May 26, 2021

Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection

Pedro Belda-Ferre[1], Lisa Marotz[1], Greg Humphrey[1], Sydney C Morgan[1], Rob Knight[1]

[1]University of California, San Diego
Pedro Belda-Ferre: Method Development
Lisa Marotz: Method Development
Greg Humphrey: Method Development
Sydney C Morgan: Admin/Writing
Rob Knight: PI

 dx.doi.org/10.17504/protocols.io.brcvm2w6

Coronavirus Method Development Community

Sydney Morgan
University of California, San Diego

ABSTRACT

The purpose of this protocol is to describe a semi-automated process for viral RNA isolation from anterior nares (AN) swabs in molecular transport medium (MTM), Mawi medium, or 5% sodium dodecyl sulfate (SDS), using the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit.

This procedure applies specifically to samples received for SARS-CoV-2 testing. The MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit uses magnetic bead technology to rapidly isolate, wash, purify, and elute viral RNA from viral samples in transport media. This process is developed for semi-automated systems and performs RNA extraction in a 96-well plate format. The RNA extracted will be used for SARS-CoV-2 detection via a TaqPath COVID-19 Multiplex Real-Time RT-PCR assay (see related protocol).

Viral samples are obtained from anterior nares (AN) swabs and placed into MTM, Mawi, or 5% SDS solutions. Viral RNA from these samples is extracted using an Eppendorf epMotion 5075 automated liquid handling workstation and a KingFisher Flex benchtop automated sample preparation machine.

DOI

dx.doi.org/10.17504/protocols.io.brcvm2w6

PROTOCOL CITATION

Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight 2021. Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.brcvm2w6

KEYWORDS

Viral RNA Extraction, SARS-CoV-2, COVID-19, RNA, Virus, RNA Isolation

CREATED

Jan 11, 2021

LAST MODIFIED

May 26, 2021

PROTOCOL INTEGER ID

46197

MATERIALS TEXT

Definitions and Acronyms

A	B
Term	**Definition**
Equilibrate	Bring to equilibrium to ambient room temperature.
EtOH	80% Ethanol.
Nuclease-Free Water	Nuclease-free water.
Freezer	Temperature range of -25°C to -15°C.
Lysis Plate	96-well KingFisher deep-well plate containing lysis solution and 200 µL sample from nasopharyngeal/anterior nares swabs.
Mawi	Refers to iSwab Microbiome collection media (ISWAB-MD-1200, Mawi DNA Technologies).
MTM	Refers to Molecular Transport Media preservation solution.
MS2 Phage Control	Control used to monitor RNA extraction.
Negative Control	Nuclease-free Water. Used to monitor cross-contamination during nucleic acid extraction and reaction setup.
AN Swab	Anterior nares swab.
PCR	Polymerase Chain Reaction.
Positive Control	TaqPath™ COVID-19 control kit. RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assay. Used to monitor RT-PCR reaction setup and reagent integrity.
QC	Quality Control.
Refrigerator	Temperature range of 2°C to 8°C.
Room Temperature (RT)	Temperature range of 15°C to 25°C.
RT-PCR	Reverse transcription polymerase chain reaction.
SDS	Refers to 5% Sodium Dodecyl Sulfate solution.
SEP	Sample Extraction Plate. Plate where viral RNA is extracted.
REP	RNA Elution Plate. Output from the KingFisher containing purified nucleic acid.
RWP	RNA Working Plate. Aliquot of REP that will be used as input to the RT-qPCR
Ultra-Low Freezer	Temperature range of -90°C to -70°C.

Equipment

A
KingFisher Flex (ThermoFisher Scientific, PN: 5400610)
epMotion® 5075 Liquid Handling Workstation (Eppendorf, PN: 5075)
Vortexer (VWR, Vortex Genie-2)
Benchtop Centrifuge (Eppendorf, PN: 5910R)
Cap press
Barcode Scanner
Freezer (-20°C)
Deep freezer (-80°C)

Supplies

1. KingFisher deep-well 96 plates (PN: 95040450), or equivalent
2. BioRad 384-well PCR plates (PN: HSP3901), or equivalent
3. epTIPS, filtered (Eppendorf, PN: 30014413), or equivalent
   3.1. epMotion 1000 µL filter tips reloads (Eppendorf, PN: 0030014510), or equivalent
   3.2. epMotion 50 µL filter tip reloads(Eppendorf, PN: 0030014472), or equivalent

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

4. EpMotion Reservoirs
   4.1. 400 mL reservoirs (Eppendorf, PN: 5075751364), or equivalent
   4.2. 100 mL reservoir (Eppendorf, PN: 960051017), or equivalent
   4.3. 10 mL reservoir (Eppendorf, PN: 0030126521), or equivalent
5. Pipette (P1000)
6. Pipette tips
   6.1. 1000 µL BioClean Ultra (Rainin, PN: 30389211), or equivalent
7. Serological Pipette
   7.1. 25 mL pipette (Genesee Scientific, PN: 12-106), or equivalent
8. Transparent plate seals (VWR, PN: 75853-868), or equivalent
9. Aluminum plate seals (AlumaSeal, PN: 152-68403), or equivalent
10. Spray bottle with 70% EtOH
11. Barcodes - unique, scannable stickers for each plate below:
   11.1. Sample Storage Bag
   11.2. Sample Extraction Plate (SEP) barcode
   11.3. RNA Elution Plate (REP) barcode
   11.4. RNA working plate (RWP) barcode
12. 50 mL sterile conical tube
13. Tube rack to hold 50 mL conical tubes
14. 2.0 mL sterile Eppendorf tubes (PN: 022600044), or equivalent
15. 500 mL graduated cylinder.
16. Flange plug caps (Globe Scientific, PN: 118127L) or equivalent

Reagents
1. MVPII Viral Isolation Kit (Thermo, PN: A48383)
   1.1. Proteinase K Solution
   1.2. Binding Solution
   1.3. Total Nucleic Acid Binding Beads
   1.4. Wash Buffer
   1.5. Elution Solution
2. MS2 Phage Control (Thermo, PN: 100092698)
3. Molecular-grade 100% (200-proof) ethanol (Sigma, PN: E7023-4X4L) or equivalent

SAFETY WARNINGS

Because this protocol is intended for the detection of SARS-CoV-2 in a clinical setting, face coverings (masks) are to be worn at all times. Lab coats/disposable lab gowns, eye protection, and gloves must be worn at all times when when handling samples containing SARS-CoV-2. Gloves should extend to cover wrists of lab coat/gown. Equipment and surfaces must be decontaminated regularly, especially before and after performing this procedure.

Sample Collection

1 **Anterior nares swabs** are obtained via self-collection.

Individuals are given an individually wrapped, sterilized swab (Nantong Renon Laboratory Equipment, PN: NLD602-1) and instructed to:
1. Insert the swab approximately 1.5 cm into the nostril,
2. Twist the swab inside the nostril three (3) times, and
3. Hold the swab against the septum for a total of 15 seconds, then
4. Repeat these steps on the other nostril with the same swab.

The swab is then inserted into a into a 5 mL sample tube containing **1.5 mL** molecular transport media (MTM), Mawi preservation solution, or 5% sodium dodecyl sulfate (SDS) (Table 1). If required (depending on the length of the swab handle), the handle of the swab is broken off at a pre-scored area directly above the tip so that the tip remains in the solution. MTM, Mawi, and SDS are able to inactivate the SARS-CoV-2 virus while preserving the viral RNA needed for detection.

Table 1. Acceptable sample storage temperatures and durations.

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

A	B	C
Specimen Type	**Preservation Solution**	**Stability and Storage**
Anterior Nares Swab	Mawi	Store at Room Temperature up to 1 month. Store at -90 C to -70 C indefinitely.
Anterior Nares Swab	MTM	Store at Room Temperature up to 7 days. Store in Refrigerator (2 C to 8 C) up to 28 days. Store at -90 C to -70 C indefinitely.
Anterior Nares Swab	SDS (5%)	Store at Room Temperature up to 1 year. 5% concentration.

This protocol includes steps to follow to track patient data using the INSPECT application, a sample data tracking tool developed for SARS-CoV-2 testing. These steps are not essential to the RNA extraction process and are noted as such.

Sample Extraction Plate (SEP) Preparation

2 Transfer proteinase K into KingFisher deep-well 96-well plates using the epMotion 5075:

epMotion 5075
Liquid Handling Workstation
Eppendorf **5075**

- Turn on the epMotion 5075.
- Open the epBlue software (version 40.7) on the associated laptop, enter username and password.
- Within the epBlue program, double click to open the 'Application Runner'.
- Open the following program: **Application_MVPII_ProK_10uL_1_Plate_210120_144344.export7**

2.1 Set up consumables on the epMotion table:

- Place a box of 50 µL epTIPs with at least 1 full column of tips into Position A2.
- Label a clean 10 mL reservoir with "ProtK" and the current date.

"ProtK" reservoir can be used throughout the day if multiple plates are being run (create a new plate each morning).

- In the EpMotion hood, add **1.4 mL** Proteinase K
  MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit **Thermo Fisher Scientific Catalog #A48383**
  into the labelled 10 mL "ProtK" reservoir and place in the left-most slot of the reservoir rack (Position 1).
- Label a clean KingFisher deep-well 96-well plate with a SEP barcode and place in Position C2.

2.2 Run the program:

- Available device will be selected by default - click "Next".
- Under the volume settings, select "Use required minimum volumes".
- Under worktable settings, leave "Detected tips" selected but uncheck "Check labware placement" and

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

"Check tube lid removed".
- Click "Next".
- Double check that all consumables are firmly in the correct position and all lids have been removed.
- Click "Run".

Sample Accessioning and Plating using Hamilton STAR

3

In this step, **200 µl** from each nasal swab sample is added to the wells of the Sample Extraction Plate (SEP) prepared in Step 2 above using a Hamilton Microlab STAR liquid handling system.

Samples are plated into 96-well sample extraction plates (SEP) in batches of 94, with 2 clean media controls in positions *A4* and *H3* of the SEP (these wells will become the Negative and Positive Control wells, respectively).

Microlab STAR
Liquid Handling System
Hamilton **Microlab STAR**

Alternatively, this step can be done by hand, by adding **200 µl** from each sample to the wells of the SEP (containing **10 µl** Proteinase K) prepared in Step 2. If conducting this procedure by hand, remember to add **200 µl** clean media to wells *A4* and *H3* (the control wells) instead of adding any sample and track the samples in a platemap.

3.1 **Set up the Hamilton STAR:**

Retrieve one Sample Extraction Plate (SEP) from Step 2 containing **10 µl** proteinase K per well for each batch of 94 samples to be processed (maximum of 5 batches per Hamilton run). Ensure that the plate has a barcode sticker placed on the right side of the plate, centered at the upper right edge of the skirt.

- Wipe down the sample carriers and Hamilton deck with 70% EtOH.
- On the computer desktop associated with the Hamilton STAR, open "Sample Transfer".
- In the pop-up window, enter the number of samples and controls to be plated (max 480).
- Select the "Sample Source Type".
- If running only MTM samples, choose "bottom barcode".
- If running only Mawi samples, choose "round barcode".
- If running another type or a combination of sample types, choose "round bottom".
- Enter your initials in capital letters.
- Hit "Continue".

NOTE: The "round barcode" designation is used for 5 mL tubes with barcode labels added to the

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

side of the tube (these tubes have a round bottom). The "bottom barcode" designation is used for Thermo bottom-barcoded tubes (these tubes have a flat bottom). See Step 1 for more tube details.

3.2 Load the carriers accordingly, checking off each box on the screen in order:

1. *Load tip carriers:* Ensure sufficient filter tips (1000 µL) are loaded. To fill each 96-well SEP, 96 tips are needed (94 samples and 1 negative and 1 positive control tube each containing 1 mL collection media only).
2. *Load sample carriers:* Load the 32-position Sample Carriers with the sample tubes collected in Step 1, starting from the carrier that will be placed at the far right of the Hamilton deck (Deck Position 34), filling from the top to the bottom (Carrier Positions 1 through 32), then moving to the next carrier (Deck Position 33), filling from the top to the bottom (Carrier Positions 1 through 32), and so on. [See note below for more details.]
3. *Load plate carrier:* Load the appropriate number of SEPs starting with the cavity furthest toward the back and double-check that the SEP barcode sticker is attached to the right face of the plate towards the top. Make sure that well A1 for each SEP is located in the upper left corner.

- Begin by inserting barcoded, blank tubes (tubes containing collection **1 mL** collection media but no patient swab sample) into the carrier positions that will go to SEP well *H3* (positive control) and *A4* (negative control). This translates to tube positions 24 and 25 of the first of the three racks for each single SEP. For one SEP plate this is deck position 34, for 2 SEP plates this is deck positions 34 and 31, for 3 plates this is deck positions 34, 31 and 28, for 4 plates this is deck positions 34, 31, 28, and 25, for 5 plates this is deck positions 34, 31, 28, 25, and 22.
- Insert sample tubes to the remaining positions on the carriers, starting with the right-most carrier (Deck Position 34), and moving to the left once full.
- Decap tubes (either manually or using an assisted tube decapper) and ensure that tube barcodes are facing the opening on the right side of the racks so they can be scanned by the Hamilton.

Once the autoload trays are loaded, click "Continue".

3.3 Run the program:

- A screen will pop up to determine where in the first carrier the tips are loaded: drag and drop to highlight where tips are loaded (gray = no tip, brown = tip).
- Click "OK".
- The Hamilton will pull in all carriers, if any barcodes are not read, the instrument will return an error. If this happens, check to make sure the barcode is facing the window on the right side of the carrier, then click “Repeat” → “Execute”.
- Once no errors are found, the instrument will transfer **200 µl** from each sample tube into the loaded SEP(s).

3.4 Once Hamilton run is finished:

- When sample transfer is complete, Hamilton will display a "Method Complete" sign.
- Pull out the carrier tray and remove the SEP(s). Seal SEP with aluminum plate seal, using a plate roller to ensure a tight seal.
- Recap each of the 94 sample tubes with a Globe Scientific Polyethylene (PE) Stopper Flange Cap (PN: 118127R, or equivalent)
- Place each set of capped 94 sample tubes into a 4" freezer storage box and leave at room temperature for up to one week.
- Wipe down sample carriers and Hamilton deck with 70% EtOH.

Sample tubes can be discarded once the original samples are no longer needed.

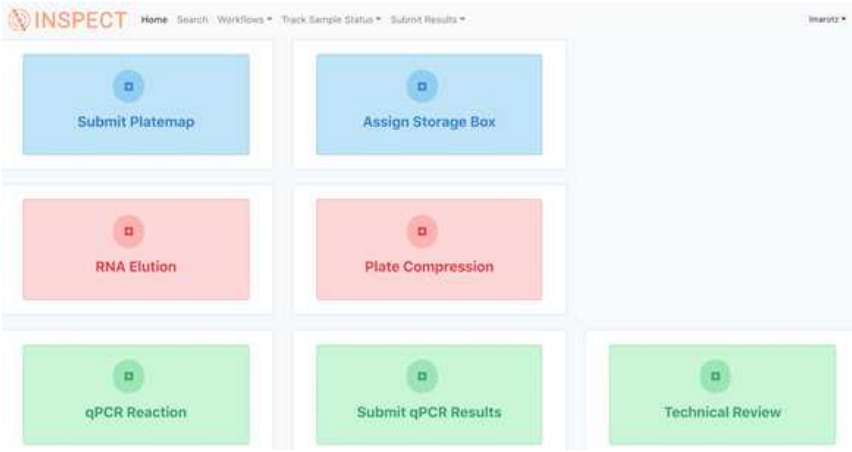
Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

3.5 Upload the plate map data generated by the Hamilton into the INSPECT application:

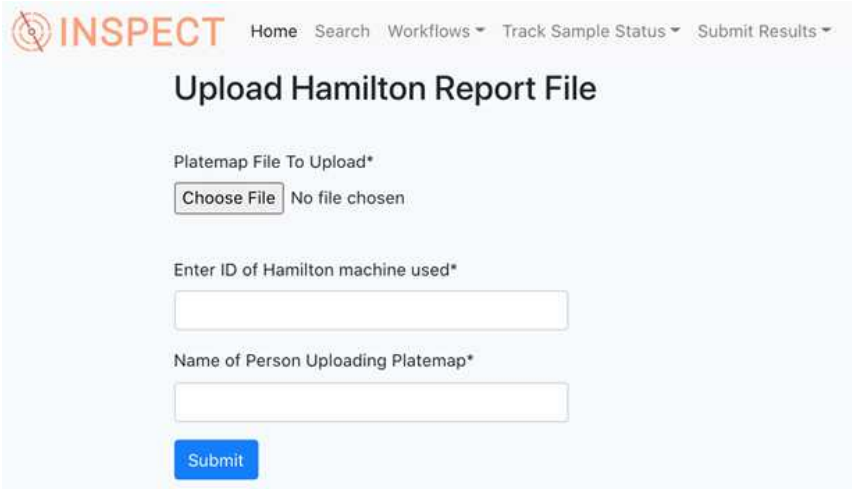
Steps involving the INSPECT application are optional and are not essential for performing RNA extraction.

- On the Hamilton desktop computer, find the most recent .csv file created during this sample transfer. This file will list the sample barcode and the well in the SEP that each sample was added to. This is the "Platemap File".
- In the INSPECT app, click the "qRT-PCR" button on the left side:

- On the following page, click "Submit Platemap".



- Click "Choose File" to upload the Platemap file from the Hamilton.



Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

- To enter the ID of the Hamilton machine used, use a barcode scanner to scan the Hamilton ID blue sticker on the right-hand side of the instrument.
- Type your initials into the "Name of Person Uploading Platemap" field.
- Click "Submit".

Wash and RNA Elution Plate (REP) Preparation for RNA Extraction

4

For each SEP containing 94 samples + 2 controls (full 96-well plate), you will need: one (1) KingFisher 96-well deep well plate containing Wash Buffer; one (1) KingFisher 96-well deep well plate containing 80% EtOH; one (1) KingFisher 96-well deep well plate containing Nuclease-Free Water (this will be the RNA Elution Plate [REP]). These plates can be prepared at the same time as the patient sample plating (section 4) above.

Prepare wash and elution reservoirs:
Prepare the following 100 mL reservoirs in a Biological Safety Cabinet:

- Label one 100 mL reservoir "Wash Buffer" and fill with **50 mL** wash buffer
  MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit **Thermo Fisher Scientific Catalog #A48383**
  . Load into Rack 1, spot 1 of epMotion 5075.
- Label one 100 mL reservoir "EtOH" and fill with **100 mL** 80% ethanol. Load into Rack 1, spot 2 of epMotion 5075.
- Label one 10 mL reservoir "H2O" and fill with **10 mL** nuclease-free water stored at **Room temperature** . Load into Rack 1, spot 3 of epMotion 5075.

4.1 Use epMotion to transfer wash and elution buffers into plates:

- If necessary, turn on the epMotion 5075 and open the eBlue software on the associated laptop, enter username and password.
- Load the attached epMotion protocol:
  Application_MVPII_wash_elution_1_plate_210120_144405.export7 .

4.2 Set up consumables on the epMotion table:

- Place a box of 1000 uL epTIPs with at least 3 full columns of tips into position A2 of the epMotion.
- Place a reservoir rack in position B2 with three reservoirs in the three slots as described above in Step 5.
- Label one clean KingFisher 96-well deep well plate "Wash Buffer" and place into position C2 on the epMotion.
- Label one clean KingFisher 96-well deep well plate "EtOH" and place into position C3 on the epMotion.
- Label one clean KingFisher 96-well deep well plate with an "REP" plate barcode and place into position C4 on the epMotion.

4.3 Run the program:

- Click the "Play" button on the top of the screen.
- Available device will be selected by default - click "Next".
- Under volume settings, select "Use minimum required volumes."
- Under worktable settings, leave "Detected tips" selected, but un-select options "Check labware placement" and "Check tube lid removed".
- Click "Next".
- Double check that all consumables (reservoirs, tips, etc.) are firmly in the correct position and all lids have been removed.
- Click "Run".

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

Safe stopping point:
After plates are filled, seal with transparent plate seals and use a roller press to seal down firmly, OR proceed directly to RNA extraction. The "Wash Buffer" plate will have **500 µl** wash buffer in each well, the "EtOH" plate will have **1000 µl** EtOH in each well, and the "REP" plate will have **50 µl** nuclease-free water in each well.

Binding Bead Mix Solution Addition to SEP

5

Add binding bead mix only after sample has been added to SEP (section 4 above).

MVPII Binding Beads tend to clump, and must be thoroughly vortexed until no clumps adhere to walls of bottle when rotated. This is to ensure even distribution of beads among the samples.

Prepare binding bead mix solution:

- Place a 100 mL epMotion reservoir into the Reservoir Rack in the top-left slot (position 1) and add **27 mL** of MagMAX Binding Solution
  MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit **Thermo Fisher Scientific Catalog #A48383**
  using a serological pipette. Thoroughly vortex the MVPII Binding Beads and add **1020 µl** using a P1000 pipette. Mix the solution by pipetting up and down while moving pipette tip sideways back and forth, until solution is homogenous.

5.1 Use the epMotion to transfer binding bead mix solution to SEP:

- If necessary, turn on the epMotion 5075 and open the epBlue software on the associated laptop, enter username and password.
- Load the following epMotion protocol:
  Application_MVPII_BindingBead_addition_to_SEP_1Plate_275uL_210120_144324.export7

5.2 Set up consumables on the epMotion table:

- Place a box of 1000 uL epTIPs with at least 1 full column of tips into Position A2 on the epMotion.
- Place a reservoir rack in Position B2, and add the **100 mL** reservoir with binding bead solution to the left-most slot (Position 1).
- Place the SEP containing samples into Position C2.

5.3 Run the program

- Click the "Play" button on the top of the screen.
- Available device will be selected by default - click "Next".
- Under volume settings, select "Use minimum required volumes."
- Under worktable settings, leave "Detected tips" selected, but un-select options "Check labware placement" and "Check tube lid removed".
- Click "Next".
- Double check that all consumables (reservoirs, tips, etc.) are firmly in the correct position and all lids have been removed.
- Click "Run".

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

After SEP plate is filled, proceed to MS2 phage control addition (step 6 below) immediately.

MS2 Phage Control Addition

6

The MS2 phage is an internal positive control that serves as an extraction, reverse transcription, and qPCR positive control for each well. If only the MS2 phage amplifies, the sample is negative. If nothing amplifies (not even the MS2 phage control), this means that something went wrong with the extraction or with the RT-qPCR, and the sample is labelled as “invalid” and must be extracted again.

This step will add **10 µl** of MS2 to every well of the SEP except for the positive control well, A4.

Add MS2 Phage to the SEP:

If necessary, prepare the 1:160 diluted MS2 phage aliquots:

- Retrieve MS2 phage from the freezer and the nuclease-free water from benchtop storage.
- In a biosafety cabinet, make a **40 mL** solution of 1:160 diluted MS2 phage in a sterile 50 mL conical tube by combining **39.750 mL** nuclease-free water and **250 µl** MS2 phage control (Thermo, PN: 100092698).
- Transfer **1.2 mL** of the diluted MS2 phage solution from the 50 mL conical tube into sterile 2.0 mL microcentrifuge tubes labelled with "MS2" and the aliquot preparation date. This solution will make 33 aliquots.
- Store MS2 phage aliquots in the freezer at **-80 °C** .

- Thaw one aliquot of 1:160 diluted MS2 phage control.
- If necessary, turn on the epMotion 5075 and open the eBlue software on the associated laptop, enter username and password.
- Load the following epMotion protocol:
  Application_NEW_A4Pos_MS2_control_addition1lysisPlate_1_PLate_210120_144412.export7

6.1 **Set up consumables on the epMotion table:**

- Place a box of 50 µL epTIPs with at least 1 full column of tips into Position A2.
- Label a clean 10 mL reservoir with “MS2” and current date.
- Set a reservoir rack into Position B2 of epMotion 5075. Place the "MS2" reservoir into the left-most slot (Position 1) of the reservoir rack. Transfer **1200 µl** of the thawed (1:160 diluted) MS2 phage aliquot into the "MS2" reservoir, using a P1000 pipette and filter tips.
- Set the SEP plate containing sample and binding beads in Position C2.

"MS2" reservoir can be used throughout the day if multiple plates are being run (create a new plate each morning).

6.2 **Run the program:**

- Click the "Play" button on the top of the screen.
- Available device will be selected by default - click "Next".
- Under volume settings, select "Use minimum required volumes."
- Under worktable settings, leave "Detected tips" selected, but un-select options "Check labware placement" and "Check tube lid removed".

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

- Click "Next".
- Double check that all consumables (reservoirs, tips, etc.) are firmly in the correct position and all lids have been removed.
- Click "Run".

Viral RNA Extraction using KingFisher Flex

7

This protocol describes the extraction of RNA from samples plated in the lysis buffer (Step 4 above), utilizing magnetic bead nucleic acid purification and using a KingFisher Flex benchtop automated extraction instrument. This protocol also involves scanning barcodes and entering information into the INSPECT application for record-keeping.

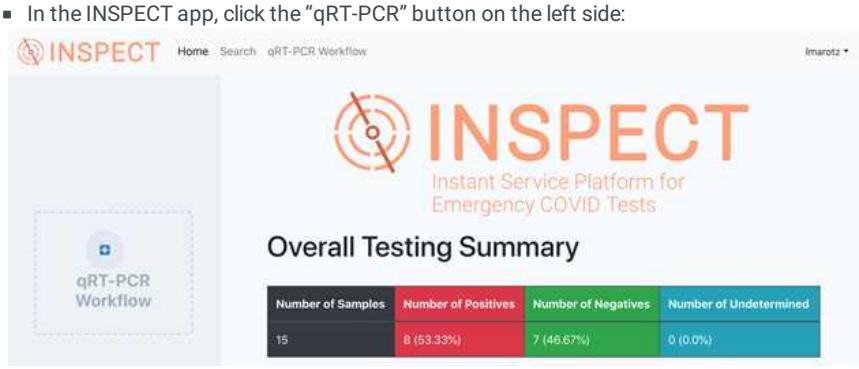
Steps involving the INSPECT application are optional and are not essential for performing RNA extraction.

Open INSPECT application if set up for your lab.

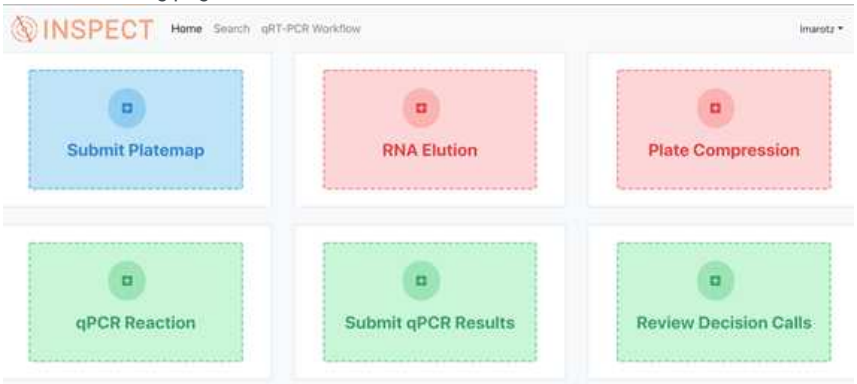
- If a data tracking application for SARS-CoV-2 tests is desired, this application can be set up for any lab following the attached protocol: dx.doi.org/10.17504/protocols.io.bis8kehw

Scan in plates and reagent lot numbers to INSPECT App:

- In the INSPECT app, click the “qRT-PCR” button on the left side:

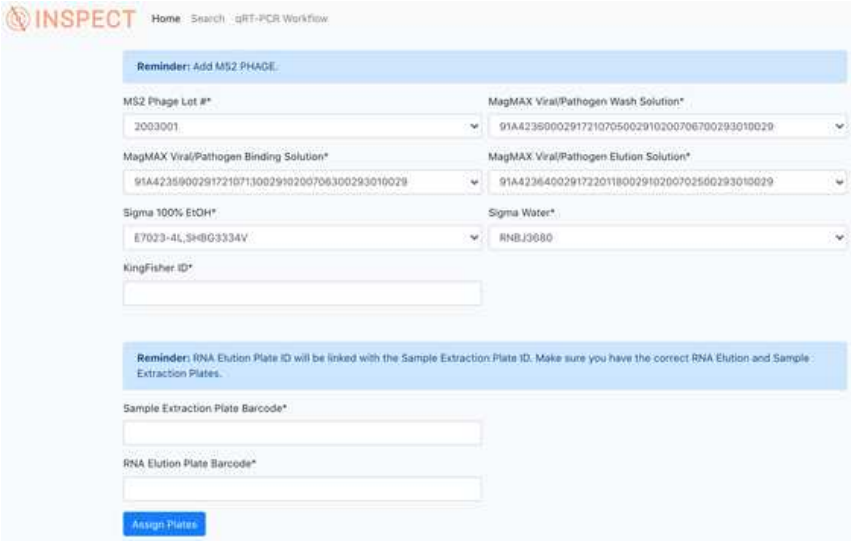


- In the following page, click “RNA elution”:



- In the following page, use the pull-down menu to select the appropriate lot numbers used for the MS2 Phage, Wash buffer, Binding Solution, EtOH (used in the second wash plate), and nuclease-free water (used in the elution plate).

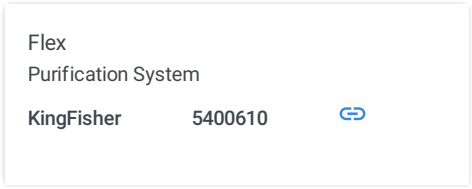
Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6



- Place your cursor in the “Sample Extraction Plate Barcode” section and scan in the plate barcode affixed to the SEP.
- Place your cursor in the “RNA Elution Plate Barcode” section and scan in the plate barcode affixed to the REP.
- Click “Assign Plates”.

7.1 Extract RNA using the KingFisher Flex:

- Turn on the KingFisher Flex (power button located on the left side of the instrument at the bottom).



- Push the right arrow to enter User folder (second tab), scroll down to "RNA", click "OK" button, and select the following protocol: **50uL_elu_MVP_2Wash_200_Flex.bdz**
- Click "Start".

7.2 The instrument will prompt you to load the following plates in order:

- *Tip Comb Plate*: there should be a clean, 96-well plate in this position, place a fresh ‘KingFisher 96 Tip Comb for DW Magnets’ into the plate so that each plastic comb fits into each well in the plate.
- Hit “START”.
- *Elution Plate*: load the REP, ensuring well A1 is next to the A1 designation in upper-left-hand corner.
- Hit "START".
- *80 Ethanol*: load the EtOH plate.
- Hit "START".
- *Wash Buffer Plate*: load the Wash plate.
- Hit “START”.
- *Sample Plate*: load the SEP, ensuring well A1 is next to the A1 designation in upper-left-hand corner.
- Hit “START”.
- The instrument will automatically begin to process samples and list a countdown timer in minutes in the lower right-hand corner of the screen.

7.3 When finished, the instrument will prompt you to take out the REP.

Safe Stopping Point:
The RNA Elution Plate (REP) now contains purified RNA from the samples. Each well should

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

contain **50 µl** . Seal REP with an AlumaSeal, using a plate roller to ensure tight adherence.

If proceeding to the "Semi-Automated and Miniaturized Detection of SARS-CoV-2 using the TaqPath COVID-19 Multiplex Real-Time RT-PCR Assay" protocol, proceed to Step 8 to compress samples into 384-well plate format. This compression process allows for up to four (4) REPs to be compressed into one 384-well RNA Working Plate (RWP); if more than one plate is being processed, place finished REPs in the fridge at **4 °C** , while waiting for the rest to finish, and then proceed with all REPs to Step 8.

Remove used consumables from Flex:

- Hit "START".
- Remove the SEP and place to the side.
- Hit "START".
- Remove the Wash plate and place to the side.
- Hit "START".
- Remove the EtOH plate and place to the side.

Pour the liquid from the SEP, Wash, and EtOH plates into a labelled chemical waste container, then discard the plates into an appropriate waste container.

Plate Compression from 96-Well Format to 384-Well Format for RT-qPCR

8

This section describes the process of compressing up to four (4) RNA Extraction Plates (REPs) created in Step 7 into one 384-well RNA Working Plate (RWP) that will be used as input for the RT-qPCR detection of SARS-CoV-2, described here: **20 µl** of each sample is transferred from the REPs to the 384-well RWP.

Centrifuge:

- Select one to four (1-4) 96-well REPs and centrifuge **1000 x g, 4°C, 00:01:00** .

8.1 **Load plates onto epMotion 5075:**

- If necessary, turn on the epMotion 5075 and open the eBlue software on the associated laptop, enter username and password.
- Load the following epMotion protocol:
  Application_4x96_1x384_bycolumn_compression_210120_144251.export7
- Load 1-4 boxes of 50 µL pipette tips into positions A2, A3, A4, and A5, depending on how many REPs are being processed (one box of pipette tips per REP), starting with position A2.
- Place the REPs into positions B2, B3, B4, and B5, depending on how many REPs are being processed, starting with position B2.
- Load a clean, barcoded Bio-Rad Hard-Shell 384-well barcoded PCR plate into position C4.

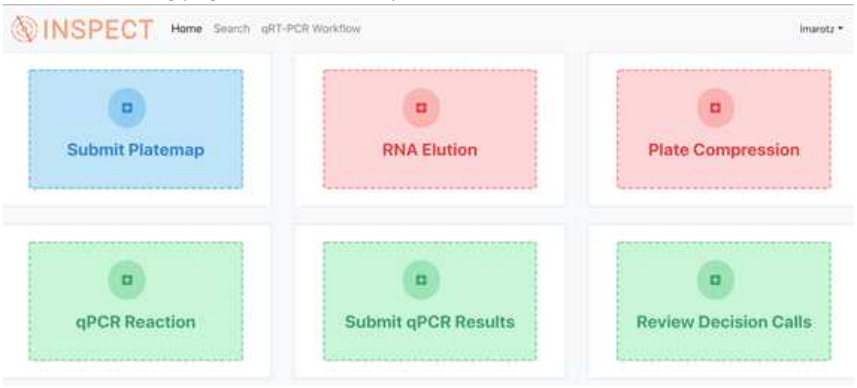
Hard-Shell 384-Well PCR plates
Barcoded PCR Plate
Bio-Rad HSP3901

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

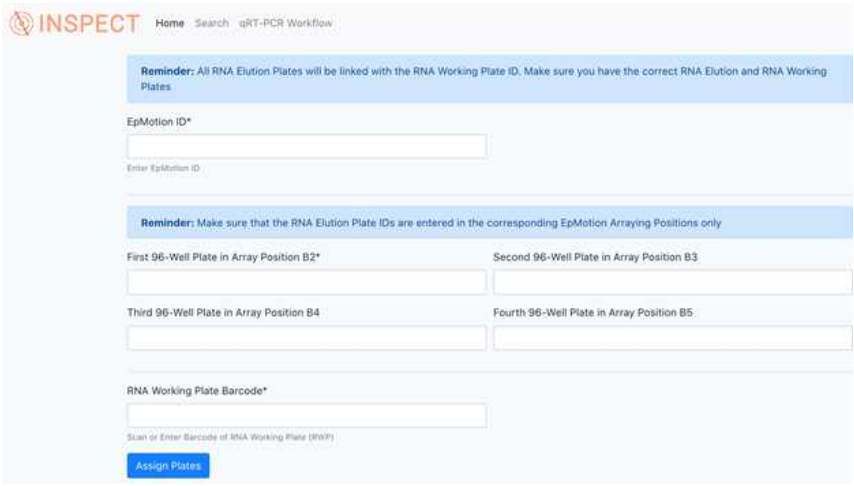
8.2 **Scan plate barcodes into INSPECT application:**

- In the INSPECT app, click the "qRT-PCR" button on the left side:

- On the following page, click "Plate Compression":



- Set the mouse clicker on the "EpMotion ID" section and scan the blue "EXCITE LAB" label on the EpMotion.
- Set the mouse clicker on the "First 96-Well Plate and Plate into Array Position B2" and scan the barcode of the REP in position B2.
- Repeat above step for any other REPs in positions B3, B4, and B5.



- Set the mouse clicker on the "RNA Working Plate Barcode" section and scan the barcode on the side of the Bio-Rad Hard-Shell 384-Well PCR plate in position C4.
- Click "Assign Plates".

Steps involving the INSPECT application are optional and are not essential for performing RNA extraction.

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6

8.3 **Run the program:**

- Remove the seals from the REPs and remove the tip box covers.
- Click the "Play" button on the top of the screen.
- Available device will be selected by default - click "Next".
- Under volume settings, select "Use minimum required volumes."
- Under worktable settings, leave "Detected tips" selected, but un-select options "Check labware placement" and "Check tube lid removed".
- Click "Next".

A "Process Control" window will appear, asking for the number of samples present in each of the 96 well REP(s). Follow the instructions below depending on the number of REPs being processed:

- You will be prompted to enter the number of samples on the first plate; Enter "96" and click "OK"
- Another window will pop up with the same prompt for the second plate; If compressing only one plate enter "0", other wise enter "96" and click "OK"
- Another window will pop up with the same prompt for the third plate; If compressing less than 3 plates, enter "0", otherwise enter "96" and click "OK"
- Another window will pop up with the same prompt for the fourth plate; If compressing less than 4 plates, enter "0", otherwise enter "96" and click "OK"

- Double check that all consumables (plates, tips) are firmly in the correct position and all lids/seals have been removed.
- Click "Run".

Once the plates are compressed into the 384-well RWP, it is ready for RT-qPCR detection of SARS-CoV-2. After running the RT-qPCR the RWP can be stored at **-80 °C** .

Semi-Automated and Miniaturized SARS-CoV-2 Detection using TaqPath COVID-19 Multiplex Real-Time RT-PCR Assay
by Sydney Morgan,
University of California, San Diego

PREVIEW RUN

Citation: Pedro Belda-Ferre, Lisa Marotz, Greg Humphrey, Sydney C Morgan, Rob Knight (05/26/2021). Semi-Automated Extraction of Viral RNA using the MagMax Viral Pathogen (MVPII) 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brcvm2w6