May 16, 2020

Kit-free automated RNA extraction for SARS-CoV-2 testing

In 1 collection

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bfb5jiq6

Crick COVID-19 Consortium


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ABSTRACT

This protocol is part of the Crick COVID-19 RT-PCR Testing Pipeline collection.

THIS DOCUMENT MUST BE READ AND UNDERSTOOD BY STAFF USING IT AND DOCUMENTED EVIDENCE PROVIDED THEREOF.

Purpose of examination / Clinical relevance

At the end of 2019, several pneumonia cases were reported in Wuhan, China and the pathogen was confirmed as a new viral strain. World Health organization has named the newly identified coronavirus as 2019-nCoV, also known as SARS-Cov-2. The disease developed into a dangerous pandemic, posing major challenges to the NHS. Although more research is necessary to better understand the virus, in response to the emergency, simple and rapid testing is essential to identify the virus in infected individuals. This will aid the implementation of efficient interventions to contain the spread, and distinguish healthcare workers who have been infected, and are required to self-isolate, from those showing similar symptoms but which are not 2019-nCoV associated. The latter category may continue to work, alleviating stress on hard-pressed healthcare resources. 2019-nCoV is an RNA virus, and the diagnostic tests detect viral RNA in swabs from patient airways using a reverse transcriptase PCR assay. Samples are submitted to HSL, an accredited reporting laboratory, and transferred to the Crick for testing. The first step of the process is sample receipt at the Crick. This SOP describes the extraction of RNA from the inactivated sample.

Principles of Examination

This procedure involves extraction of COVID-19 RNA from inactivated virus samples that have been aliquoted into plates. This protocol follows a method developed in-house that is automated on the Biomek FX liquid handling platform. All reagents and buffers prepared in house are batch tested and certified.

GUIDELINES

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Grade of operator

All qualified members of staff who have been signed off as competent and supervised trainees.

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Disposal

If the need arises to dispose of tubes or plates (e.g. due to spillage), all contaminated tubes and reagents used are discarded into plastic disposable jars and then into autoclave waste bins. All sharps are placed in a sharps container prior to incineration.

Automation

This protocol describes the automation of this method on the Biomek FX liquid handling platform but it could be performed manually or programmed on other instruments. We have also installed this method on an Agilent Bravo and obrain the same performance.

MATERIALS

NAME	CATALOG #	VENDOR
Honeywell Riedel-de Haen™ Water CHROMASOLV™ for HPLC	15692870 / 270733-2.5L	Fisher Scientific
Tris buffer pH 8.0 (1 M) for molecular biology	A4577,0500	AppliChem
Guanidine hydrochloride	G3272-1KG	Sigma Aldrich
2-Propanol	1096342500	Merck Millipore
Sodium acetate buffer solution 3M pH 5.2	S7899	Sigma Aldrich
Buffer PE	19065	Qiagen
Ethanol	1009832500	Merck Millipore
TWEEN® 20	T2700-100ML	Sigma Aldrich
EDTA solution pH 8.0 (0.5 M) for molecular biology	A4892,1000	AppliChem
Silica Magnetic Beads	786-915	G-Biosciences
Eppendorf twin.tec® PCR plate 96 LoBind	0030129512	Eppendorf
Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates	260251	Thermo Fisher Scientific
Axygen™ Storage Microplates	P-2ML-SQ-C	Fisher Scientific
Reagent reservoirs	613-1175	VWR International
Adhesive PCR Plate Seals	AB0558	Thermo Fisher Scientific
Biomek P250 Sterile Tips with Filter	717253	Beckman Coulter
Biomek Tips P50 Sterile Filtered	A21586	Beckman Coulter

MATERIALS TEXT

Note: Some of the reagents are used to make the buffers below.

Sample Requirements

Samples will be in a 96 deep well NUNC plate following virus inactivation and automated aliquoting.

Equipment / Consumables

Consumables	Used/run
Eppendorf 96-well skirted plates LoBind (Eppendorf, cat.no. 0030129512)	1
NUNC 96 deep well plates (ThermoScientific, cat.no. 260251)	3
Axygen squared 96-well deep well plates (Fisher Scientific cat.no. P-2ML-SQ-C)	1
Reservoir (VWR, cat. no. 613-1175)	3
Adhesive PCR Plate Seals (Thermo Fisher, cat no. AB0558)	1
BIOMEK FX 250 µl tips, Filtered, Sterile (cat. 717253)	4
BIOMEK FX 50 µl tips, Filtered, Sterile (cat. A21586)	3
Equipment	

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BIOMEK FX workstation and associated equipment	1
Alpaqua Magnum FLX (cat. A000400)	1

Magnum FLX® Enhanced Universal Magnet Plate
Magnet Plate
Alpaqua A000400

Buffers

TET buffer [10 ml] (for recipe see here)
PRE-PREPARED AND BATCH CERTIFIED

Reagent	Volume	Final concentration
Water	~49.4 ml	
0.5 M EDTA, pH 8.0	100 µl	1 mM
1 M Tris-HCl, pH 8.0	500 µl	10 mM
Tween-20	25 µl	0.05%

Binding Buffer [BB] [50 ml] (for recipe see here)
PRE-PREPARED AND BATCH CERTIFIED

Reagent	Volume/amount	Final concentration
Guanidine hydrochloride	23.88 g	5 M
Water	to 30 ml	
Isopropanol	to 50 ml	40%
Tween 20	25 µl	0.05%
3 M sodium acetate	2 ml	115 mM

PE-buffer, 60 ml needed for 1 BIOMEK FX extraction run
REMEMBER TO ADD ETHANOL TO QIAGEN REAGENT AS PER QIAGEN INSTRUCTIONS ON THE PE BOTTLE

Reagent	Volume
PE buffer by Qiagen	60 ml

EQUIPMENT

NAME	CATALOG #	VENDOR
Magnum FLX® Enhanced Universal Magnet Plate	A000400	Alpaqua

SAFETY WARNINGS

Health and Safety

All practices must be carried out in accordance with the current health and safety policies and procedures. If in any doubt about the aspects of health and safety concerning this procedure, seek advice from the departmental Safety Officer or the health and safety team.
Guanidine hydrochloride is harmful if inhaled, swallowed or if it comes into contact with skin and causes severe skin burns and eye damage. SARS-CoV-2 is currently classified as a Hazard Group Three (HG3) agent by Advisory Committee on Dangerous Pathogens (ACDP). As such this work falls within the Control of Substances Hazardous

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to Health (COSHH) Regulations.
The Government has provided guidance for clinical diagnostic laboratories safety, sampling and packaging of specimens associated with COVID-19. For hazards, risks and appropriate control measures identified in the risk assessment relevant to this procedure.

PPE
General personal protective equipment (PPE) Control Measures for laboratory work include the wearing of closed toe footwear, laboratory coat, appropriate disposable gloves (nitrile for general work or specified gloves for chemical work), and safety spectacles should be worn throughout this procedure.

Spillage
The spill kits provided for use in the department can be used for both biohazard and chemical spills. If a spill does occur, follow the procedure within the spill kit.

BEFORE STARTING

Prepare buffers according to section 'Materials'.

Biomek FX Setup

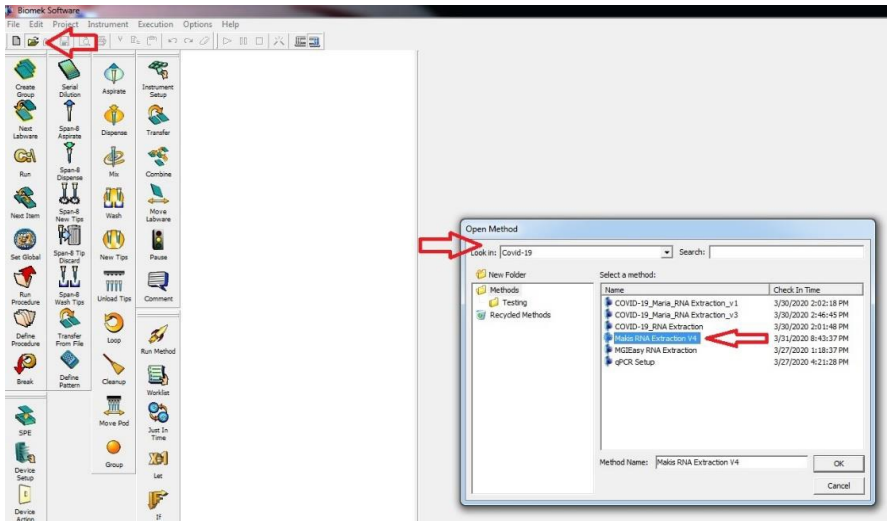
1

RNA extraction automated on Biomek FX using IN HOUSE protocol.

This protocol descibes the processing of one 96-well plate (93 samples and 3 controls).

[Note: At the Crick we have two platforms with slightly different decks (FX1 and FX2) and this protocol descibes setup on FX2]

Open the Biomek software, and select the program “RNA Extraction vFINAL” which is located in the “Covid-19” project:



2 Click on “Instrument Setup” as shown below to view the deck layout:

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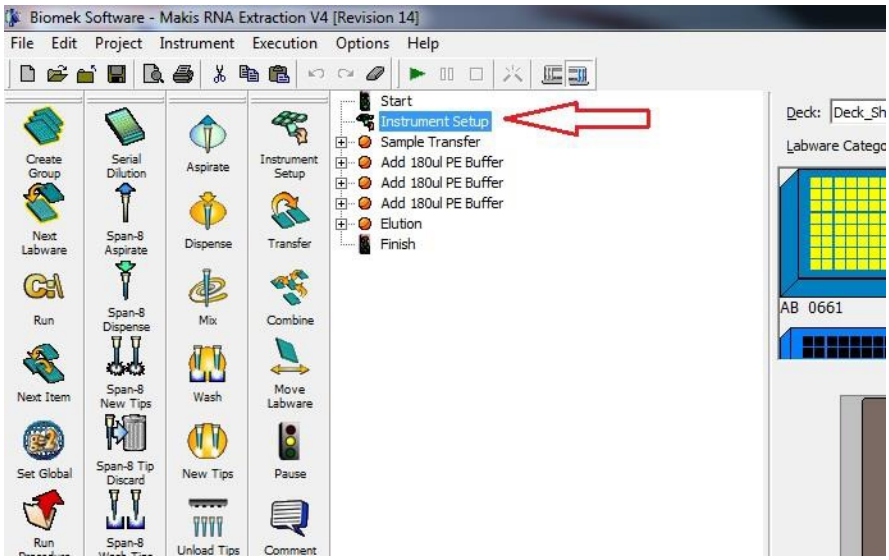

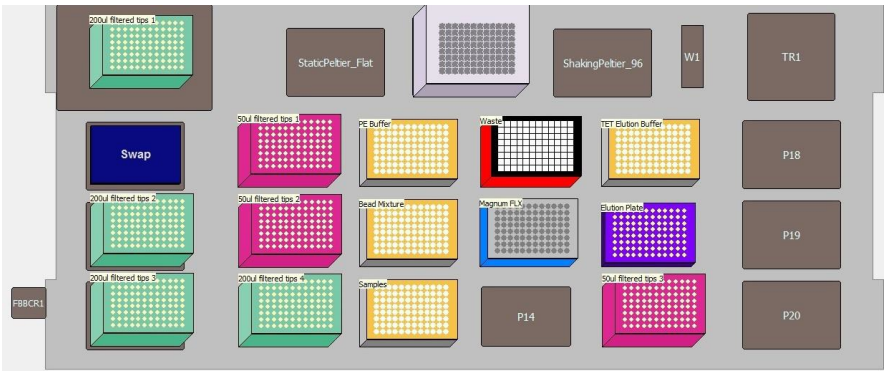

FX2 DECK LAYOUT

3 Place 4 boxes (lid off) of 250 µl filter tips (green boxes) as shown above.

4 Place 3 boxes (lid off) of 50 µl filter tips (pink boxes) as shown above.

5 Place an Axygen Deep well plate in position P12.

6 At the bench, prepare **Beads**, **Binding buffer/bead mix**, **PE Buffer**, **TET Buffer**, **Sample Plate**, **Elution Plate** and **Magnetic Plate** as follows:

Beads clean-up

7 Take a 1.5 ml tube of pre-aliquoted **silica beads** (**550 µl**) from the fridge and vortex well.

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We routinely use Silica Magnetic Beads from G-BioScience (see Materials) but we have also tested and validated SeraSil Mag 400 beads (GE Healthcare/Cytiva) as a potential substitite.

8 Record beads manufacturer and LOT number.

9 Place on the single tube magnet for **00:01:00** or until supernatant is clear.

10 Remove and discard supernatant (into the tip waste bin).

11 Pulse spin the tube and place on the magnet for **00:01:00** or until supernatant is clear.

12 Remove tube from magnet and add **550 µl TET** . Resuspend the beads by vortexing.

13 Remove and discard supernatant.

14 Repeat bead washing for a total of 3 times.

14.1 Add **550 µl TET** . Resuspend the beads by vortexing.
Pulse spin the tube and place on the magnet for **00:01:00** or until supernatant is clear.
Remove and discard supernatant. (2/3)

14.2 Add **550 µl TET** . Resuspend the beads by vortexing.
Pulse spin the tube and place on the magnet for **00:01:00** or until supernatant is clear.
Remove and discard supernatant. (3/3)

15 Remove the tube from the magnet, resuspend the beads in **550 µl TET** , vortex and spin down.

Binding buffer/bead mix

16 In the Falcon tube containing the **50 ml binding buffer** (labelled BB, pre-aliquoted) add the beads prepared..

17 Mix well by inverting.

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18 Pour into a reservoir and aliquot **400 µl** immediately *in each well* of a 96 deep well Nunc plate, using a multichannel pipette. Place on the deck in position **P9**.

PE Buffer

19 Add **PE buffer** to a new reservoir and aliquot **600 µl** into a new Nunc plate and place plate in position **P8**.

TET Buffer

20 Add **TET** to new reservoir and aliquot **6 ml TET** (from batch certified aliquot) into a new Nunc plate and place in position **P15**.

Sample Plate

21 Place the sample plate (seal removed) in position **P10**.

N.B.: If samples have been temporarily stored, briefly spin in the plate centrifuge.

Elution Plate

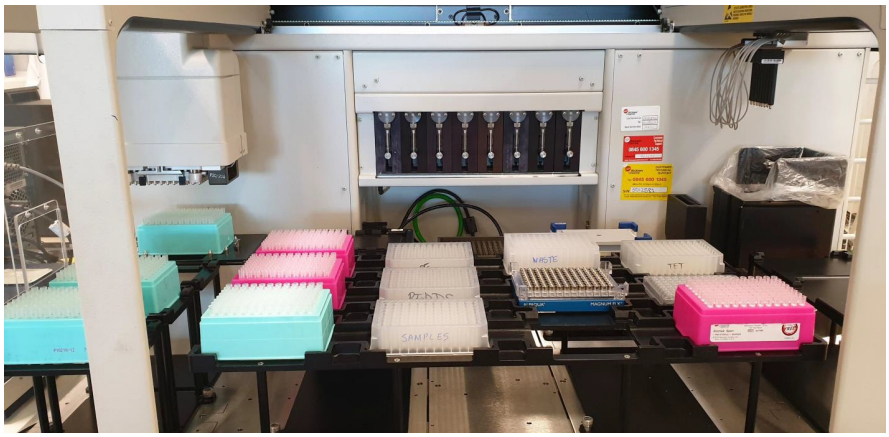
22 Label an empty Eppendorf LoBind 96-well skirted plate with a pre-printed SPL barcode (e.g. SPL00000) on the front side, and place in position **P16**.

Magnetic Plate

23 Place the Alpaqua Magnum FLX magnetic plate in position **P13**.

Final Setup

24 The final set up should look like the photo below:



FX2 DECK LAYOUT

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25 Using the laptop beside the FX, push the samples through the Extraction step in ClarityLIMS (refer to 'COVID-19 LIMS walkthrough' document on COVID-19 sample handling landing page).

26 Make sure you switch ON the Inheco (blue box next to the FX monitor).

27 To START the program press the Run button (green triangle) located in the software.

The robot carries out the following automated protocol

1. Transfer 150 µl of samples to 400 µl (2.6x Vol) of Binding Buffer (BB) containing the Silica beads and mixe 10 times by pipetting up and down
2. Move the plate on the Shaker for 5 min
3. Move plate to position P8 and mix 20 times
4. Move plate to Shaker for 2 min
5. Move plate to P8 and incubate for 3 min
6. Move plate to Magnet for 5 min
7. Remove supernatant
8. Move plate from magnet to position P8

Wash with PE (1)
9. Transfer 180 µl of PE Buffer in each well of the Bead Mixture plate
10. Move plate to the Shaker for 20 sec
11. Move plate to the Magnet for 60 sec
12. Remove supernatant
13. Move plate from the magnet to position P8

Wash with PE (2)
14. Transfer 180 µl of PE Buffer in each well of the Bead Mixture plate
15. Move plate to the Shaker for 20 sec
16. Move plate to the Magnet for 60 sec
17. Remove supernatant
18. Move plate from the magnet to position P8

Wash with PE (3)
19. Transfer 180 µl of PE Buffer in each well of the Bead Mixture plate
20. Move plate to the Shaker for 20 sec
21. Move plate to the Magnet for 60 sec
22. Remove supernatant
23. Move plate from the magnet to position P8
24. Wait for 2 min (drying)

Elution
25. Transfer 40 µl of TET Buffer to the Bead Mixture plate and mix 10 times
26. Move plate to the Shaker for 5 min
27. Move plate to the magnet for 60 sec
28. Transfer 35 µl from the Bead Mixture plate to the Eppendorf twin.tec plate

28 When then program is complete, remove the Eppendorf 96-well skirted plate from the deck, and seal with a Thermo Adhesive PCR plate seal.

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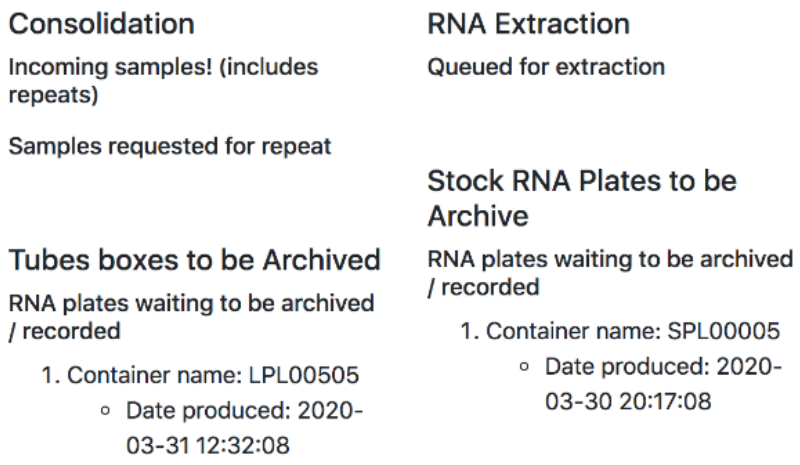
29 Continue with the qPCR RNA Transfer or place in the **-80 °C** until it is used for qPCR setup.

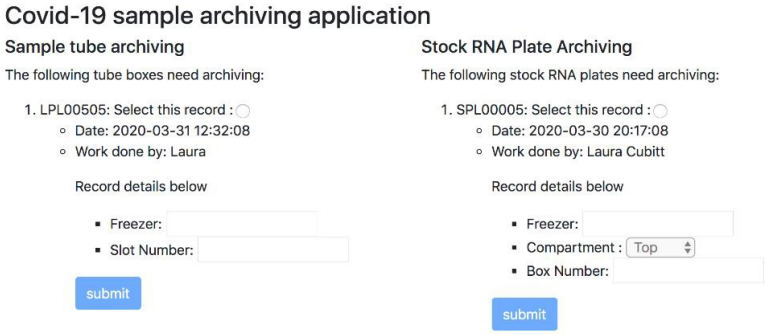
Archiving

30 Archiving of Inactivated viral samples would have already been performed after sample plating. For archiving the stock RNA plates using the app in Clarity LIMS, a database table is updated with the information.

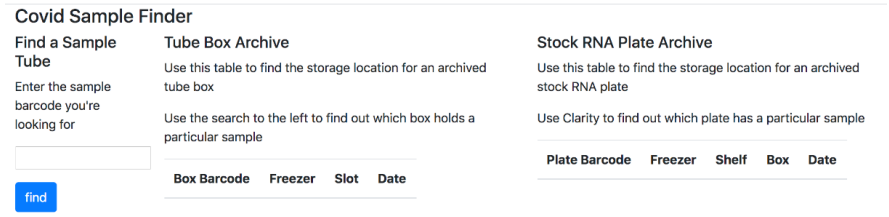
31 This results in an entry appearing on the dashboard to let the team know the samples are ready to be archived:



32 An archiving app then displays the boxes and plates that are waiting to be archived, and gives the team the chance to enter the storage information:



33 Once logged, the record disappears from this screen, and the archiving information will appear in the archive search app:



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