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Semi-Automated Extraction of Viral RNA using the Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit for SARS-CoV-2 Detection

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Coronavirus Method Development Community

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ABSTRACT

The purpose of this protocol is to describe a semi-automated process for viral RNA isolation from nasopharyngeal (NP) swabs in viral transport medium (VTM) using the Omega Bio-tek Mag-Bind Viral RNA Xpress Kit.

This procedure applies specifically to samples received for SARS-CoV-2 testing. The Omega Bio-tek Mag-Bind Viral DNA/RNA 96 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 nasopharyngeal (NP) swabs and placed into VTM. Viral RNA from these samples is extracted using a KingFisher Flex benchtop automated sample preparation machine and an Eppendorf epMotion 5075 automated liquid handling workstation.

DOI

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PROTOCOL CITATION

Lisa Marotz, Pedro Belda-Ferre, Mark Zeller, Catelyn Anderson, Sydney C Morgan, Rob Knight, Kristian Andersen 2021. Semi-Automated Extraction of Viral RNA using the Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit for SARS-CoV-2 Detection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.brqhm5t6

KEYWORDS

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

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MATERIALS TEXT

Definitions and Acronyms

A	B
Term	**Definition**
Equilibrate	Bring to equilibrium to ambient room temperature.
EtOH	80% Ethanol.
Freezer	Temperature range of -25°C to -15°C.
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.
NP Swab	Nasopharyngel swab.
Nuclease-Free Water	Nuclease-free water.
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.
REP	RNA Elution Plate. Output from the KingFisher containing purified nucleic acid.
Room Temperature (RT)	Temperature range of 15°C to 25°C.
RT-PCR	Reverse transcription polymerase chain reaction.
RWP	RNA Working Plate. Aliquot of REP that will be used as input to the RT-qPCR
SDS	Refers to 5% Sodium Dodecyl Sulfate solution.
SEP	Sample Extraction Plate. Plate where viral RNA is extracted.
Ultra-Low Freezer	Temperature range of -90°C to -70°C.
VTM	Refers to Vital Transport Medium preservation solution.

Equipment

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

Supplies

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1. KingFisher deep-well 96 plates (PN: 95040450)
2. KingFisher standard (200 µL) 96-well plates (PN: 97002540)
3. Eppendorf 384-well PCR plates (PN:)
4. epTIPS 40-1000 µL (Eppendorf, PN: 30014413)
4.1. epMotion 1000 µL filter tips (Eppendorf, PN: 0030014499)
4.2. epMotion 50 µL tip boxes (Eppendorf, PN: 30014413)
5. 100 mL reservoirs (Eppendorf, PN: 5075751364)
6. Bio-Rad Microseal 'B' plate seals (PN: MSB1001)
7. Pipettes (P10 - P1000)
7.1. P10 - P1000 single channel pipettes
7.2. P300 multichannel pipette
8. Pipette tips
8.1. P10-P1000 pipette tips
8.2. Extended 1000 µL pipette tips (Mettler-Toledo Rainin, PN: 30389223)
9. Lab tape
10. 96-well deep-well sealing mat (square) (Thermo Scientific, PN: AB0675)
11. 96-well storage plate (0.8 mL) (Thermo Scientific, PN: AB0765)
12. 96-well sealing mat (round) (Thermo Scientific, PN: AB0566)
13. Transparent plate seals (VWR, PN: 75853-868)
14. Aluminum plate seals (AlumaSeal, PN: 152-68403)
15. Spray bottle with 70% EtOH
16. Spray bottle with 10% bleach
17. Plate barcodes
18. 50 mL sterile conical tube
19. Tube rack to hold 50 mL conical tubes
20. 2.0 mL sterile Eppendorf tubes
21. 500 mL graduated cylinder

Reagents

1. Mag-Bind® Viral DNA/RNA 96 Kit (Omega Bio-tek, PN: M6246-03)
1.1. TNA Lysis Buffer
1.2. Proteinase K Solution
1.3. Mag-Bind® Particles CNR
1.4. Carrier RNA
1.5. VHB Buffer
1.6. SPR Wash Buffer
1.7. Nuclease-free water
2. Isopropanol
3. MS2 Phage Control (Thermo, PN: 100092698)
4. Molecular-grade 100% (200-proof) ethanol

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 **Nasopharyngeal (NP) swabs** are collected by licensed personnel by sampling the posterior nasopharynx through both nares using an individually-wrapped and sterilized swab, according to CDC instructions for nasopharyngeal swab collection. The swab is then inserted into a 5 mL sample tube containing **3 mL** viral transport media (VTM). 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.

Table 1. Acceptable sample storage temperatures and durations.

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A	B	C
Specimen Type	**Preservation Solution**	**Stability and Storage**
Nasopharyngeal Swab	VTM	Store in Refrigerator (2 C to 8 C) for up to 1 year.

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.

If pairing this protocol with the INSPECT app, tubes should contain a barcode which can be scanned into the app.

Sample Extraction Plate (SEP) Preparation

2 Prepare Sample Extraction Plate (SEP):

This protocol describes the steps to follow for preparing a single 96-well plate containing 94 NP swab samples and two (2) controls.

- In a biological safety cabinet, prepare the lysis master mix in a 100 mL Eppendorf reservoir (PN: 960051017). Add the components in the order listed in Table 2, using the appropriate size of pipette/pipette tips for each volume:

Table 2. Reagents for Lysis Master Mix.

A	B	C
Component	**Volume per extraction**	**Volume for 96-well plate**
Isopropanol	280 µL	31.5 mL
TNA Lysis Buffer	240 µL	27.0 mL
Proteinase K Solution	10 µL	1.125 mL
Mag-Bind Particles CNR*	10 µL	1.125 mL
Carrier RNA	8 µL	0.9 mL
Total Volume:	*548 µL*	*61.65 mL*

*Mag-Bind Particles CNR tend to clump, and must be thoroughly vortexed (until no clumps appear when the bottle is rotated). This is to ensure an even distribution of beads among the samples.

2.1 Transfer the Lysis Master Mix into KingFisher deep-well 96-well plate 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:

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Application_2b_Lysis_Plates_1plate_210205_083127.export7

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

- Place a box of 1000 µL epTIPs with at least one (1) full column of tips into position A2.
- Place a reservoir rack in position B2 with the 100 mL reservoir containing the lysis MM in the first slot.
- Label a clean KingFisher deep well 96-well plate (PN: 95040450) with the name "SEP" for "Sample Extraction Plate" and the current date and place in position C2.

2.3 **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 "Check tube lid removed".
- Click "Next".
- Process control will pop up and ask for number of samples, enter 96 and click "OK".
- Double check that all consumables are firmly in the correct position and all lids have been removed.
- Click "Run".

Seal the SEP with a Bio-Rad Microseal 'B' and store in fridge at **4 °C** until needed.

Sample Plating

3 **Prepare Sample Storage Plate (SSP):**

- Select a batch of 94 NP swab samples and bring into biosafety cabinet.
- Label a KingFisher deep well 96-well plate (PN: 95040450) "SSP" for "Sample Storage Plate" and add the current date.
- Tape the clean SSP in the following way: Starting from column 12, place a strip of lab tape over the column, then move one column to the left and place a strip of lab tape over column 11 so that it partially overlaps the column 12 tape strip, and continue this way until only column 1 is left open (Figure 1).

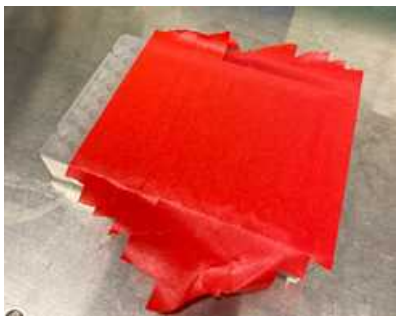

Figure 1. Initial lab tape covering of SSP.

- Add **700 µl** VTM into the Negative Control and Positive Control wells of the SSP (wells A1 and H1, respectively), using a P1000 pipette and changing pipette tips between the two wells.
- If pairing this protocol with the INSPECT application, scan each of the 94 NP swab sample tubes with the barcode scanner to enter into the app.
- Briefly vortex the NP swab sample tubes for 3-5 seconds using a benchtop vortexer.
- Transfer **700 µl** from each sample tube to the wells of the SSP, using a P1000 pipette with extended 1000 µL pipette tips (to avoid contaminating the pipette), and changing pipette tips between each sample. Do not touch the lab tape on the SSP with your pipette tip.

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- Start from well A1 and continue down vertically, with one sample per well of the SSP. Once the first column has been filled, move the tape covering column 2 so that it covers column 1 but exposes column 2 (Figure 2).

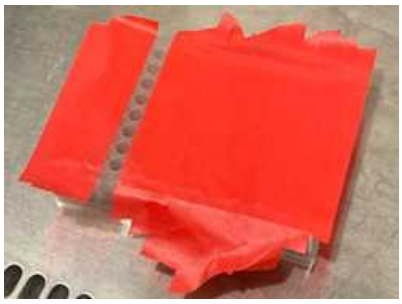

Figure 2. Moving lab tape on the SSP to expose one column at a time.

The purpose of the lab tape is to reduce the chance of cross-contamination between wells by exposing only a small section of the plate at one time.

If not using the INSPECT application to organize the samples, make sure to record which samples are transferred to which wells of the SSP. Take care to skip wells *A1* and *H1*, which are reserved for Negative and Positive Controls.

- Continue adding samples in this way, uncovering one column at a time on the SSP, until the plate is filled.

3.1 Transfer samples from SSP to SEP:

- Briefly centrifuge the SEP prepared in Step 2 **4400 x g, 4°C, 00:00:10** to remove any liquid from the seal.
- Bring the SEP into the biosafety cabinet where the SSP is.
- Starting from column 1 of the SEP, place a strip of lab tape over the column, then move one column to the right and place a strip of lab tape over column 2 so that it partially overlaps the column 1 tape, and continue this way until only column 12 is left open (similar to the process described for the SSP above [Figure 1] but in reverse) (Figure 3).

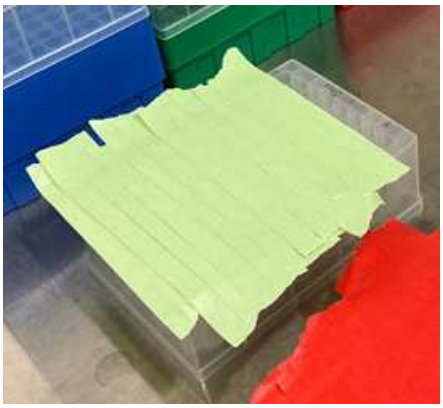

Figure 3. Initial lab tape covering of SEP.

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- Carefully, using a P300 multichannel pipette, transfer **200 µl** from each well of the SSP into each well of the SEP (Figure 4).

Be careful not to overflow the wells of the SSP when you enter the pipette tips into the wells. Add the sample directly to the Lysis Master Mix (not against the side of the well above). Pipette up and down once to mix.

Be careful not to touch the tape with your pipette tip.

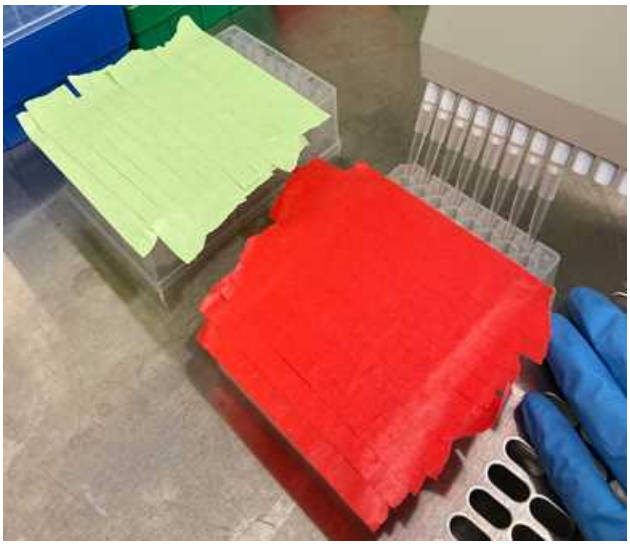

Figure 4. Transferring samples from SSP to SEP using a P300 multichannel pipette.

- Once one column of samples has been transferred, discard the pipettes, remove the tape from the next column to the left, and continue until every sample has been transferred from the SSP to the SEP (Figure 5).

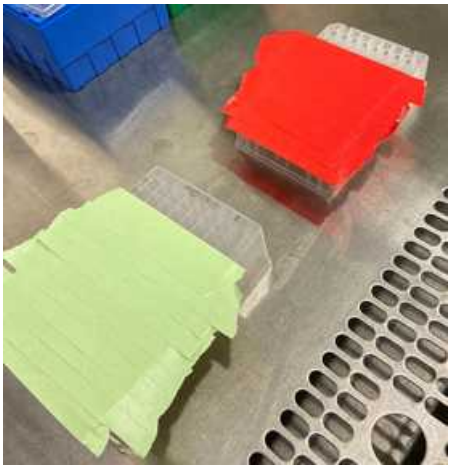

Figure 5. Tape removal from neighboring columns during sample transfer from SSP to SEP.

- Once sample transfer is complete, seal both plates with 96-well sealing mats (Thermo Scientific, PN: AB0566). Do not shake the plates after sealing to avoid well-to-well contamination.

Spray both plates generously with 70% EtOH. Spray all sides of plate including bottom. Use a

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paper towel to remove excess EtOH. Do not place these plates back down in the biosafety cabinet after spraying - your plates are now considered decontaminated and can be moved outside the biosafety cabinet.

- Apply two aluminum seals perpendicular to the sealing mat on the SEP (Figure 6).

Figure 6. Two aluminum seals applied to the SEP in a perpendicular orientation.

Collect all 15 mL sample tubes that were used in this step and place in a transparent resealable zipper storage bag. Spray all sides of the bag with 70% EtOH. Remove the bag from the biosafety cabinet and use a paper towel to remove any excess EtOH.

3.2 Add barcode labels:

- Apply a barcode to the sample bag. Scan barcode into INSPECT app.
- Apply a barcode to the SSP. Scan barcode into INSPECT app.
- Apply a barcode to the SEP. Scan barcode into the INSPECT app.

Store plates and sample bag in fridge at 4 °C .

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

MS2 Phage Control Addition to SEP

4 Set up:

The MS2 phage is an internal positive control that serves as an extraction, reverse transcription, and qPCR 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 run again.

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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 **-20 °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_4_MS2_additiontolysisplate.export7 copy**

4.1 Set up the consumables on the epMotion table:

- Place a box of 10 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 metal reservoir rack into Position B5 of the 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.
- Load the SEP into Position B4 of the epMotion 5075.
- Remove the seal from the SEP and remove the tip box covers.

4.2 Run the program:

- Close the front door of the epMotion 5075 and click "Play" on the program.
- 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.
- When prompted to input the volume, enter "1200 µL".
- Click "Run".

When program is complete, seal SEP with a temporary plate seal and centrifuge **4400 x g, 4°C, 00:00:10** .

Wash Plate Preparation for

5

This step describes the preparation of wash plates needed for viral RNA extration of 94 samples + 2 controls (full 96-well SEP), including: one (1) KingFisher deep well 96-well plate containing VHB buffer; two (2) KingFisher deep

Citation: Lisa Marotz, Pedro Belda-Ferre, Mark Zeller, Catelyn Anderson, Sydney C Morgan, Rob Knight, Kristian Andersen (05/26/2021). Semi-Automated Extraction of Viral RNA using the Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brqhm5t6

well 96-well plates containing SPR wash buffer; one (1) KingFisher standard 96-well plate containing nuclease-free water (this will become the RNA Elution Plate [REP]).

Prepare the following reservoirs in the biosafety cabinet:

- Label one 100 mL reservoir "VHB" and the current date and fill with **40 mL** VHB wash buffer.
- Label one 100 mL reservoir "SPR" and the current date and fill with **100 mL** SPR wash buffer.
- Label one 100 mL reservoir "H2O" and the current date and fill with **100 mL** nuclease-free water.

5.1 Set up consumables on the epMotion table:

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

  Application_5a_wash_aliquoting_1plate_210205_183714.export7
- Place a box of 1000 µL epTIPs with at least 3 full columns of tips into Position A2 on the epMotion.
- Place a reservoir rack in Position B3 with the three reservoirs created in step above placed in the rack in the following order:
- Load "VHB" reservoir into Position 1 of the rack.
- Load "SPR" reservoir into Position 2 of the rack.
- Load "H2O" reservoir into Position 3 of the rack.
- Label one clean KingFisher deep well 96-well plate "VHB" and the current date and place into Position C1 on the epMotion.
- Label two clean KingFisher deep well 96-well plates "SPR" and the current date and place into Positions C2 and C3 on the epMotion.
- Label one clean KingFisher standard 96-well plate "REP" and place into Position C4 on the epMotion.

5.2 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".
- Click "Next".
- Process control will pop up and ask how many samples - type "96" and click "Enter".
- Double check that all consumables (reservoirs, tips, etc.) are firmly in the correct position and all lids have been removed.
- Click "Run".

This program transfers **400 µl** VHB buffer into each well of the VHB plate, **500 µl** SPR buffer into each well of both SPR plates, and **75 µl** nuclease-free water into each well of the REP plate.

Once the program is finished and the plates are filled, seal plates with aluminum foil OR proceed directly to RNA extraction.

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This protocol describes the extraction of RNA from samples plated in the lysis buffer (Step 2 above), 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.

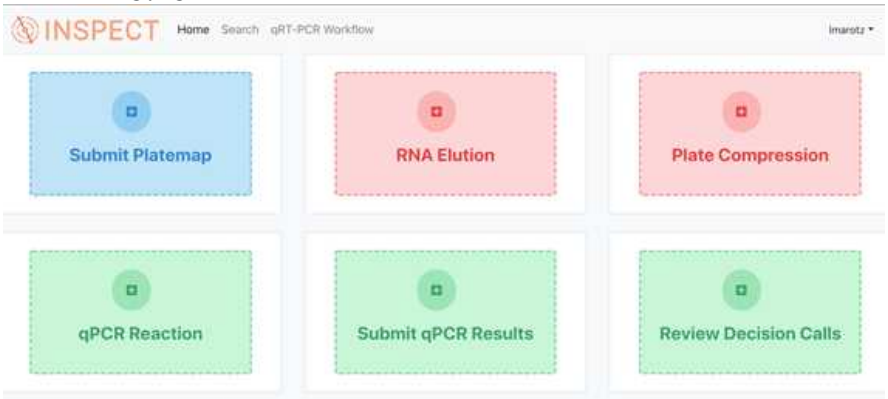
Open INSPECT application if set up for your lab.

- 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

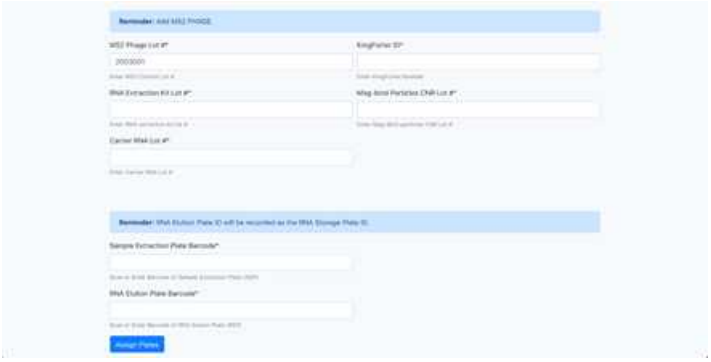
Scan in plates and reagent lot numbers to INSPECT App:

- 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 buffers, and nuclease-free water (used in the elution plate).



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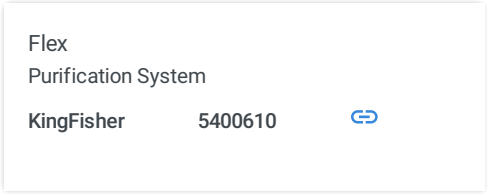
- Place your cursor in the “Sample Extraction Plate Barcode” section and scan in the plate barcode affixed to the SEP.
- Place your cursor in the “RNA Elution Plate Barcode” section and scan in the plate barcode affixed to the REP.
- Click “Assign Plates”.

6.1 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 program: **Omega_M6246_KFFlex.bdz**
- Click "Start".

6.2 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".
- *SPR1*: load the first SPR plate.
- Hit "START".
- *SPR2*: load the second SPR plate.
- Hit “START”.
- *VHB*: load the VHB 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.

6.3 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 contain **75 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 7 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 7.

Remove used consumables from Flex:

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

Citation: Lisa Marotz, Pedro Belda-Ferre, Mark Zeller, Catelyn Anderson, Sydney C Morgan, Rob Knight, Kristian Andersen (05/26/2021). Semi-Automated Extraction of Viral RNA using the Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit for SARS-CoV-2 Detection. https://dx.doi.org/10.17504/protocols.io.brqhm5t6

- Hit “START”.
- Remove the VHB plate and place to the side.
- Hit “START”.
- Remove the second SPR plate and place to the side.
- Hit “START”.
- Remove the first SPR plate and place to the side.

Pour the liquid from the SEP, VHB, and SPR 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

7

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** .

7.1 **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**

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

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

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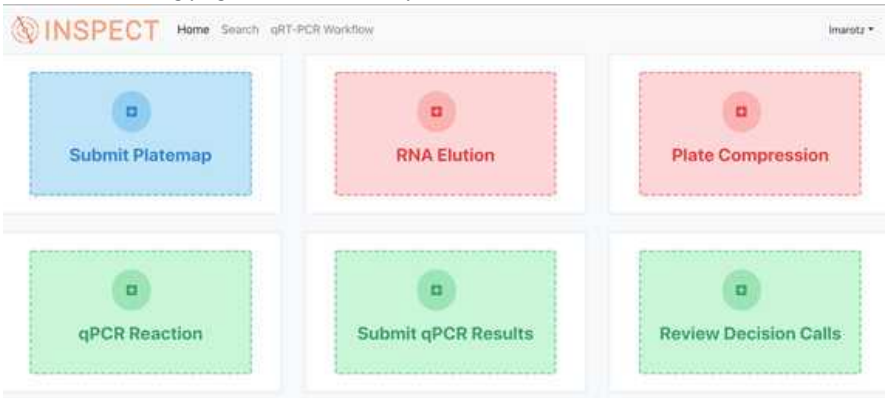
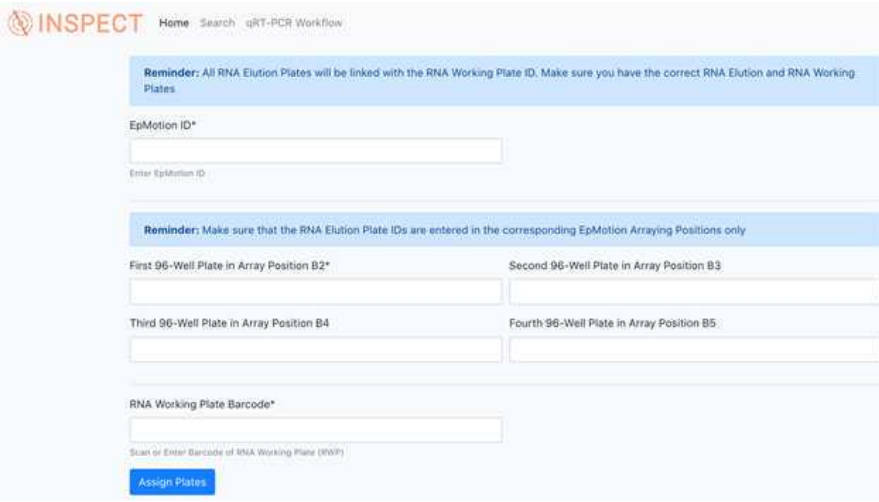
- On the following page, click "Plate Compression":



- 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.

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7.3 **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:

- Enter "96" and click "OK"
- If compressing only one plate enter "0", other wise enter "96" and click "OK"
- If compressing less than 3 plates, enter "0", otherwise enter "96" and click "OK"
- 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. 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

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