**)
- Air dry (**00:05:00**)
- Elute: vortex (**00:01:00**) & collect beads (**00:01:00**)
- Raise the magnetic bars out of the eluates

After **00:20:00** the machine will beep to indicate the run completed successfully.

19 Remove the 96-well plates from the Ender VX-500 machines.

20 Power off the Ender VX-500 machines.

21 Place the 96-well plates on a 96 well magnetic rack and use a multichannel pipette to transfer **50 µl** of eluate onto a fresh 96-well plate that will combine the eluates of the 4 extraction runs.

30 µl of eluate is sacrificed to prevent any bead SPRI bead carryover.

Machine clean-up

22 Carefully remove the "Tube holder" from the EnderVX-500 “Adaptor”.

23 Use paper tissues to release the three 8-tube PCR strips from the "Tube holder" and decontaminate the attachment in a bath containing a 1% sodium hypochlorite solution for min **00:10:00** .