Oxford Nanopore DIAGNOSTICS

Instructions for Use

Oxford Nanopore Diagnostics LamPORE COVID-19 Test Kit

OND-SQK-LP0096S | OND-SQK-LP0096M | OND-SQK-LP0096M-A | OND-EXP-SCT001 | OND-EXP-SCT002 | OND-EXP-SCT002-A | OND-EXP-FRK001

For use with

OND-FLO-MIN106D | OND-GRD003

FOR *IN VITRO* DIAGNOSTIC USE ONLY

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Oxford Nanopore Diagnostics
LamPORE COVID-19 Test Kit

1. Intended use

Oxford Nanopore Diagnostics LamPORE COVID-19 Test Kit 96 Plex (S, M or M-A) is a test intended for qualitative detection of nucleic acid from the SARS-CoV-2 virus in oropharyngeal and nasopharyngeal swabs.

Positive results indicate the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Oxford Nanopore Diagnostics LamPORE COVID-19 Test Kit 96 Plex is intended for use by laboratory personnel experienced in the conduct of *in vitro* diagnostic procedures.

2. Principle of the examination method

LamPORE has been developed by Oxford Nanopore Diagnostics to enable the simple and rapid detection of target regions of single or multiple genomes in a highly multiplexed manner.

The LamPORE COVID-19 Test Kits are designed to detect the presence of the SARS-CoV-2 viral RNA in oropharyngeal and nasopharyngeal swabs.

LamPORE combines barcoded multi-target isothermal amplification, rapid barcoded library preparation and real-time nanopore sequencing. By placing molecular barcodes in the Loop-mediated isothermal amplification (LAMP) reaction and coupling these with Oxford Nanopore's rapid barcoding adapters, a dual indexing approach is achieved, enabling many barcode combinations to be generated and analysed.

LamPORE deploys a reverse transcription LAMP (RT-LAMP) specific to three regions of the SARS-CoV-2 genome:

- N gene
- E gene
- ORF1a gene

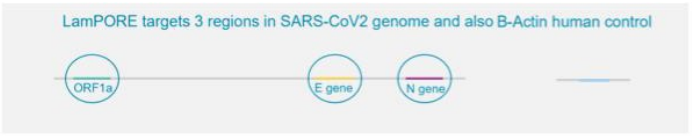
Additionally, a set of primers to amplify human β-actin mRNA is included as an internal process control. Failure to detect β-actin RNA in a swab sample strongly suggests inappropriate sample collection, storage or processing, and identifies a sample as invalid and requiring repeat testing.

To perform amplification, a strand-displacing polymerase is added to extracted RNA in the presence of primers targeting the regions of interest, and the reaction is incubated at 65°C for 35 minutes. In the initial stages of the reaction, the enzyme produces a series of dumbbell-shaped cDNA molecules and these are then exponentially amplified. Amplification of the dumbbell-shaped molecules results in long DNA strands consisting of concatenated copies of the original 180 bp target region.

To enable pooling of multiple samples into a single sequencing run, the LamPORE SARS-CoV-2 primers also include a 10-nucleotide sequence that acts as an identifying barcode for the sample placed in each well. Following amplification, several copies of the barcode are incorporated into each DNA concatemer.

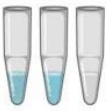
At the end of the reaction, the enzymes are inactivated by incubation at 80°C for 5 minutes. LAMP produces a variety of products including multimeric DNA with inverted repeats. These complex amplification products are converted into nanopore sequencing libraries using the rapid barcoding chemistry. A second barcode is added at this stage to further increase the number of samples that can be tested in parallel.

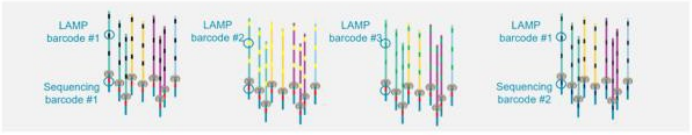
1 **RNA is extracted from a swab**



2 **Isothermal amplification step**

3 **Rapid library preparation step**





4 **Sequence and analyse**

3. Materials provided

LamPORE COVID-19 Test Kit 96 Plex S (OND-SQK-LP0096S)

Kit component	Quantity	Volume per plate or tube	Storage	Shelf life
LAMP Master Mix (LMM)	1x 96-well plate	30 µl per well (25 µl per test)	≤ -20°C	As marked
LAMP Primer Mixes (LPM)	1x 96-well plate	7 µl per well (5 µl per test)	≤ -20°C	As marked
Rapid Barcodes (RB)	1x 96-well plate	5 µl per well (2.5 µl per 8 tests)	≤ -20°C	As marked
SPRI Beads (SPRI)	1 tube	140 µl (96 µl per flow cell)	≤ -20°C	As marked
Elution Buffer (EB)	1 tube	200 µl (15 µl per flow cell)	≤ -20°C	As marked
Sequencing Buffer (SQB)	1 tube	300 µl (37.5 µl per flow cell)	≤ -20°C	As marked
Loading Beads (LB)	1 tube	360 µl (25.5 µl per flow cell)	≤ -20°C	As marked
Rapid Adapter (RAP)	1 tube	10 µl (1 µl per flow cell)	≤ -20°C	As marked
Flush Buffer (FB)	6 tubes	1.17 ml (1.17 ml per flow cell)	≤ -20°C	As marked
Flush Tether (FLT)	1 tube	200 µl (30 µl per flow cell)	≤ -20°C	As marked

SARS-CoV-2 Control Kit S (OND-EXP-SCT001)

Kit component	Quantity	Volume per tube	Description	Storage	Shelf life
Positive Control (CTL)	6 tubes	2 µl (<0.1 µl per reaction). The Positive Control is diluted substantially by the customer by addition of water directly to the vial.	Positive control containing synthetic SARS-CoV-2 RNA	≤ -80°C	As marked
No Template Control (NTC)	4 tubes	300 µl (20 µl per reaction)	Negative control containing nuclease-free water	≤ -20°C	As marked

LamPORE COVID-19 Test Kit 96 Plex M (OND-SQK-LP0096M)

Kit component	Quantity	Volume per plate or tube	Storage	Shelf Life
LAMP Master Mix (LMM)	8x 96-well plate	30 µl per well (25 µl per test)	≤ -20°C	As marked
LAMP Primer Mixes (LPM)	8x 96-well plate	7 µl per well (5 µl per test)	≤ -20°C	As marked
Rapid Barcodes (RB)	1 x 96-well plate	5 µl per well (2.5 µl per 8 tests)	≤ -20°C	As marked
SPRI Beads (SPRI)	1 tube	1 ml (96 µl per flow cell)	≤ -20°C	As marked
Elution Buffer (EB)	1 tube	200 µl (15 µl per flow cell)	≤ -20°C	As marked
Sequencing Buffer (SQB)	1 tube	400 µl (37.5 µl per flow cell)	≤ -20°C	As marked
Loading Beads (LB)	1 tube	360 µl (25.5 µl per flow cell)	≤ -20°C	As marked
Rapid Adapter (RAP)	1 tube	13 µl (1 µl per flow cell)	≤ -20°C	As marked
Flush Buffer (FB)	8 tubes	1.17 ml (1.17 ml per flow cell)	≤ -20°C	As marked
Flush Tether (FLT)	1 tube	400 µl (30 µl per flow cell)	≤ -20°C	As marked

SARS-CoV-2 Control Kit M (OND-EXP-SCT002)

Kit component	Quantity	Volume per tube	Description	Storage	Shelf life
Positive Control (CTL)	8 tubes	2 µl (<0.1 µl per reaction). The Positive Control is diluted substantially by the customer by addition of water directly to the vial.	Positive control containing synthetic SARS-CoV-2 RNA	≤ -80°C	As marked
No Template Control (NTC)	11 tubes	300 µl (20 µl per reaction)	Negative control containing nuclease-free water	≤ -20°C	As marked

LamPORE COVID-19 Test Kit 96 Plex M-A (OND-SQK-LP0096M-A)

Kit component	Quantity	Volume per plate or tube	Storage	Shelf Life
LAMP Master Mix (LMM)	1x 96-well plate	240 µl per well (25 µl per test)	≤ -20°C	As marked
LAMP Primer Mixes (LPM)	1x 96-well plate	56 µl per well (5 µl per test)	≤ -20°C	As marked
Rapid Barcodes (RB)	1 x 96-well plate	5 µl per well (2.5 µl per 8 tests)	≤ -20°C	As marked
SPRI Beads (SPRI)	1 tube	1 ml (96 µl per flow cell)	≤ -20°C	As marked
Elution Buffer (EB)	1 tube	320 µl (15 µl per flow cell)	≤ -20°C	As marked
Sequencing Buffer (SQB)	1 tube	700 µl (37.5 µl per flow cell)	≤ -20°C	As marked
Loading Beads (LB)	1 tube	560 µl (25.5 µl per flow cell)	≤ -20°C	As marked
Rapid Adapter (RAP)	1 tube	25 µl (1 µl per flow cell)	≤ -20°C	As marked
Flush Buffer (FB)	1 tube	11 ml (1.17 ml per flow cell)	≤ -20°C	As marked
Flush Tether (FLT)	1 tube	400 µl (30 µl per flow cell)	≤ -20°C	As marked

SARS-CoV-2 Control Kit M-A (OND-EXP-SCT002-A)

Kit component	Quantity	Volume per tube	Description	Storage	Shelf life
Positive Control (CTL)	8 tubes	2 µl (<0.1 µl per reaction). The Positive Control is diluted substantially by the customer by addition of water directly to the vial.	Positive control containing synthetic SARS-CoV-2 RNA	≤ -80°C	As marked
No Template Control (NTC)	8 tubes	300 µl (20 µl per reaction)	Negative control containing nuclease-free water	≤ -20°C	As marked

Flow Cell Recalibration Kit (OND-EXP-FRK001)

Kit component	Quantity	Volume per tube	Storage	Shelf life
Wash Mix (WMX)	1 tube	15 µl (2 µl per reaction)	≤ -20°C	As marked
Wash Diluent (DIL)	2 tubes	1.3 ml (398 µl per reaction)	≤ -20°C	As marked
Storage Buffer (S)	2 tubes	1.6 ml (500 µl per reaction)	≤ -20°C	As marked

Flow Cell (OND-FLO-MIN106D)

Component	Quantity	Storage	Shelf life
MinION / GridION flow cell	1	2–8°C	As marked

Product MSDS is available on www.oxfordnanoporedx.com

4. Storage and stability

The LamPORE COVID-19 Test Kit 96 Plex (S, M or M-A) is shipped with ice packs at –20°C and stored at –20°C. The materials provided are resilient to up to four freeze-thaw cycles and 1 week at room temperature.

The SARS-CoV-2 Control Kit (S, M or M-A) is shipped on dry ice at –80°C and stored at –80°C. Freeze-thaw cycles of the Positive Control (CTL) are not recommended (do not store CTL at –20°C). The No Template Control (NTC) is nuclease-free water and is stable at –80°C to +25°C and can be freeze-thawed as required.

The Flow Cell Recalibration Kit is shipped at –20°C and must be stored at –20°C. The materials provided are resilient to up to 10 freeze-thaw cycles and 1 week at room temperature.

The Flow Cell is shipped at 2–8°C and stored at 2–8°C. The flow cell is stable for up to 1 month at room temperature. Freezing the flow cell irreversibly damages the product and must not be done. There is a flow cell check performed at the start of each experiment to verify the integrity and correct functioning of the flow cell.

5. Instruments and materials required but not provided

	Product name	Manufacturer	Cat. No.
Instruments/ equipment	Thermal cycler or 96-well heat block	-	-
	8-channel pipette capable of handling volumes of 0.5–10 µl and 5–50 µl, and a 12-channel pipette capable of handling volumes of 0.5–10 µl	-	-
	Single-channel pipettes capable of handling volumes from 2 µl to 1000 µl	Gilson	FA10001M FA10002M FA10003M FA10004M FA10005M FA10006M
	Bench-top centrifuge with plate adapter	-	-
	Vortex mixer	-	-
	Magnetic rack	-	-
	Automated sample and reagent handling equipment (if used)	-	-
RNA extraction kit (Manual)	Extraction system suitable for the directed sample type	-	-
Consumables	1.5 ml DNA LoBind tubes	Eppendorf	022431081
	5 ml DNA LoBind tubes	Eppendorf	0030108310
	0.2 ml 96-well PCR plates	-	-
	96-well plate lids or seals	-	-
	0.2 ml thin-walled PCR tubes	-	-
	Nuclease-free water	ThermoFisher	AM9937
	Freshly-prepared 80% ethanol in nuclease-free water	-	-
	Nuclease-free pipette filter tips	-	-

Always read 3rd party materials/equipment supplier's documentation prior to use and follow the instruction for correct operation and any required maintenance.

We recommend purchasing non-flexible pipette tips to facilitate plate foil piercing.

6. Reagent preparation

Reagent	Thaw	Mix/spin	Store
LAMP Master Mix (LMM)	At room temperature	Spin down the plate	On ice
LAMP Primer Mixes (LPM)	At room temperature	Spin down the plate	On ice
Positive Control (CTL)	On ice	Vortex and spin down	On ice
No Template Control (NTC)	At room temperature	Vortex and spin down	On ice
Rapid Barcodes (RBxx)	At room temperature	Spin down the plate	On ice
Rapid Adapter (RAP)	At room temperature	Vortex and spin down	On ice
SPRI Beads (SPRI)	At room temperature	Vortex and spin down. Mix well by vortexing immediately before use	At room temperature
Elution Buffer (EB)	At room temperature	Vortex and spin down	On ice
Sequencing Buffer (SQB)	At room temperature	Vortex and spin down	On ice
Loading Beads (LB)	At room temperature	Mix well by pipetting immediately before use	On ice
Flush Buffer (FB)	At room temperature	Vortex and spin down	On ice
Flush Tether (FLT)	At room temperature	Vortex and spin down	On ice

Kit components must be thawed at room temperature (RNA samples and Positive Control thawed on ice) and placed on ice as soon as the contents are thawed. Reagents should be returned to frozen storage immediately after use. Do not freeze-thaw test reagents more than 4 times. Do not freeze-thaw the CTL.

7. Warnings and precautions

7.1. Limitations of the LamPORE assay

1. Prevent contamination of reagents by following standard contamination prevention procedures including but not limited to the use of personal protective equipment, separation of sample handling and extraction locations from the post amplification stage of LamPORE, using filter pipette tips for handling RNA and using dedicated pipettes for pre-amplification areas and post amplification areas.

2. Reagents must be stored under the indicated storage conditions before and after use.

3. Waste should be disposed of in accordance with infectious waste disposal regulations.

4. Do not mix different lots of reagents.

5. We recommend using DNase & RNase-free pipette tips. Never reuse the consumables (for example, tips, gloves, and test tubes).

6. Always wear protective gloves, protective goggles, etc. when handling the samples and test reagents. Take care to prevent the reagents from contacting your skin, eyes, mucous membranes, etc. If contact occurs, immediately wash the affected area with water. Thoroughly wash your hands after handling samples and test reagents.

7. Do not use the test reagent if the package is damaged or reagent tubes are leaking (reagents may be compromised, leading to an incorrect result). Please contact technical support. When dealing with multi-reaction volumes, users should not re-seal opened plates.

8. Do not use test reagents after their expiration date.

9. Decontaminate surfaces with 0.5% sodium hypochlorite (bleach) diluted with deionized or purified water. For instructions on cleaning the GridION, refer to the GridION User Manual.

10. LAMP can produce large numbers of easily-amplifiable molecules after a successful reaction, and therefore due to the risk of cross-contamination the LamPORE assay may be unsuitable for testing samples obtained from operators of the LamPORE system.

7.2. Limitation of the experimental procedure

1. Specimens must be collected, transported, and stored using procedures and conditions determined by the user to preserve the integrity of specimens for use in molecular assays for the detection of viral RNA. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target molecules.

2. Extraction of nucleic acid from clinical specimens must be performed using methods that have been validated by the user to be appropriate for use in molecular assays for the detection of viral RNA.

3. Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.

4. The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this document.

5. False-negative results may arise from:

    - Improper specimen collection
    - Degradation of the viral RNA during shipping/storage
    - Using inappropriate extraction or assay reagents
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
    - Using expired reagents

6. False-positive results may arise from:

    - Cross-contamination from incorrect handling of Positive Control
    - Cross-contamination during specimen handling or preparation
    - Cross-contamination between patient samples
    - Specimen mix-up
    - RNA contamination during product handling

7. As with any molecular test, mutations within the target regions of the SARS-CoV-2 assay could affect primer binding, resulting in failure to detect the presence of virus.

8. The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

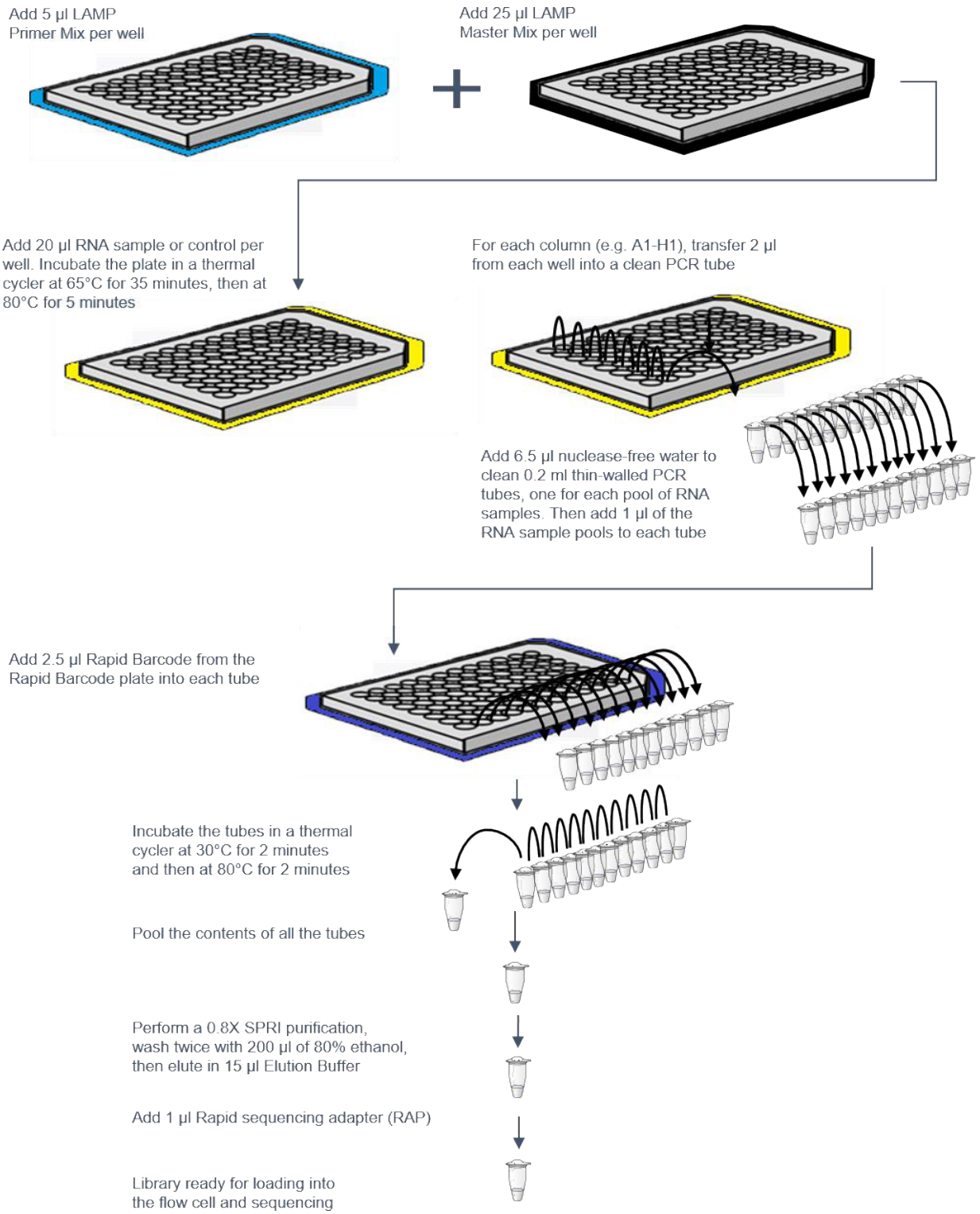
9. Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision. Follow-up testing should be performed according to the current CDC recommendations.

10. A positive result indicates the detection of nucleic acid from SARS-CoV-2.

11. Nucleic acid may persist even after the virus is no longer viable.

12. The SARS-CoV virus is closely related to SARS-CoV-2, and if it is in active circulation, this may give a positive result.

8. Overview of procedure



9. Sample preparation

9.1. Specimen collection

The LamPORE workflow starts with RNA extracted from nasopharyngeal or oropharyngeal swabs.

Inadequate or inappropriate specimen collection, storage and transport are likely to yield invalid or incorrect test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

1. Refer to local guidance or guidance from the WHO on sampling for diagnostic laboratories

2. In the UK, refer to the UK Government COVID-19 guidance for sampling and for diagnostic laboratories: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories

3. Follow specimen collection devices manufacturer instructions for proper collection methods.

Specimens must be packaged, shipped and transported according to the local government regulations.

9.2. RNA extraction from samples

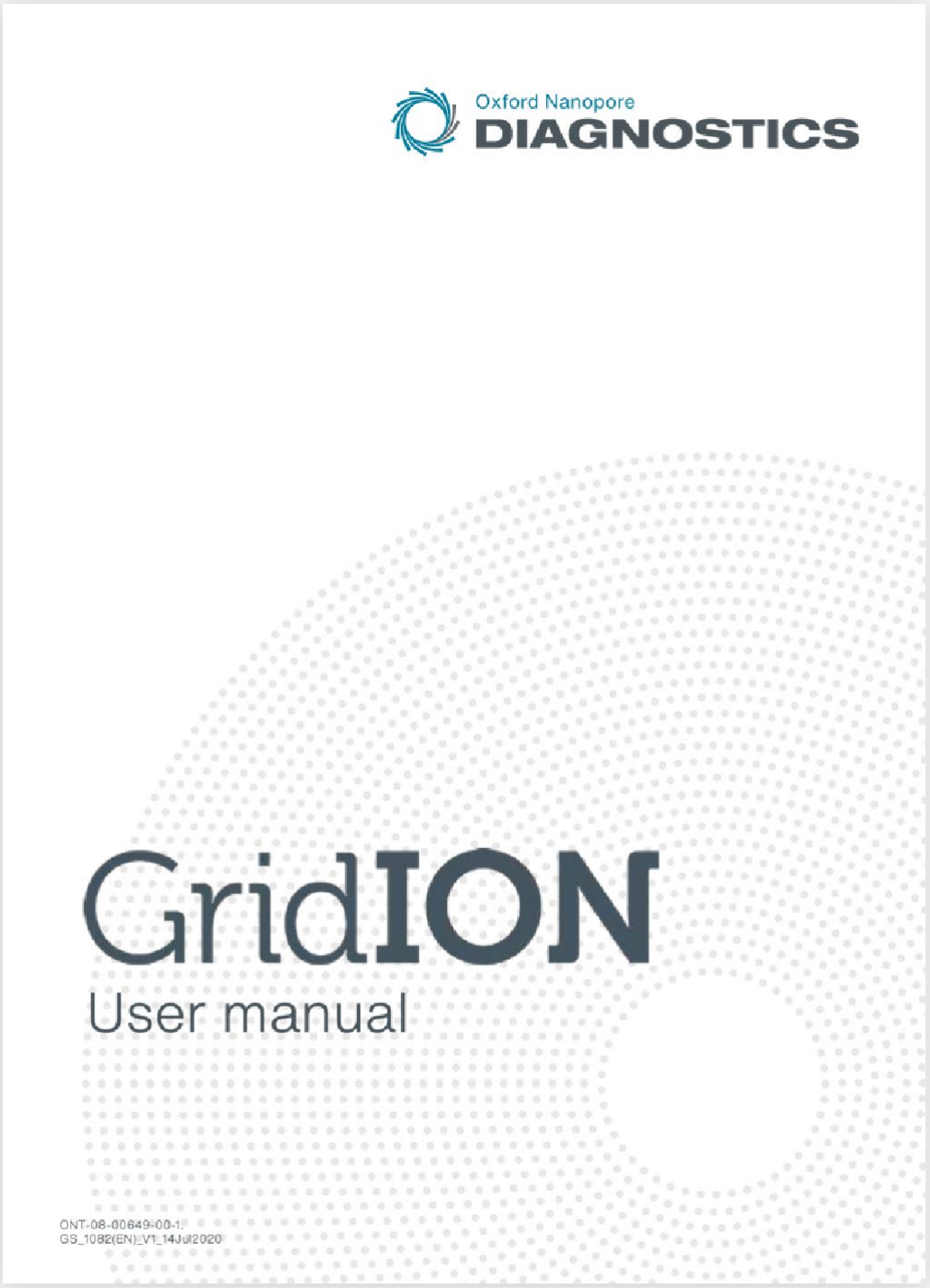
Please consult the relevant Instruction For Use (IFU) and Materials Safety Data Sheet (MSDS), available from the manufacturer of your selected RNA extraction method, before use.

As a reference only, kits used during the LamPORE product evaluations include:

- QIAGEN QiaAMP Viral RNA Mini kit (52906)
- Roche Life Sciences MagNA Pure 96 DNA and Viral NA Small Volume Kit (06543588001)
- QIAGEN QIAsymphony DSP Virus/Pathogen Kit (937036)

10. GridION installation and configuration

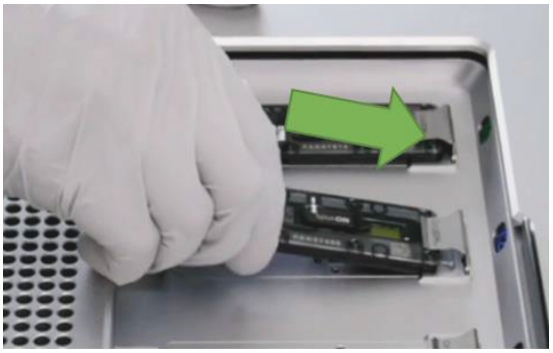
Please refer to the GridION User Manual for instructions for how to install and configure your device for use.

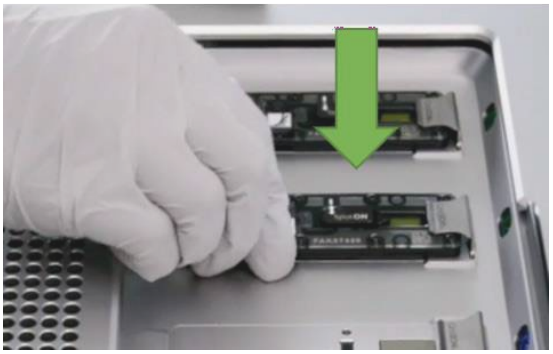


11. Checking the flow cell

1. Switch on the GridION device. When the login screen appears, enter the password and log in.

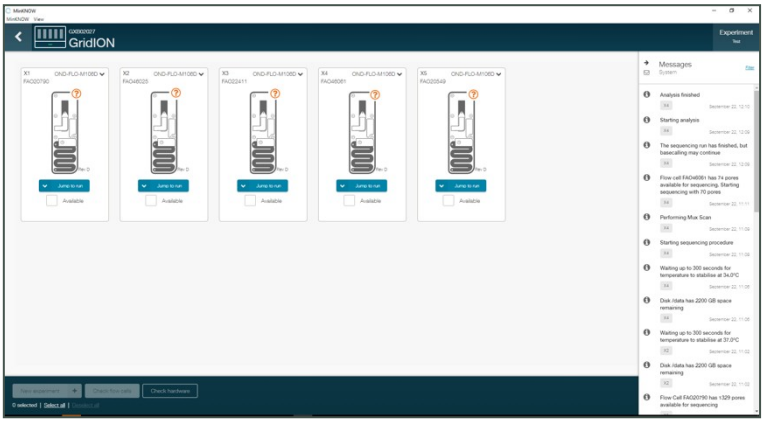
2. Slide open the GridION lid and insert the flow cell. Press down firmly on the flow cell to ensure correct thermal and electrical contact.





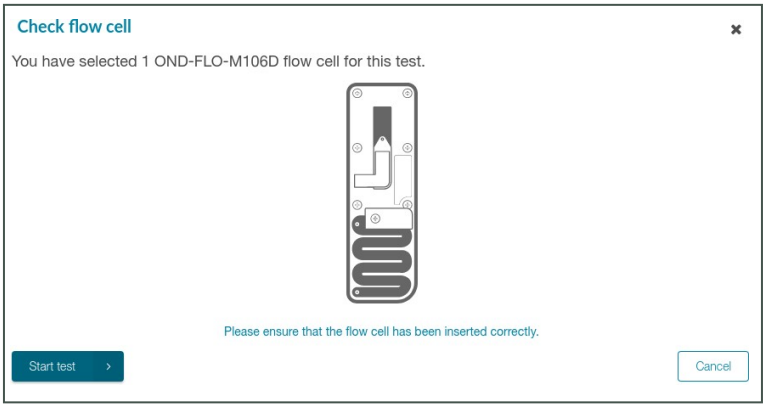
3. Click the Nanopore wheel icon on the desktop to load the MinKNOW software. You will see the MinKNOW user interface appear.

4. Check the "Available" box for all the flow cells you want to run.



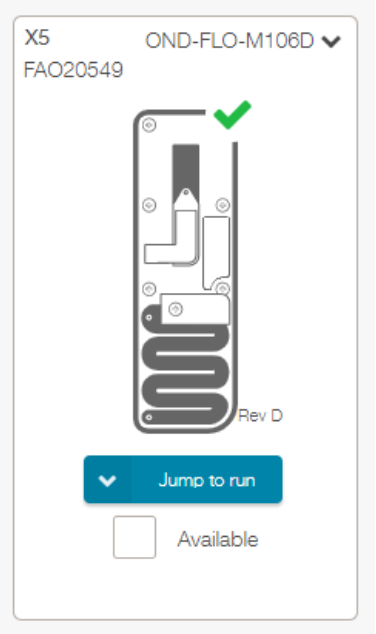
5. Click "Check flow cells" at the bottom of the screen.

6. A screen will load displaying the number of flow cells selected for your test. Click "Start test". The flow cell check will take approximately 10 minutes.



The quality of the flow cell will be shown as one of the two outcomes:

A green tick - The flow cell is within warranty and can be used for a LamPORE test.



A yellow exclamation mark - The flow cell is below warranty and should not be used for the LamPORE test. Please contact **support@oxfordnanoporedx.com** to arrange a replacement.

Note: The indicator of quality (exclamation mark or tick) will only remain visible during a MinKNOW session. Once the MinKNOW service has ended, the status of the flow cell will be erased.

12. LamPORE Control Experiment

The LamPORE control experiment is designed for users who have never run LamPORE before to gain familiarity with the process and to ascertain their performance before running clinical samples.

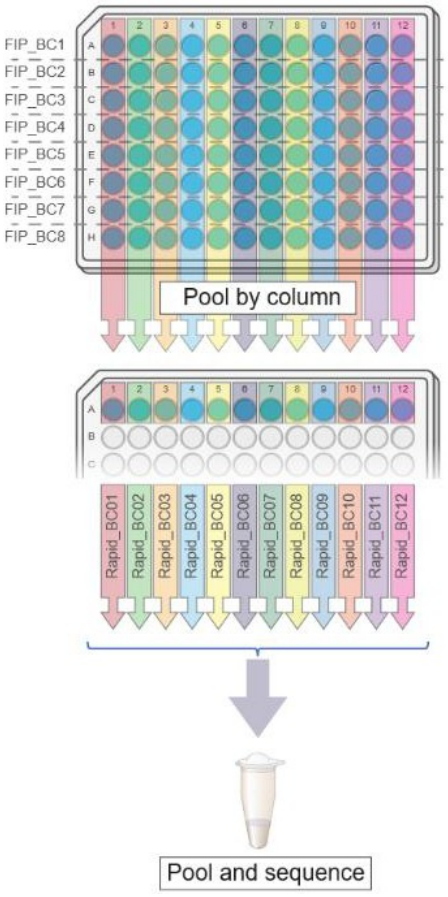
12.1. RT-LAMP procedure

LAMP can produce large numbers of easily-amplifiable molecules after a successful reaction, and therefore the risk of cross-contamination between samples must be mitigated.

- Preparation for amplification (RT-LAMP) must be performed in a PCR hood or bubble in a completely separate area to the library preparation
- Use dedicated pipettes for liquid handling, with filter pipette tips for the RNA processing and LAMP
- Wear a fresh pair of gloves that are immediately discarded after this step
- After the LAMP reaction is complete, remove the seal from the PCR plate very carefully to avoid splashing/cross-contamination between wells
- Clean down hoods and surfaces with an appropriate cleaning agent (e.g. bleach) before and after the RT-LAMP step and between reagent plates.

Barcoding of samples

This method uses a combinatorial barcoding approach. One set of barcodes is contained in the FIP primers, used in the LAMP reaction, and an additional set of Rapid Barcodes is also supplied. All controls in a given row of a 96-well plate of controls will receive the same FIP barcode, and all controls in a given column of the plate will ultimately receive the same Rapid Barcode, as shown in the figure below.



Here, samples in row A of a 96-well plate of samples receive FIP barcode 01, samples in row B receive FIP barcode 02, etc. Meanwhile, once pooled, samples in column 1 receive the Rapid Barcode 01, samples in column 2 receive the Rapid Barcode 02, etc. This way, each sample will receive a unique combination of FIP barcode and Rapid Barcode.

1. Prepare the reagents as follows:

Reagent	Thaw	Mix/spin	Store
LAMP Master Mix (LMM)	At room temperature	Spin down the plate	On ice
LAMP Primer Mixes (LPM)	At room temperature	Spin down the plate	On ice
Positive Control (CTL)	On ice	Vortex and spin down	On ice
No Template Control (NTC)	At room temperature	Vortex and spin down	On ice

2. Immediately before beginning the RT-LAMP, carry out the following steps:

    a. Add 1 ml of nuclease-free water to a 5 ml Eppendorf DNA LoBind tube.

    b. Transfer 2 µl of CTL into the water in the 5 ml tube. If insufficient volume is obtained from a single CTL tube, pool together the contents of two CTL tubes and transfer 2 µl from the pool.

    c. Vortex for at least 30 seconds and spin down if possible.

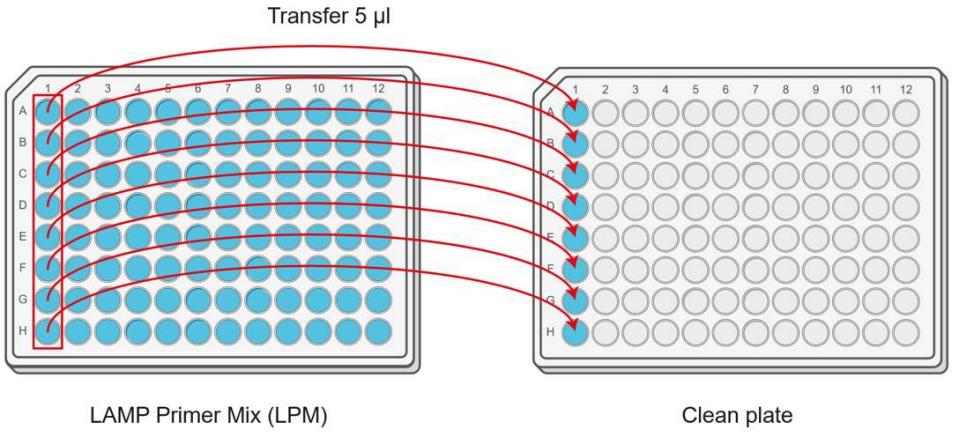
    d. Carefully open the tube to avoid splashing, then add 1 ml nuclease-free water

into tube and mix by pipetting up and down 10 times.

e. Vortex the tube again for at least 30 seconds and spin down if possible.

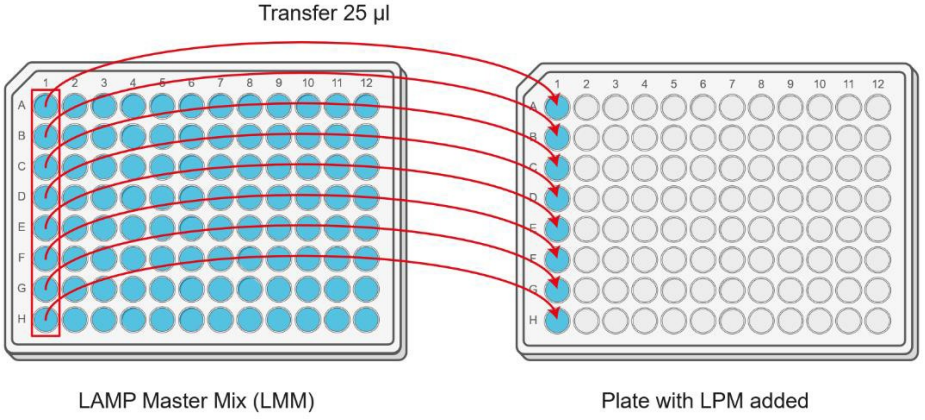
3. Set a multichannel pipette to 5 µl. Mix the contents of column 1 of the LAMP Primer Mix (LPM) plate by pipetting up and down several times. Then transfer 5 µl of LAMP Primer Mix from column 1 of the LPM plate into column 1 of a clean 96-well plate. Repeat the mixing and pipetting steps for all the remaining columns.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.

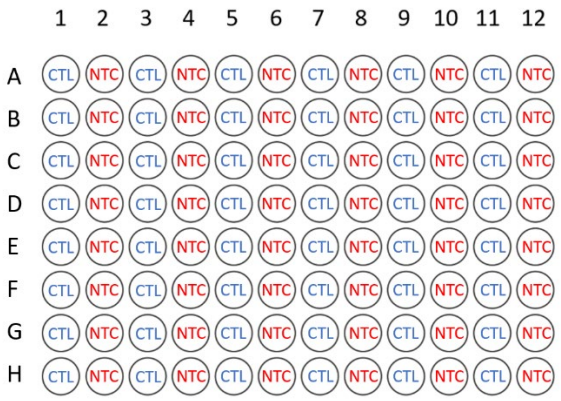


4. Set a multichannel pipette to 25 µl. Mix the contents of column 1 of the LAMP Master Mix (LMM) plate thoroughly by pipetting up and down several times. Then transfer 25 µl of LAMP Master Mix from column 1 of the LMM plate into column 1 of the 96-well plate. Mix the contents of the wells by pipetting up and down. Repeat the mixing and pipetting steps for all the remaining columns.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



5. Add CTL and NTC to alternating columns of the plate, as shown in the diagram. Add 20 µl NTC to each well in the row. For the CTL rows, add 5 µl of CTL to each well followed by 15 µl nuclease-free water to bring the total volume to 20 µl.



6. Mix the contents of each well by pipetting up and down, taking care not to cross-contaminate different wells.

7. Seal the plate and spin down in a microfuge for 10 seconds.

8. Incubate the plate in a thermal cycler at 65°C for 35 minutes, then at 80°C for 5 minutes.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

12.2. Library preparation

Library preparation should be carried out in a separate area of the laboratory to the RT-LAMP PCR hood/bubble.

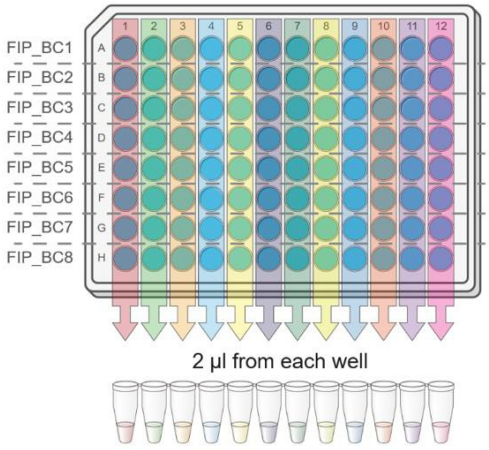
1. Prepare the remaining reagents as follows:

Reagent	Thaw	Mix/spin	Store
Rapid Barcodes (RBxx)	At room temperature	Spin down the plate	On ice
SPRI Beads (SPRI)	At room temperature	Vortex and spin down	At room temperature
Rapid Adapter (RAP)	At room temperature	Vortex and spin down	On ice
Elution Buffer (EB)	At room temperature	Vortex and spin down	On ice

2. Spin down the plate with the LAMP reactions in a microfuge for 10 seconds to bring all samples to the bottom of the wells. Remove the seal from the plate carefully, avoiding sample splashing and cross-contamination between wells.

3. For each column of the plate (e.g. wells A1-H1), pool the reactions by transferring 2 µl of LAMP products from each well into a clean 0.2 ml thin-walled PCR tube. Pipette very carefully to avoid cross-contamination between wells.

Eco tip: As all samples in the same column are to be pooled, it is possible to use 12 pipette tips to transfer the plate contents and reuse these tips for each row rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.

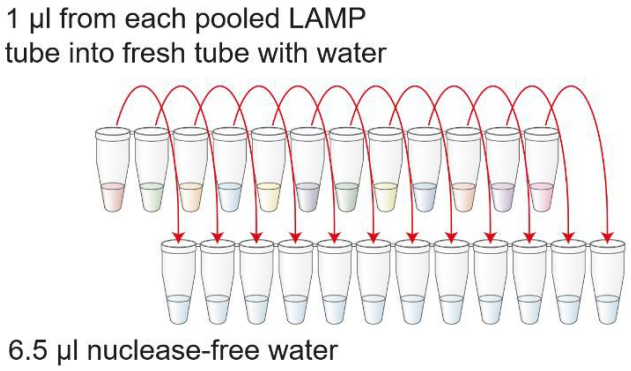


4. Mix the contents of each tube by pipetting up and down using a multichannel pipette, and spin down in a microfuge for 10 seconds.

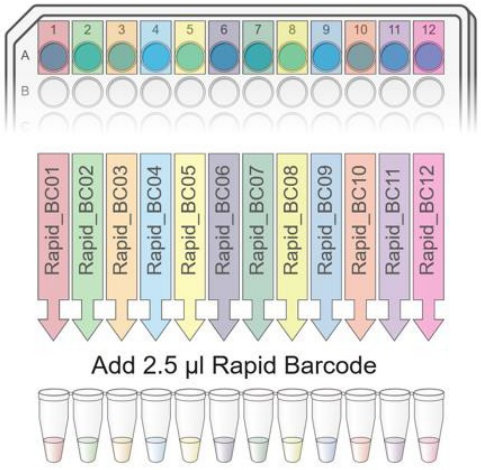
5. Add 6.5 µl nuclease-free water to clean 0.2 ml thin-walled PCR tubes, one for each pool of

reverse-transcribed and amplified controls. Then add 1 µl of the control pools to each tube:

Reagent	Volume
Nuclease-free water	6.5 µl
1 µl of each pool of processed controls	1 µl
Total	**7.5 µl**



6. To each tube, add 2.5 µl of Rapid Barcodes (one for each pool of LAMP products). These are in row A of the Rapid Barcode plate: RB01-12 are in wells A1-A12.



7. Mix gently by flicking the tubes, and spin down in a microfuge for 10 seconds.

8. Incubate the tubes in a thermal cycler at 30°C for 2 minutes and then at 80°C for 2 minutes. Put the tubes on ice for one minute to cool them down, and spin down in a microfuge for 10 seconds.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

9. Combine all reactions into a single 1.5 ml Eppendorf DNA LoBind tube for a total volume of 120 µl.

10. Resuspend the tube of SPRI Beads (SPRI) by vortexing for 5 seconds.
11. To the entire pooled barcoded sample from Step 9, add 96 µl of resuspended SPRI Beads, and mix by pipetting up and down.
12. Incubate for 5 minutes at room temperature.
13. Prepare 500 µl of fresh 80% ethanol in nuclease-free water.
14. Spin down the sample and pellet the beads on a magnetic rack for a minimum of 2 minutes, or until the supernatant is clear. Keep the tube on the magnet and pipette off and discard the supernatant.
15. Take the tube off the magnet and wash the beads by resuspending them thoroughly with 200 µl of freshly-prepared 80% ethanol. Place on the magnet and pellet the beads for 2 minutes, or until the solution is clear. Remove the ethanol using a pipette and discard.
16. Repeat the previous step.
17. Spin down and place the tube back on the magnetic rack for 2 minutes, or until any remaining supernatant is clear. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking.
18. Remove the tube from the magnetic rack and resuspend the pellet by pipetting in 15 µl Elution Buffer (EB). Spin down and incubate for 5 minutes at room temperature.
19. Pellet the beads on the magnet for 2 minutes, or until the eluate is clear and colourless.
20. Transfer 11 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
21. Add 1 µl of Rapid Adapter (RAP) to the barcoded DNA.
22. Mix gently by flicking the tube, and spin down in a microfuge for 10 seconds.
23. Incubate the reaction for 5 minutes at room temperature.
24. The prepared library is used for loading onto the flow cell. Store the library on ice until ready to load.

13. LAMP and library preparation when testing 92 samples and 4 controls using the LamPORE COVID-19 Test Kit 96 Plex S (OND-SQK-LP0096S) or the LamPORE COVID-19 Test Kit 96 Plex M (OND-SQK-LP0096M)

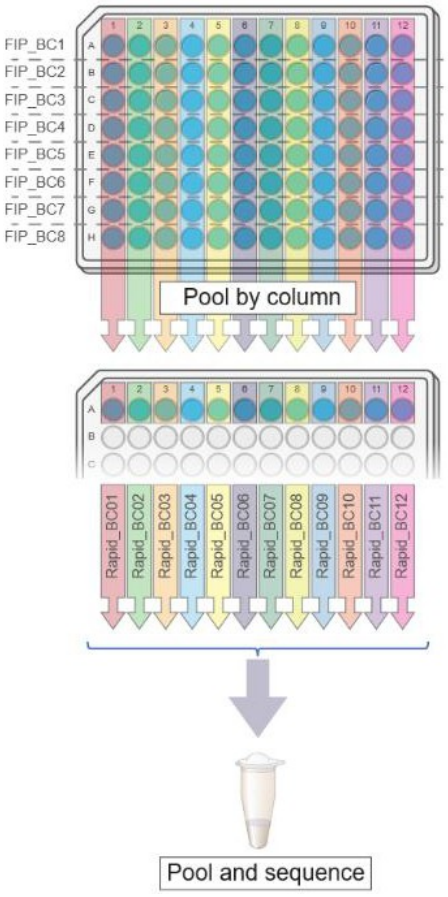
13.1. RT-LAMP procedure

LAMP can produce large numbers of easily-amplifiable molecules after a successful reaction, and therefore the risk of cross-contamination between samples must be mitigated.

- Preparation for amplification (RT-LAMP) must be performed in a PCR hood or bubble in a completely separate area to the library preparation
- Use dedicated pipettes for liquid handling, with filter pipette tips for the RNA processing and LAMP
- Wear a fresh pair of gloves that are immediately discarded after this step
- After the LAMP reaction is complete, remove the seal from the PCR plate very carefully to avoid splashing/cross-contamination between wells
- Clean down hoods and surfaces with an appropriate cleaning agent (e.g. bleach) before and after the RT-LAMP step and between reagent plates.

Barcoding of samples

This method uses a combinatorial barcoding approach. One set of barcodes is contained in the FIP primers, used in the LAMP reaction, and an additional set of Rapid Barcodes is also supplied. All samples and controls in a given row of a 96-well plate of samples will receive the same FIP barcode, and all samples and controls in a given column of the plate will ultimately receive the same Rapid Barcode, as shown in the figure below.



Here, samples in row A of a 96-well plate of samples receive FIP barcode 01, samples in row B receive FIP barcode 02, etc. Meanwhile, once pooled, samples in column 1 receive the Rapid Barcode 01, samples in column 2 receive the Rapid Barcode 02, etc. This way, each sample will receive a unique combination of FIP barcode and Rapid Barcode.

It is necessary to include at least two wells of No Template Control and at least two wells of Positive Control per plate, which leaves a maximum of 92 samples per plate.

1. Prepare the RNA samples and reagents as follows:

Reagent	Thaw	Mix/spin	Store
RNA samples	On ice	Vortex and spin down	On ice
LAMP Master Mix (LMM)	At room temperature	Spin down the plate	On ice
LAMP Primer Mixes (LPM)	At room temperature	Spin down the plate	On ice
Positive Control (CTL)	On ice	Vortex and spin down	On ice
No Template Control (NTC)	At room temperature	Vortex and spin down	On ice

2. Immediately before beginning the RT-LAMP, carry out the following steps:
    a. Add 1 ml of nuclease-free water to a 5 ml Eppendorf DNA LoBind tube.
    b. Transfer 2 µl of CTL into the water in the 5 ml tube. If insufficient volume is obtained from a single CTL tube, pool together the contents of two CTL tubes and transfer 2 µl from the pool.

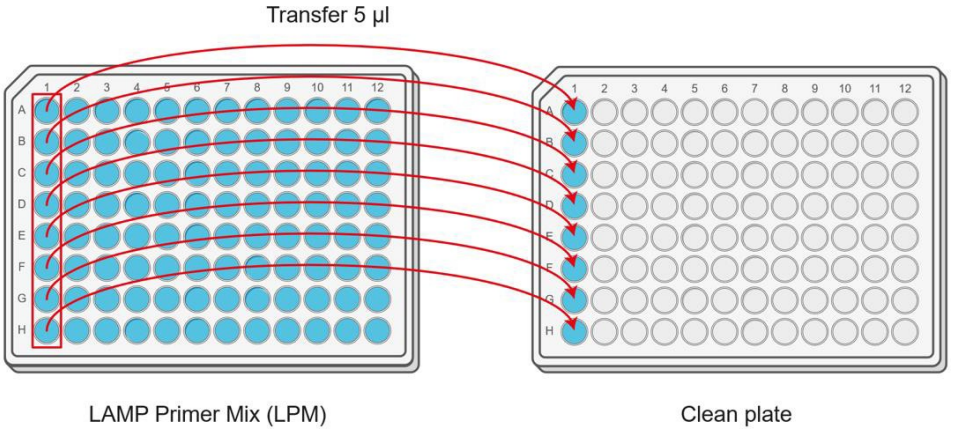
c. Vortex for at least 30 seconds and spin down if possible.

d. Carefully open the tube to avoid splashing, then add 1 ml nuclease-free water into tube and mix by pipetting up and down 10 times.

e. Vortex the tube again for at least 30 seconds and spin down if possible.

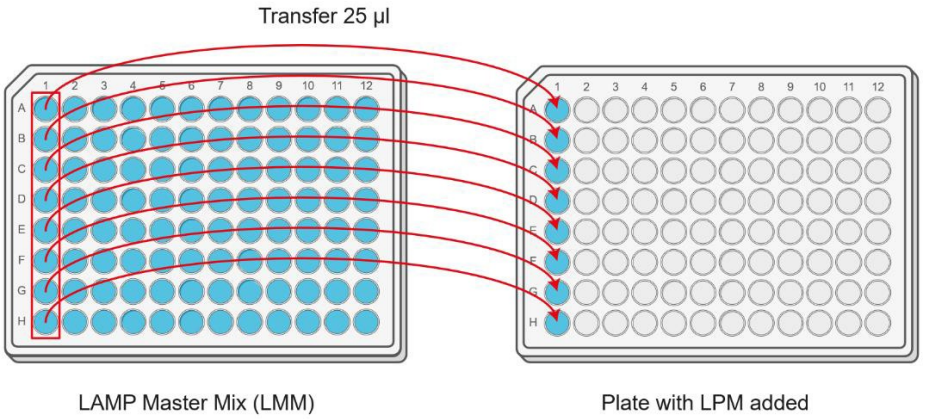
3. Set a multichannel pipette to 5 µl. Mix the contents of column 1 of the LAMP Primer Mix (LPM) plate by pipetting up and down several times. Then transfer 5 µl of LAMP Primer Mix from column 1 of the LPM plate into column 1 of a clean 96-well plate. Repeat the mixing and pipetting steps for all the remaining columns.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



4. Set a multichannel pipette to 25 µl. Mix the contents of column 1 of the LAMP Master Mix (LMM) plate thoroughly by pipetting up and down several times. Then transfer 25 µl of LAMP Master Mix from column 1 of the LMM plate into column 1 of the 96-well plate. Mix the contents of the wells by pipetting up and down. Repeat the mixing and pipetting steps for all the remaining columns.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



5. Add 20 µl of RNA sample to 92 of the wells. Add 20 µl NTC to two other wells. For the remaining two wells, add 5 µl of CTL to each well followed by 15 µl nuclease-free water to bring the total volume to 20 µl. In the diagram below only four RNA samples are annotated, however the remaining wells will also be filled with RNA samples.

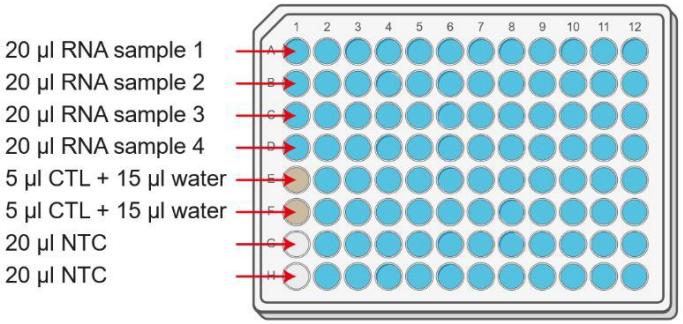


Plate with LPM and LMM added

6. Mix the contents of each well by pipetting up and down, taking care not to cross-contaminate different wells.
7. Seal the plate and spin down in a microfuge for 10 seconds.
8. Incubate the plate in a thermal cycler at 65°C for 35 minutes, then at 80°C for 5minutes.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

13.2. Library preparation

Library preparation should be carried out in a separate area of the laboratory to the RT-LAMP PCR hood/bubble.

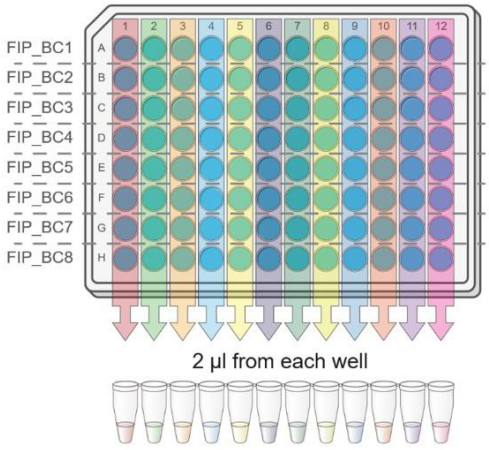
1. Prepare the remaining reagents as follows:

Reagent	Thaw	Mix/spin	Store
Rapid Barcodes (RBxx)	At room temperature	Spin down the plate	On ice
SPRI Beads (SPRI)	At room temperature	Vortex and spin down	At room temperature
Rapid Adapter (RAP)	At room temperature	Vortex and spin down	On ice
Elution Buffer (EB)	At room temperature	Vortex and spin down	On ice

2. Spin down the plate with the LAMP reactions in a microfuge for 10 seconds to bring all samples to the bottom of the wells. Remove the seal from the plate carefully, avoiding sample splashing and cross-contamination between wells.

3. For each column of the plate (e.g. wells A1-H1), pool the reactions by transferring 2 µl of LAMP products from each well into a clean 0.2 ml thin-walled PCR tube. Pipette very carefully to avoid cross-contamination between wells.

Eco tip: As all samples in the same column are to be pooled, it is possible to use 12 pipette tips to transfer the plate contents and reuse these tips for each row rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.

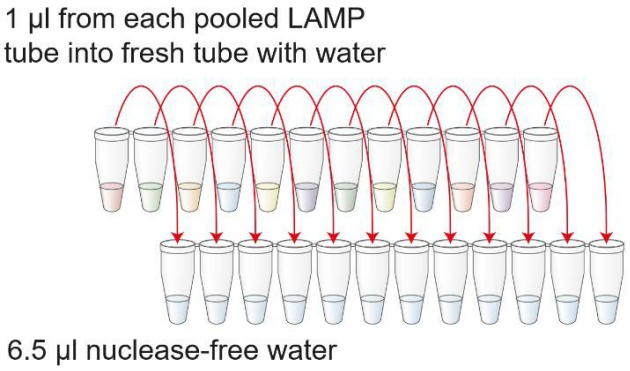


4. Mix the contents of each tube by pipetting up and down using a multichannel pipette, and spin down in a microfuge for 10 seconds.

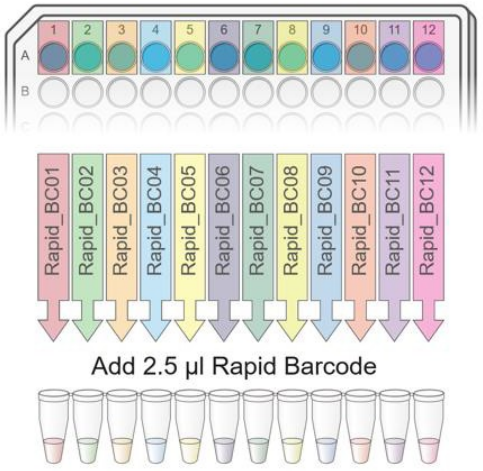
5. Add 6.5 µl nuclease-free water to clean 0.2 ml thin-walled PCR tubes, one for each pool of

reverse-transcribed and amplified RNA samples. Then add 1 µl of the RNA sample pools to each tube:

Reagent	Volume
Nuclease-free water	6.5 µl
1 µl of each pool of processed RNA samples	1 µl
Total	**7.5 µl**



6. To each tube, add 2.5 µl of Rapid Barcodes (one for each pool of LAMP products). These are in row A of the Rapid Barcode plate: RB01-12 are in wells A1-A12.



7. Mix gently by flicking the tubes, and spin down in a microfuge for 10 seconds.

8. Incubate the tubes in a thermal cycler at 30°C for 2 minutes and then at 80°C for 2 minutes. Put the tubes on ice for one minute to cool them down, and spin down in a microfuge for 10 seconds.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

9. Combine all reactions into a single 1.5 ml Eppendorf DNA LoBind tube for a total volume of 120 µl.

10. Resuspend the tube of SPRI Beads (SPRI) by vortexing for 5 seconds.

11. To the entire pooled barcoded sample from Step 9, add 96 µl of resuspended SPRI Beads, and mix by pipetting up and down.

12. Incubate for 5 minutes at room temperature.

13. Prepare 500 µl of fresh 80% ethanol in nuclease-free water.

14. Spin down the sample and pellet the beads on a magnetic rack for a minimum of 2 minutes, or until the supernatant is clear. Keep the tube on the magnet and pipette off and discard the supernatant.

15. Take the tube off the magnet and wash the beads by resuspending them thoroughly with 200 µl of freshly-prepared 80% ethanol. Place on the magnet and pellet the beads for 2 minutes, or until the solution is clear. Remove the ethanol using a pipette and discard.

16. Repeat the previous step.

17. Spin down and place the tube back on the magnetic rack for 2 minutes, or until any remaining supernatant is clear. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking.

18. Remove the tube from the magnetic rack and resuspend the pellet by pipetting in 15 µl Elution Buffer (EB). Spin down and incubate for 5 minutes at room temperature.

19. Pellet the beads on the magnet for 2 minutes, or until the eluate is clear and colourless.

20. Transfer 11 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.

21. Add 1 µl of Rapid Adapter (RAP) to the barcoded DNA.

22. Mix gently by flicking the tube, and spin down in a microfuge for 10 seconds.

23. Incubate the reaction for 5 minutes at room temperature.

24. The prepared library is used for loading onto the flow cell. Store the library on ice until ready to load.

14. LAMP and library preparation when testing 92 samples and 4 controls on each of eight 96-well plates using the LamPORE COVID-19 Test Kit 96 Plex M-A (OND-SQK-LP0096M-A)

The M-A version of the LamPORE kit is designed for users running 96 plex testing at a high throughput level and wish to do this either manually, or invest the time in automating the procedure on industry-standard liquid handlers.

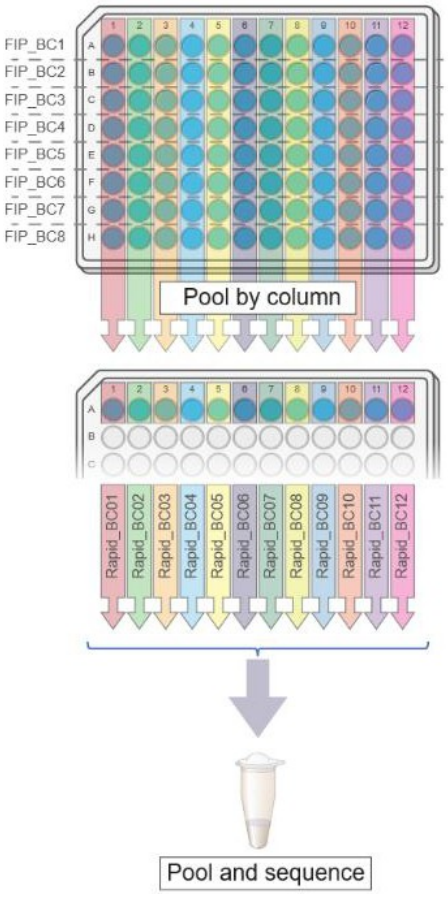
14.1. RT-LAMP procedure

LAMP can produce large numbers of easily-amplifiable molecules after a successful reaction, and therefore the risk of cross-contamination between samples must be mitigated.

- Preparation for amplification (RT-LAMP) must be performed in a PCR hood or bubble in a completely separate area to the library preparation
- Use dedicated pipettes for liquid handling, with filter pipette tips for the RNA processing and LAMP
- Wear a fresh pair of gloves that are immediately discarded after this step
- After the LAMP reaction is complete, remove the seal from the PCR plate very carefully to avoid splashing/cross-contamination between wells
- Clean down hoods and surfaces with an appropriate cleaning agent (e.g. bleach) before and after the RT-LAMP step and between reagent plates.

Barcoding of samples

This method uses a combinatorial barcoding approach. One set of barcodes is contained in the FIP primers, used in the LAMP reaction, and an additional set of Rapid Barcodes is also supplied. All samples and controls in a given row of a 96-well plate of samples will receive the same FIP barcode, and all samples and controls in a given column of the plate will ultimately receive the same Rapid Barcode, as shown in the figure below.



Here, samples in row A of a 96-well plate of samples receive FIP barcode 01, samples in row B receive FIP barcode 02, etc. Meanwhile, once pooled, samples in column 1 receive the Rapid Barcode 01, samples in column 2 receive the Rapid Barcode 02, etc. This way, each sample will receive a unique combination of FIP barcode and Rapid Barcode.

It is necessary to include at least two wells of No Template Control and at least two wells of Positive Control per plate, which leaves a maximum of 92 samples per plate.

1. Prepare the RNA samples and reagents as follows:

Reagent	Thaw	Mix/spin	Store
RNA samples	On ice	Vortex and spin down	On ice
LAMP Master Mix (LMM)	At room temperature	Spin down the plate	On ice
LAMP Primer Mixes (LPM)	At room temperature	Spin down the plate	On ice
Positive Control (CTL)	On ice	Vortex and spin down	On ice
No Template Control (NTC)	At room temperature	Vortex and spin down	On ice

2. Immediately before beginning the RT-LAMP, carry out the following steps:
    a. Add 1 ml of nuclease-free water to a 5 ml Eppendorf DNA LoBind tube.
    b. Transfer 2 µl of CTL into the water in the 5 ml tube. If insufficient volume is obtained from a single CTL tube, pool together the contents of two CTL tubes and transfer 2 µl from the pool.

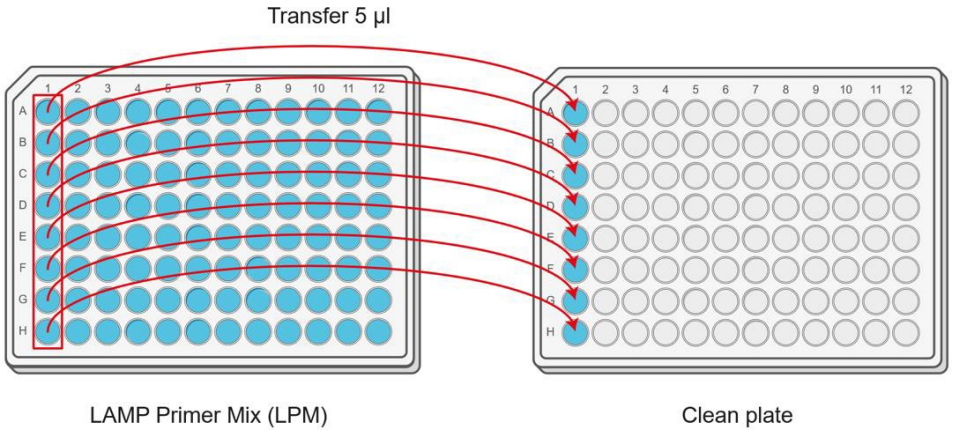
c. Vortex for at least 30 seconds and spin down if possible.

d. Carefully open the tube to avoid splashing, then add 1 ml nuclease-free water into tube and mix by pipetting up and down 10 times.

e. Vortex the tube again for at least 30 seconds and spin down if possible.

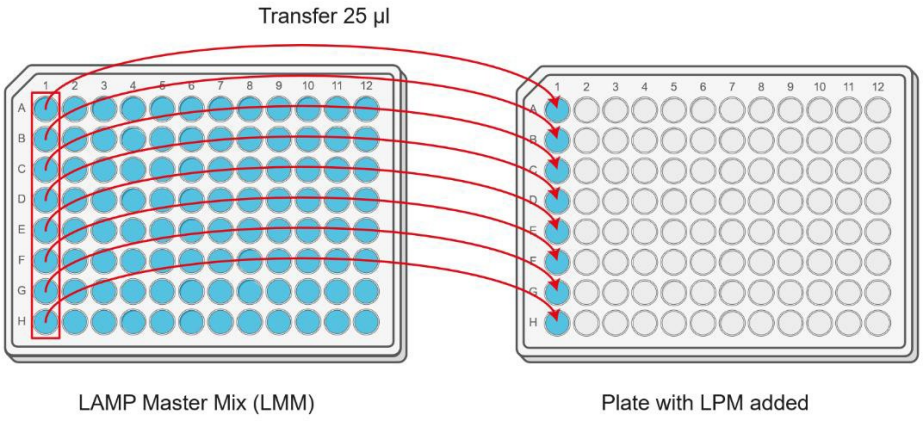
3. Set a multichannel pipette to 5 µl. Mix the contents of column 1 of the LAMP Primer Mix (LPM) plate by pipetting up and down several times. Then transfer 5 µl of LAMP Primer Mix from column 1 of the LPM plate into column 1 of eight clean 96-well plates. Repeat the mixing and pipetting steps for all the remaining columns (transferring material into respective columns of the clean plates).

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



4. Set a multichannel pipette to 25 µl. Mix the contents of column 1 of the LAMP Master Mix (LMM) plate thoroughly by pipetting up and down several times. Then transfer 25 µl of LAMP Master Mix from column 1 of the LMM plate into column 1 of the eight 96-well plates. Mix the contents of the wells by pipetting up and down. Repeat the pipetting steps for all the remaining columns.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



5. Add 20 µl of RNA sample to 92 of the wells of each of the eight 96-well plates. Add 20 µl NTC to two other wells of each plate. For the remaining two wells of each plate, add 5 µl of CTL followed by 15 µl nuclease-free water to bring the total volume added to 20 µl. In the diagram below only four RNA samples are annotated, however the remaining wells will also be filled with RNA samples.

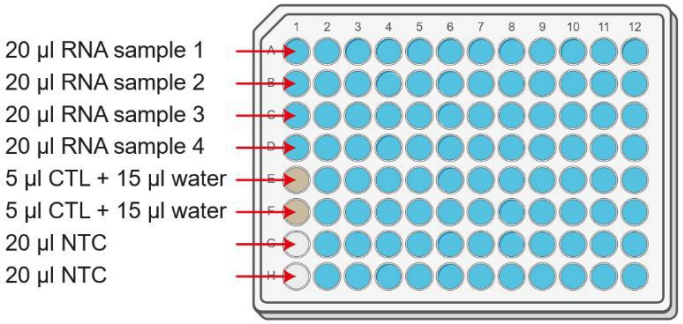


Plate with LPM and LMM added

6. Mix the contents of each well by pipetting up and down, taking care not to cross-contaminate different wells.
7. Seal the plates and spin down in a microfuge for 10 seconds.
8. Incubate the plate in a thermal cycler at 65°C for 35 minutes, then at 80°C for 5minutes.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

14.2. Library preparation

Library preparation should be carried out in a separate area of the laboratory to the RT-LAMP PCR hood/bubble.

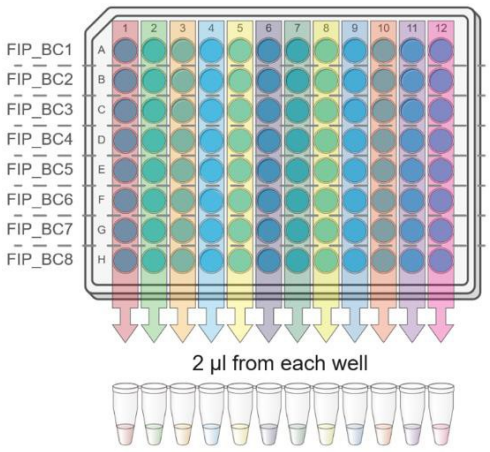
1. Prepare the remaining reagents as follows:

Reagent	Thaw	Mix/spin	Store
Rapid Barcodes (RBxx)	At room temperature	Spin down the plate	On ice
SPRI Beads (SPRI)	At room temperature	Vortex and spin down	At room temperature
Rapid Adapter (RAP)	At room temperature	Vortex and spin down	On ice
Elution Buffer (EB)	At room temperature	Vortex and spin down	On ice

2. Spin down the plates with the LAMP reactions in a microfuge for 10 seconds to bring all samples to the bottom of the wells. Remove the seal from each plate carefully, avoiding sample splashing and cross-contamination between wells.

3. For each column of each plate (e.g. wells A1-H1 from plate 1), pool the reactions by transferring 2 µl of LAMP products from each well into a clean well within a clean 96-well PCR plate. Note that pooled reactions from respective columns of different plates must remain separate. Pipette very carefully to avoid cross-contamination between wells. Continue until all sample plates have been processed and the clean 96-well PCR plate contains no empty wells.

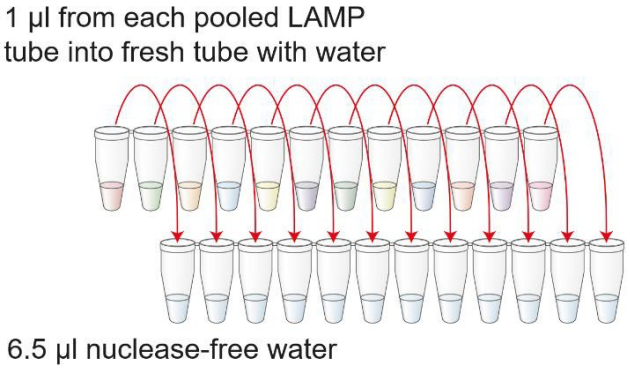
Eco tip: As all samples in the same column are to be pooled, it is possible to use 12 pipette tips to transfer the plate contents and reuse these tips for each row of the same sample plate rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



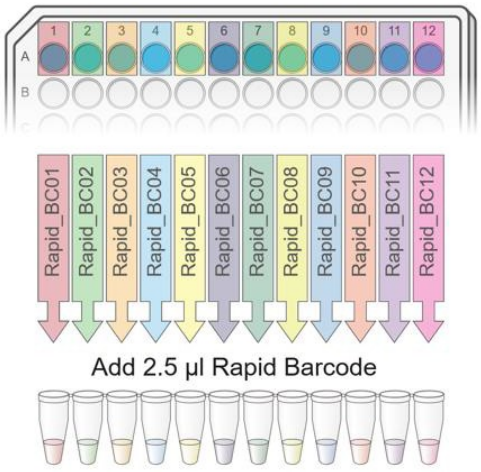
4. Mix the contents of each pool/well by pipetting up and down using a multichannel pipette, seal the plate, and spin down in a microfuge for 10 seconds.

5. Add 6.5 µl nuclease-free water to each well of a clean 96-well PCR plate. Then add 1 µl of the sample pools to each respective well:

Reagent	Volume
Nuclease-free water	6.5 µl
1 µl of each pool of processed RNA samples	1 µl
Total	**7.5 µl**



6. To each well, add 2.5 µl of Rapid Barcodes from the respective wells of the Rapid Barcodes plate. RB01 is in each well in column 1, RB02 is in each well of column 2, etc.



7. Mix gently and carefully, seal the plate, and spin down in a microfuge for 10 seconds.

8. Incubate the plate in a thermal cycler at 30°C for 2 minutes and then at 80°C for 2 minutes. Put the plate on ice for one minute to cool the samples down, and spin down in a microfuge for 10 seconds.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

9. Combine all reactions from each row into separate 1.5 ml Eppendorf DNA LoBind tubes for a total volume of 120 µl per tube.

10. Resuspend the tube of SPRI Beads (SPRI) by vortexing for 5 seconds.

11. To each 120 µl pooled barcoded sample from Step 9, add 96 µl of resuspended SPRI Beads, and mix by pipetting up and down.

12. Incubate for 5 minutes at room temperature.

13. Prepare 4 ml of fresh 80% ethanol in nuclease-free water.

14. Spin down the samples from step 12 and pellet the beads on a magnetic rack for a minimum of 2 minutes, or until the solutions are clear. Keep the tubes on the magnet and pipette off and discard the supernatant, using a clean pipette tip for each.

15. Take the tubes off the magnet and wash the beads by resuspending each pellet thoroughly with 200 µl of freshly-prepared 80% ethanol. Place back on the magnet and pellet the beads for 2 minutes, or until the solutions are clear. Remove the ethanol using a clean pipette tip for each tube, and discard.

16. Repeat the previous step.

17. Spin down and place the tubes back on the magnetic rack for 2 minutes, or until any remaining supernatants are clear. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellets to the point of cracking.

18. Remove the tube from the magnetic rack and resuspend each pellet by adding 15 µl Elution Buffer (EB). Flick the tubes or pipette mix if necessary. Spin down briefly and incubate for 5 minutes at room temperature.

19. Pellet the beads on the magnet for 2 minutes, or until the eluates are clear and colourless.

20. Transfer 11 µl of each eluate into separate, clean 1.5 ml Eppendorf DNA LoBind tubes.

21. Add 1 µl of Rapid Adapter (RAP) to each barcoded DNA sample.

22. Mix by pipetting or by flicking the tubes, and spin down in a microfuge for 10 seconds.

23. Incubate the reactions for 5 minutes at room temperature.

24. The prepared DNA libraries are used for loading onto the flow cell. Store the libraries on ice until ready to load.

15. LAMP and library preparation when testing fewer than 92 samples

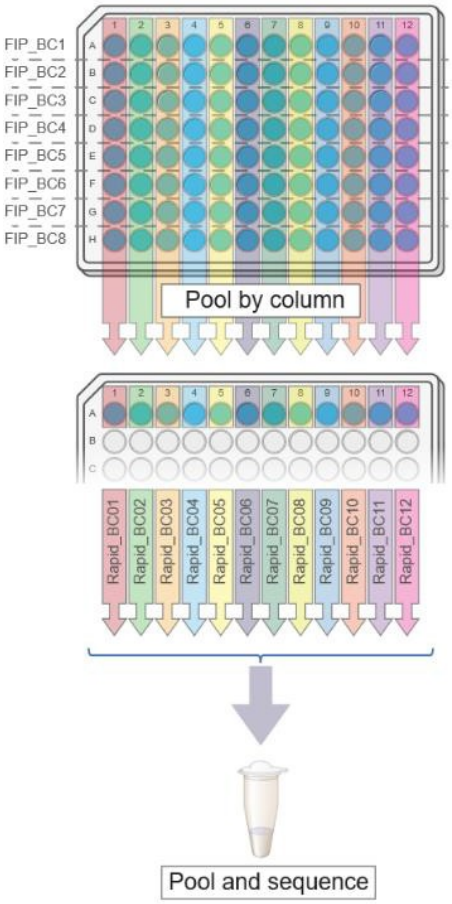
15.1. RT-LAMP procedure

LAMP can produce large numbers of easily-amplifiable molecules after a successful reaction, and therefore the risk of cross-contamination between samples must be mitigated.

- Preparation amplification (RT-LAMP) must be performed in a PCR hood or bubble in a completely separate area to the library preparation
- Use dedicated pipettes for liquid handling, with filter pipette tips for the RNA processing and LAMP
- Wear a fresh pair of gloves that are immediately discarded after this step
- After the LAMP reaction is complete, remove the seal from the PCR plate very carefully to avoid splashing/cross-contamination between wells
- Clean down hoods and surfaces with an appropriate cleaning agent (e.g. bleach) before and after the RT-LAMP step and between reagent plates.

Barcoding of samples

This method uses a combinatorial barcoding approach. One set of barcodes is contained in the FIP primers, used in the LAMP reaction, and an additional set of Rapid Barcodes is also supplied. All samples and controls in a given row of a 96-well plate of samples will receive the same FIP barcode, and all samples and controls in a given column of the plate will ultimately receive the same Rapid Barcode, as shown in the figure below.



Here, samples in row A of a 96-well plate of samples receive FIP barcode 01, samples in row B receive FIP barcode 02, etc. Meanwhile, once pooled, samples in column 1 receive the Rapid Barcode 01, samples in column 2 receive the Rapid Barcode 02, etc. This way, each sample will receive a unique combination of FIP barcode and Rapid Barcode.

It is necessary to include at least two wells of No Template Control and at least two wells of Positive Control per run, which leaves, for example, a maximum of 20 patient samples per 24 LamPORE experiments on a 96-well plate. When running fewer than 92 samples at a time, fill up columns rather than rows with samples. Any unused wells in a column partially filled with samples or controls must be filled with LAMP Master Mix/LAMP Primers and 20 µl nuclease-free water and processed in the same way as a sample.

1. Prepare the RNA samples and reagents as follows:

Reagent	Thaw	Mix/spin	Store
RNA samples	On ice	Vortex and spin down	On ice
LAMP Master Mix (LMM)	At room temperature	Spin down the plate	On ice
LAMP Primer Mixes (LPM)	At room temperature	Spin down the plate	On ice
Positive Control (CTL)	On ice	Vortex and spin down	On ice
No Template Control (NTC)	At room temperature	Vortex and spin down	On ice

2. Immediately before beginning the RT-LAMP, carry out the following steps:

    a. Add 1 ml of nuclease-free water to a 5 ml Eppendorf DNA LoBind tube.

    b. Transfer 2 µl of CTL into the water in the 5 ml tube. If insufficient volume is obtained from a single CTL tube, pool together the contents of two CTL tubes and transfer 2 µl from the pool.

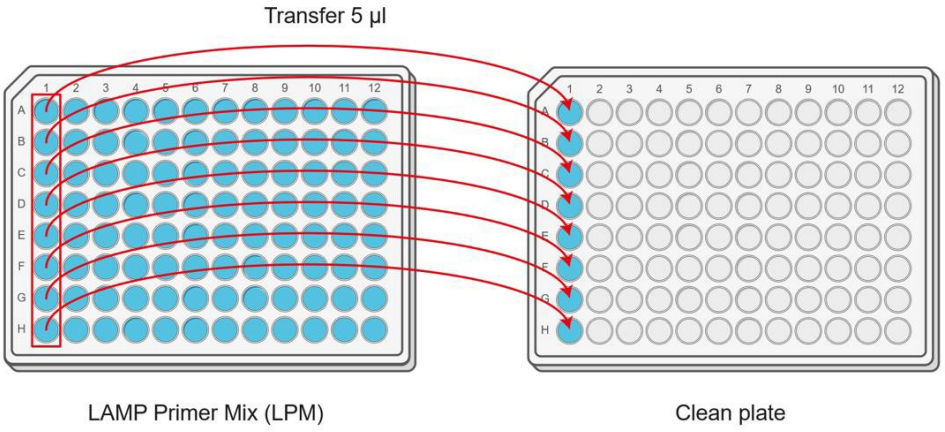
    c. Vortex for at least 30 seconds and spin down if possible.

    d. Carefully open the tube to avoid splashing, then add 1 ml nuclease-free water into tube and mix by pipetting up and down 10 times.

    e. Vortex the tube again for at least 30 seconds and spin down if possible

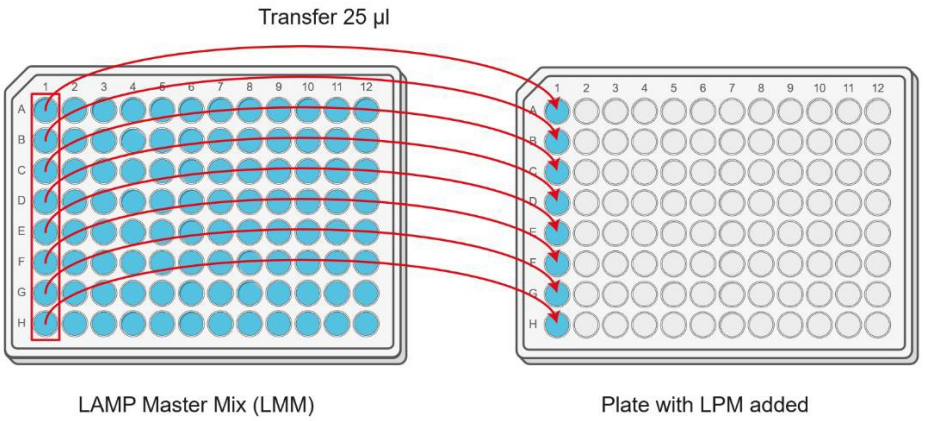
3. Set a multichannel pipette to 5 µl. Mix the contents of column 1 of the LAMP Primer Mix (LPM) plate by pipetting up and down several times. Then transfer 5 µl of LAMP Primer Mix from column 1 of the LPM plate into column 1 of a clean 96-well plate. Repeat the mixing and pipetting steps for additional columns depending on the number of samples being tested: for 24 samples (inclusive of any controls and empty wells), transfer three columns of reagents, for 48 samples transfer six columns of reagents etc., up to 96 samples (all 12 columns).

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.

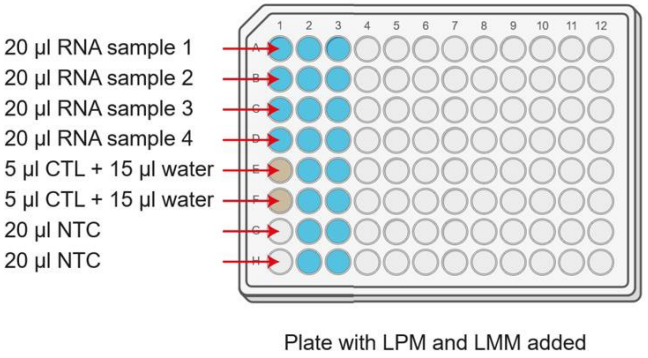


4. Set a multichannel pipette to 25 µl. Mix the contents of column 1 of the LAMP Master Mix (LMM) plate thoroughly by pipetting up and down several times. Then transfer 25 µl of LAMP Master Mix from column 1 of the LMM plate into column 1 of the 96-well plate. Mix the contents of the wells by pipetting up and down. Repeat the mixing and pipetting steps for all columns with LAMP Primer Mix added.

Eco tip: As the FIP barcodes are shared within rows, it is possible to use 8 pipette tips to transfer the plate contents and reuse these tips for each column rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



5. Add 20 µl of RNA sample or NTC to each well. For positive control wells, add 5 µl of CTL to each well, followed by 15 µl nuclease-free water to bring the total volume to 20 µl. Make up any unused wells with 20 µl nuclease-free water. The minimum number of RNA samples to be tested at a time is 20, with two wells of CTL and two wells of NTC.



6. Mix the contents of each well by pipetting up and down, taking care not to cross-contaminate different wells.

7. Seal the plate and spin down in a microfuge for 10 seconds.

8. Incubate the plate in a thermal cycler at 65°C for 35 minutes, then at 80°C for 5minutes.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

15.2. Library preparation

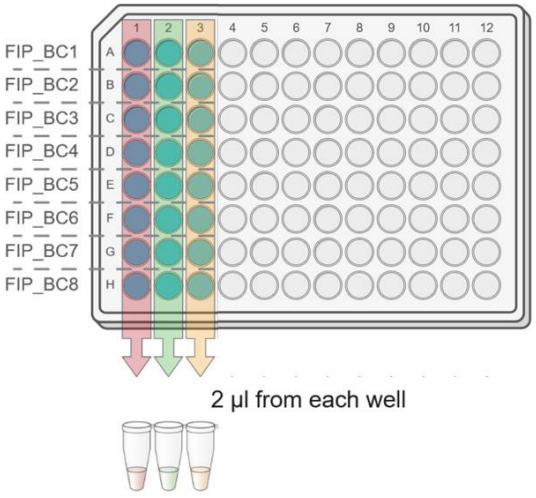
Library preparation should be carried out in a separate area of the laboratory to the RT-LAMP PCR hood/bubble.

1. Prepare the remaining reagents as follows:

Reagent	Thaw	Mix/spin	Store
Rapid Barcodes (RBxx)	At room temperature	Spin down the plate	On ice
SPRI beads (SPRI)	At room temperature	Vortex and spin down	At room temperature
Rapid Adapter (RAP)	At room temperature	Vortex and spin down	On ice
Elution Buffer (EB)	At room temperature	Vortex and spin down	On ice

2. Spin down the plate with the LAMP reactions in a microfuge for 10 seconds to bring all samples to the bottom of the wells. Remove the seal from the plate carefully, avoiding sample splashing and cross-contamination between wells.
3. For each column of the plate (e.g. wells A1-H1), pool the reactions by transferring 2 µl of LAMP products from each well into a clean 0.2 ml thin-walled PCR tube. Pipette very carefully to avoid cross-contamination between wells.

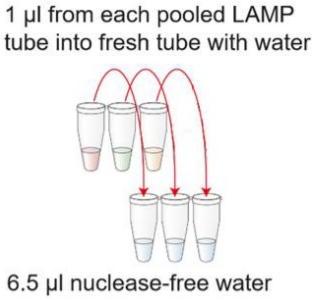
Eco tip: As all samples in the same column are to be pooled, it is possible to use 12 pipette tips to transfer the plate contents and reuse these tips for each row rather than using fresh tips. Care must be taken to ensure the multichannel pipette is in the same orientation for each transfer.



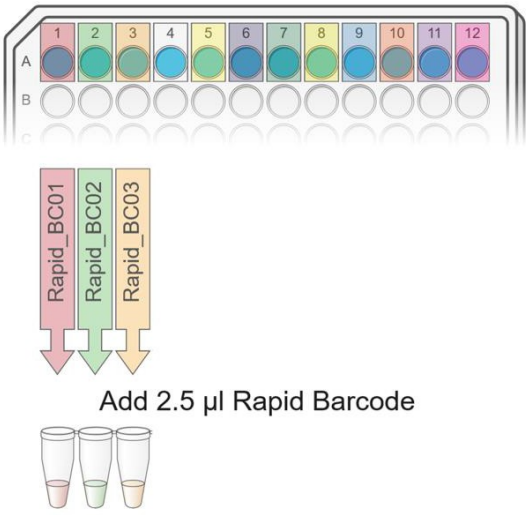
4. Mix the contents of each tube by pipetting up and down using a multichannel pipette, and spin down in a microfuge for 10 seconds.
5. Add 6.5 µl nuclease-free water to clean 0.2 ml thin-walled PCR tubes, one for each pool of reverse-transcribed and amplified RNA samples. Then add 1 µl of the RNA sample pools to

each tube:

Reagent	Volume
Nuclease-free water	6.5 µl
1 µl of each pool of processed RNA samples	1 µl
Total	**7.5 µl**



6. To each tube, add 2.5 µl of Rapid Barcodes (one for each pool of LAMP products). These are in row A of the Rapid Barcode plate: RB01-12 are in wells A1-A12.



7. Mix gently by flicking the tubes, and spin down in a microfuge for 10 seconds.

8. Incubate the tubes in a thermal cycler at 30°C for 2 minutes and then at 80°C for 2 minutes. Put the tubes on ice for one minute to cool them down, and spin down in a microfuge for 10 seconds.

(Optional) If necessary, the protocol can be paused at this point. The samples should be kept at 4°C and can be stored overnight.

9. Combine all reactions into a single 1.5 ml Eppendorf DNA LoBind tube.

10. Resuspend the tube of SPRI Beads (SPRI) by vortexing for 5 seconds.

11. To the entire pooled barcoded sample from Step 9, add 0.8x volume of resuspended SPRI Beads (e.g. add 96 µl SPRI beads to 120 µl sample), and mix by pipetting up and down.

	3 columns	6 columns	9 columns	12 columns
Pooled sample volume	30 µl	60 µl	90 µl	120 µl
SPRI beads	24 µl	48 µl	72 µl	96 µl

12. Incubate for 5 minutes at room temperature.
13. Prepare 500 µl of fresh 80% ethanol in nuclease-free water.
14. Spin down the sample and pellet the beads on a magnetic rack for a minimum of 2 minutes, or until the supernatant is clear. Keep the tube on the magnet and pipette off and discard the supernatant.
15. Take the tube off the magnet and wash the beads by resuspending them thoroughly with 200 µl of freshly-prepared 80% ethanol. Place on the magnet and pellet the beads for 2 minutes, or until the solution is clear. Remove the ethanol using a pipette and discard.
16. Repeat the previous step.
17. Spin down and place the tube back on the magnetic rack for 2 minutes, or until any remaining supernatant is clear. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking.
18. Remove the tube from the magnetic rack and resuspend the pellet by pipetting in 15 µl Elution Buffer (EB). Spin down and incubate for 5 minutes at room temperature.
19. Pellet the beads on the magnet for 2 minutes, or until the eluate is clear and colourless.
20. Transfer 11 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
21. Add 1 µl of Rapid Adapter (RAP) to the barcoded DNA.
22. Mix gently by flicking the tube, and spin down in a microfuge for 10 seconds.
23. Incubate the reaction for 5 minutes at room temperature.
24. The prepared library is used for loading onto the flow cell. Store the library on ice until ready to load.

16. Priming and loading the flow cell when using the LamPORE COVID-19 Test Kit 96 Plex S (OND-SQK-LP0096S) or the LamPORE COVID-19 Test Kit 96 Plex M (OND-SQK-LP0096M)

1. Prepare the remaining kit reagents as follows:

Reagent	Thaw	Mix/spin	Store
Sequencing Buffer (SQB)	At room temperature	Vortex and spin down	On ice
Loading Beads (LB)	At room temperature	-	On ice
Flush Tether (FLT)	At room temperature	Vortex and spin down	On ice
Flush Buffer (FB)	At room temperature	Vortex and spin down	On ice

2. To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FB), and mix by vortexing for 5 seconds.

3. Slide the priming port cover of the flow cell clockwise to open the priming port.

4. After opening the priming port, check for a small air bubble under the cover. Draw back 20-30 µl to remove any bubbles:

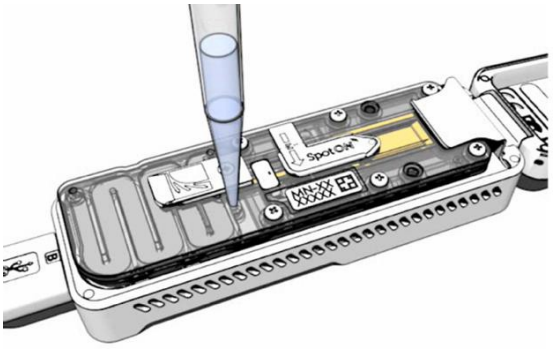
    a. Set a P1000 pipette to 200 µl

    b. Insert the tip into the priming port

    c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip Visually check that there is continuous buffer from the priming port across the sensor array.

Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

5. Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below.



6. Thoroughly mix the contents of the Loading Beads (LB) by pipetting. The Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

7. In a new tube, prepare the library for loading as follows:

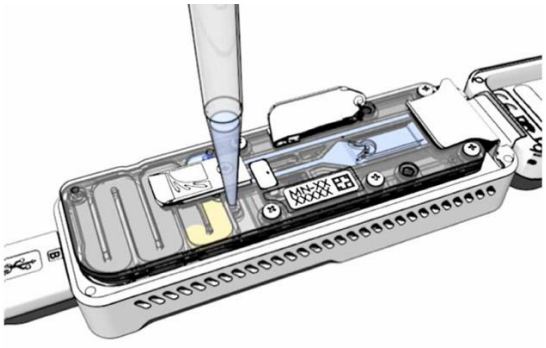
Reagent	Volume
Sequencing Buffer (SQB)	37.5 µl
Loading Beads (LB), mixed immediately before use	25.5 µl
DNA library	12 µl
Total	75 µl

8. Mix the prepared library by pipetting up and down, and spin in a microfuge for 10 seconds.

9. Complete the flow cell priming process (from step 5):

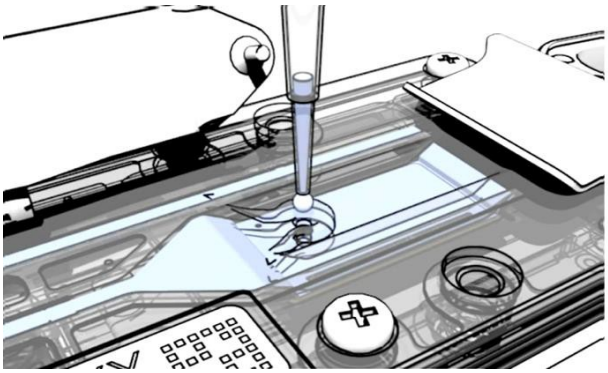
    a. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.

    b. Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.

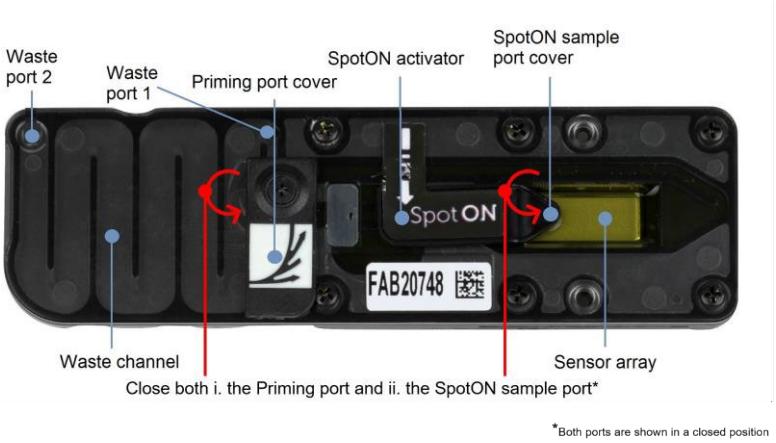


10. Mix the prepared library gently by pipetting up and down just prior to loading.

11. Add 75 µl of prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.



12. Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the GridION lid.



*Both ports are shown in a closed position

17. Priming and loading the flow cell when using the LamPORE COVID-19 Test Kit 96 Plex M-A (OND-SQK-LP0096M-A)

1. Prepare the remaining kit reagents as follows:

Reagent	Thaw	Mix/spin	Store
Sequencing Buffer (SQB)	At room temperature	Vortex and spin down	On ice
Loading Beads (LB)	At room temperature	-	On ice
Flush Tether (FLT)	At room temperature	Vortex and spin down	On ice
Flush Buffer (FB)	At room temperature	Vortex	On ice

2. To prepare the flow cell priming mix for each flow cell to be run, add 30 µl of thawed and mixed Flush Tether (FLT) to 1.17 ml of vortexed Flush Buffer (FB). Mix by vortexing for 5 seconds. Volumes can be adjusted *pro rata*: to prepare priming mix for eight flow cells, add 240 µl of FLT to 9.36 ml of FB.

3. Slide the priming port cover of each flow cell clockwise to open the priming port.

4. After opening the priming port, check for a small air bubble under the cover. Draw back 20-30 µl to remove any bubbles:

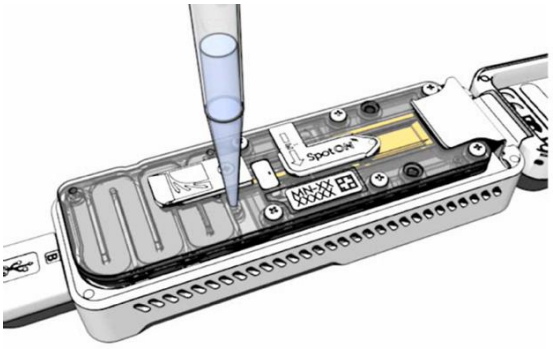
    a. Set a P1000 pipette to 200 µl

    b. Insert the tip into the priming port

    c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip Visually check that there is continuous buffer from the priming port across the sensor array.

Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

5. Load 800 µl of the priming mix into each flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare each library for loading by following the steps below.



6. Thoroughly mix the contents of the Loading Beads (LB) by pipetting. The Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

7. In separate tubes, prepare each library for loading as follows:

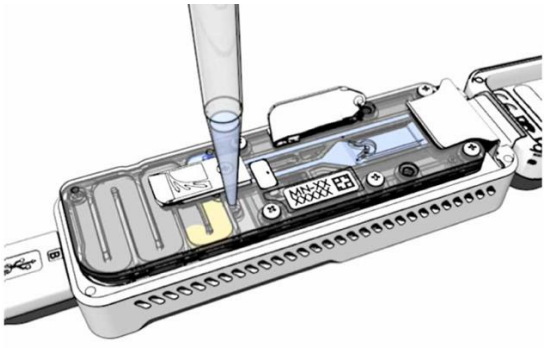
Reagent	Volume
Sequencing Buffer (SQB)	37.5 µl
Loading Beads (LB), mixed immediately before use	25.5 µl
DNA library	12 µl
Total	75 µl

8. Mix the prepared libraries by pipetting up and down, and spin in a microfuge for 10 seconds.

9. Complete the flow cell priming process (from step 5):

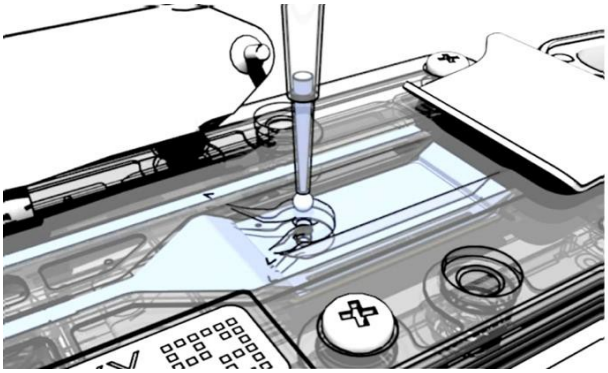
    a. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.

    b. Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.

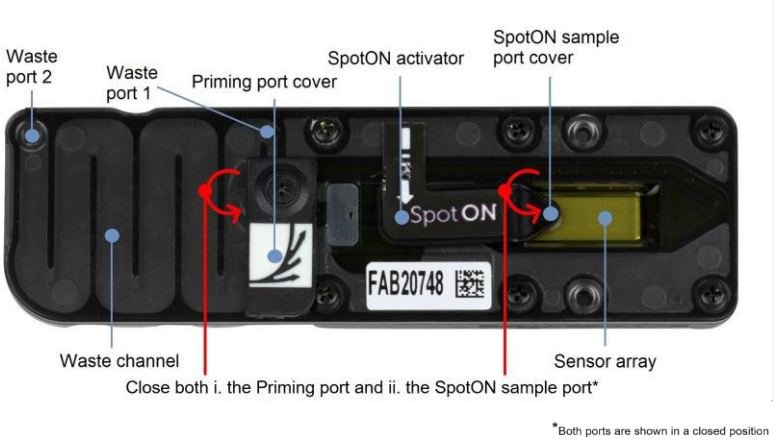


10. Mix each prepared library gently by pipetting up and down just prior to loading onto a respective flow cell.

11. Add 75 µl of prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.



12. Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the GridION lid.



18. Cleaning, decontamination and waste disposal

18.1. General decontamination

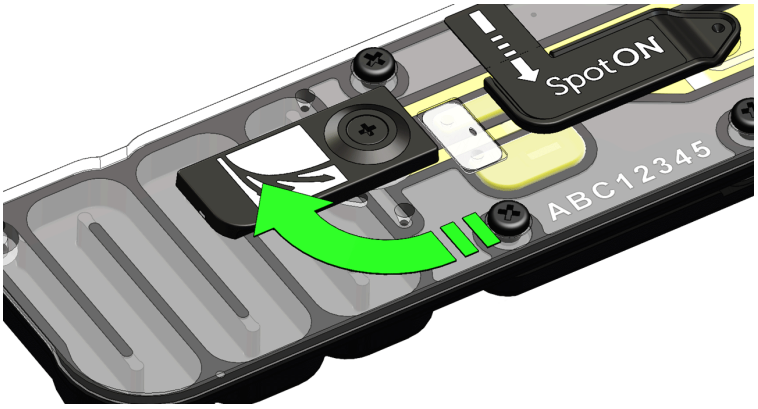
1. Safely discard unused reagents, wastes, samples, etc. in accordance with local regulations, as those are potentially infectious.
2. Decontaminate surfaces with 0.5% sodium hypochlorite (bleach) diluted with deionized or purified water.

18.2. Flow cell flushing and returns

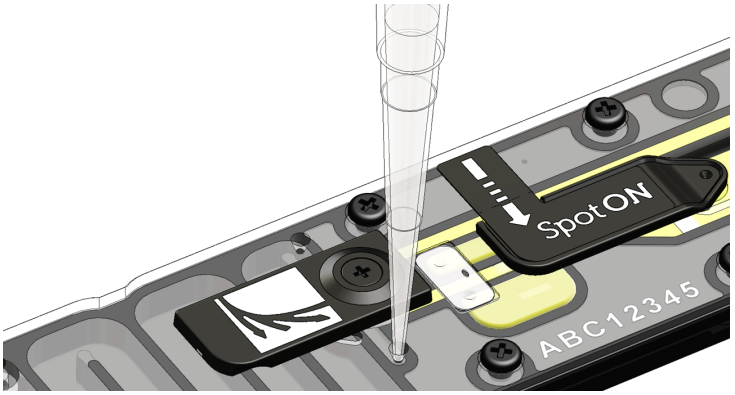
1. Request a flow cell returns box of the required size by following the link: https://community.nanoporetech.com/support/contact_us/request-nanopore-box

> When flushing flow cells ready for returning, any liquid overflow from the flow cell should **not** be disposed of down the sink. Avoid contact with strong acids or alkalis.

2. Place sufficient absorbent material on the bench to take up approximately 4000 µl of flush waste per flow cell, applied in 1000 µl aliquots.
3. Replace the SpotON port cover, ensuring the bung enters the SpotON port. Then open the priming port cover.



4. Place the flow cell onto the absorbent material at a 45° angle. The reservoir end of the flow cell should be in contact with the absorbent material, which will capture any overflow of buffer/sample leaving the flow cell.
5. Using a P1000 pipette, slowly load 4 ml water in 1000 µl aliquots via the priming port so that the liquid fills the reservoir all the way to the outlet vent port.

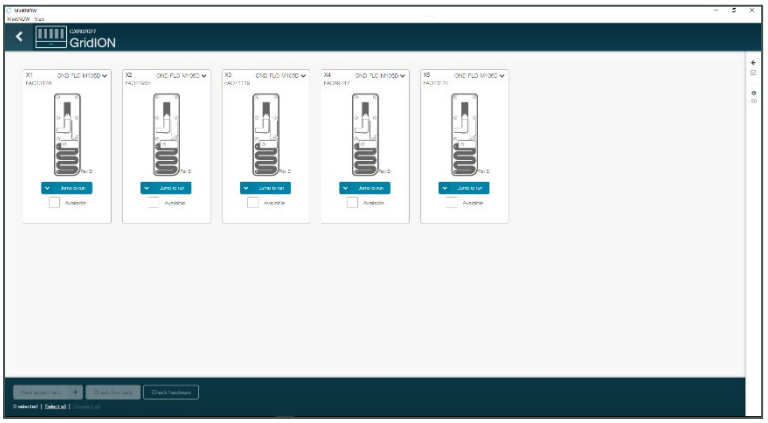


6. Once flushing is complete, close the priming port cover.
7. Using the pipette, remove the liquid from the waste reservoir via the waste port. Take care to leave sufficient liquid in the sensor chip area and neighbouring channels to ensure the sensor array remains submerged during transit.
8. Dispose of the absorbent material as biological waste, as detailed in local guidelines.
9. Wipe the bench surface with a suitable disinfectant. Do not use any strong acids or alkalis.
10. Follow the rest of the returns process and book your flow cell collection as described: https://community.nanoporetech.com/support/contact_us/return-flow-cells

19. Setting up the LamPORE assay and data analysis

1. Click the Nanopore wheel icon on the desktop to load the MinKNOW software. You will see the MinKNOW user interface appear.

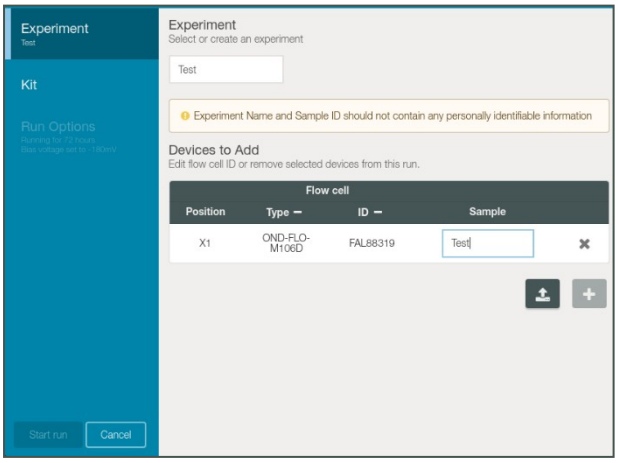
2. Select the flow cells to be run by checking the "Available" box.



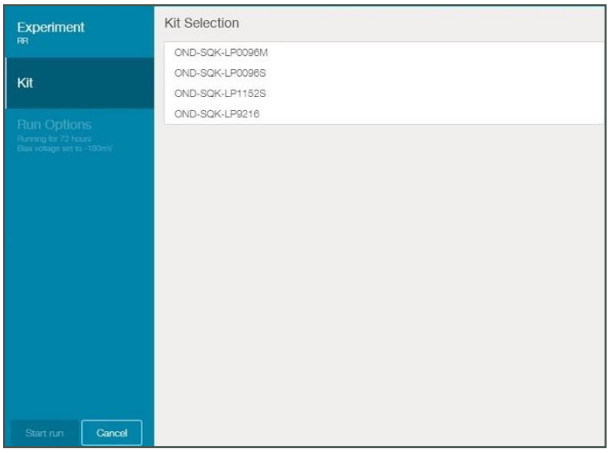
3. Click the "New Experiment" button at the bottom left of the user interface.

   On the New experiment pop-up screen, select the running parameters for your experiment from the individual tabs:

a. **Experiment**
The Experiment tab will show the flow cells chosen. An experiment name can then be assigned to all flow cells.
Fill in the **Experiment** field with the operator name or ID. Fill in the **Sample** field with a unique name. Experiment name and Sample ID should not contain personally-identifiable information. The other tabs will not become available until an experiment name has been provided.



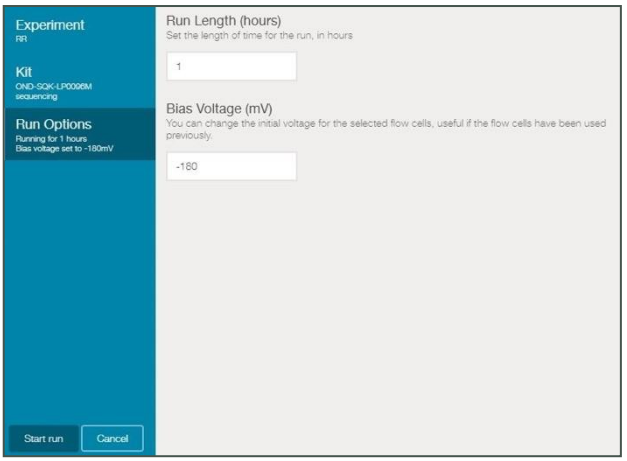
b. **Kit:** You will be presented with four kit options. Choose **OND-SQK-LP0096S or OND-SQK-LP0096M**, depending on the kit used. If you used OND-SQK-LP0096M-A, choose the **OND-SQK-LP0096M** from the dropdown menu.



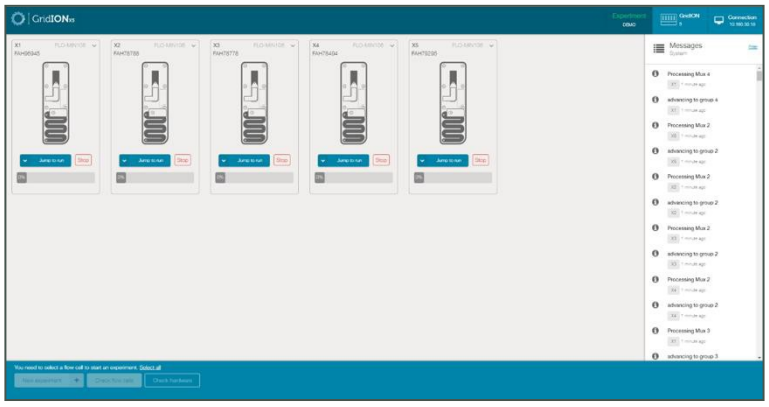
c. **Run options:** adjust the Run Length based on the number of samples you are sequencing:

Number of samples	Run length (hours)
24	0.5
48	0.5
72	1
96	1

Keep the Bias Voltage at its default value.



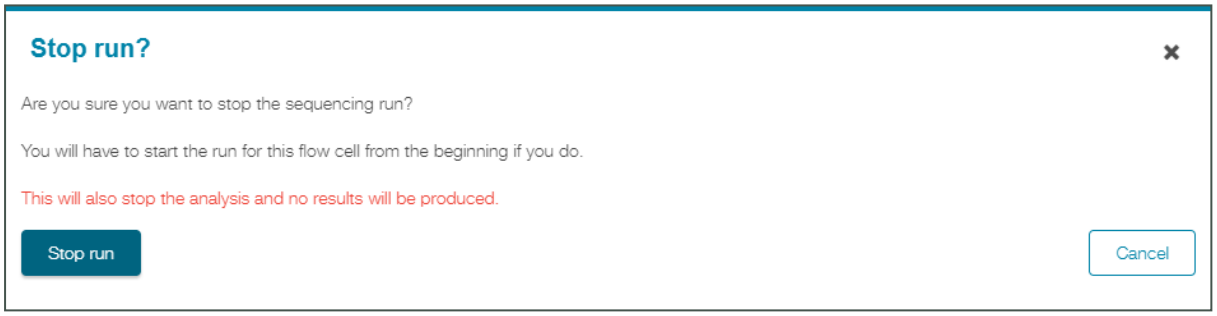
4. Click **Start run**. The pop-up box will disappear, and the flow cells will become greyed out.

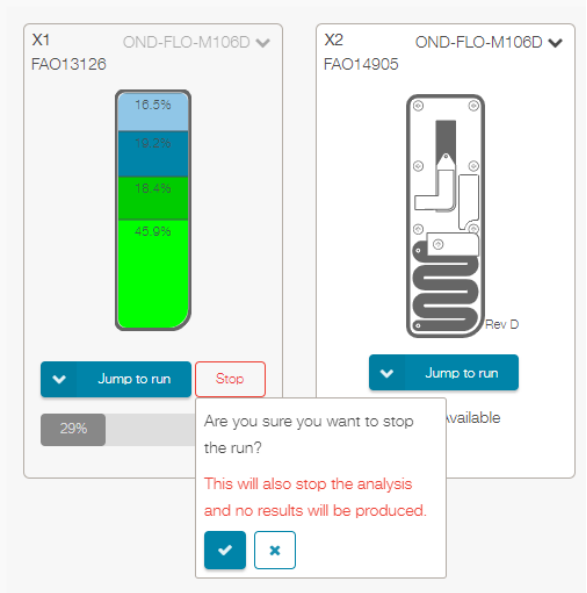


5. Allow the script to run to completion. The message panel in the user interface will inform you when the experiment is complete.

   The data analysis pipeline will start automatically at the end of the sequencing experiment.

   Note: clicking the **Stop** button during the run will cancel the downstream analysis.


Stop run?
Are you sure you want to stop the sequencing run?
You will have to start the run for this flow cell from the beginning if you do.
This will also stop the analysis and no results will be produced.
Stop run
Cancel


X1
FAO13126
OND-FLO-M106D
16.5%
19.2%
18.4%
45.9%
Jump to run
Stop
29%
Are you sure you want to stop the run?
This will also stop the analysis and no results will be produced.
X2
FAO14905
OND-FLO-M106D
Rev D
Jump to run

20. Recalibrating a flow cell

20.1. Recalibrating a flow cell

A flow cell has a very high capacity to generate data, and running fewer than the maximum number of LamPORE tests means the flow cell is not fully utilised. The recalibration kit enables a user to run a set of LamPORE tests, recalibrate the flow cell, store and run a subsequent set of LamPORE tests.

To completely remove any opportunity for cross-contamination between sets, users must keep a record of barcode combinations per flow cell. We recommend a table with at least the entries below:

Flow Cell ID	Date of run	Number of LamPORE tests	Barcode references
FAA123456	16.08.2020	24	FIP 1–8 Rapid Barcode 1–3
FAA123456	16.08.2020	24	FIP 1–8 Rapid Barcode 4–6
FAA123456	17.08.2020	48	FIP 1–8 Rapid Barcode 7–12

Users may see a small number of reads containing barcodes from previous runs when analysing their LamPORE report. These should be discounted and only the reads aligning to the barcodes used in the current test should be interpreted.

The aim is to remove most of the initial library and prepare the flow cell for the loading of a subsequent library. The Flow Cell Recalibration Kit contains all solutions required for removal of the initial library. The experiment in MinKNOW should be allowed to end before recalibrating the flow cell. After the flow cell has been recalibrated, a new library can be loaded or the flow cell can be stored at 4°C.

A maximum of three libraries can be run on one flow cell, with recalibration steps between library loads.

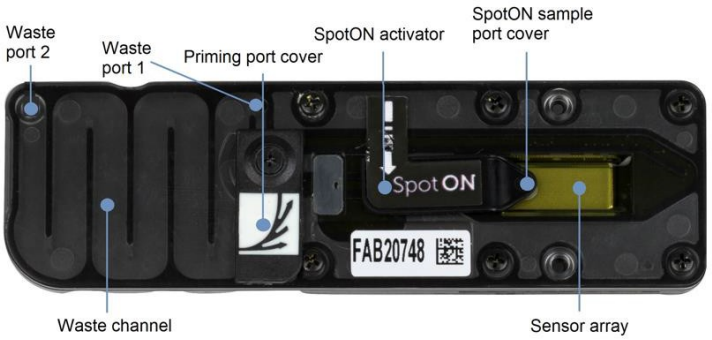
1. Place the tube of Wash Mix on ice. Do not vortex the tube.
2. Thaw one tube of Wash Diluent at room temperature.
3. Mix the contents of Wash Diluent thoroughly by vortexing, spin down briefly and place on ice.
4. In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following mix:

Component	Volume
Wash Mix (WMX)	2 µl
Wash Diluent (DIL)	398 µl

5. Mix well by pipetting, and place on ice. Do not vortex the tube.

6. Make sure the sequencing experiment in MinKNOW has finished and leave the flow cell in the device.

7. Ensure that the priming port cover and SpotON sample port cover are in the closed positions, as indicated in the figure below.



8. Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.

> **!** It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

9. Rotate the flow cell priming port cover clockwise so that the priming port is visible.

10. Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few microlitres):

    a. Set a P1000 pipette to 200 µl

    b. Insert the tip into the priming port

    c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip.

    d. Visually check that there is continuous buffer from the priming port across the sensor array.

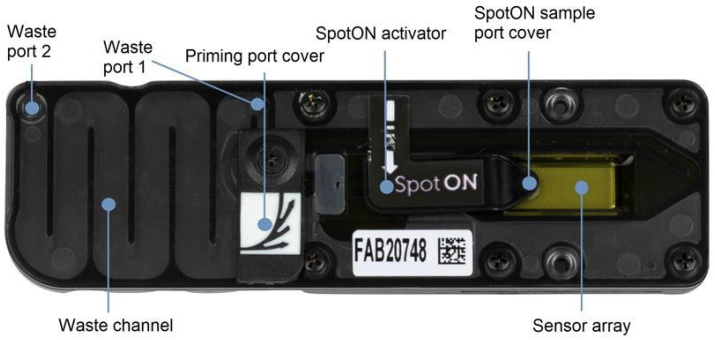
> **!** Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

11. Load 400 µl of the prepared WMX and DIL mix into the flow cell via the priming port, avoiding the introduction of air.

12. Close the priming port and wait for 30 minutes.

13. Ensure that the priming port cover and SpotON sample port cover are in the positions indicated in the figure below.

14. Using a P1000, remove all fluid from the waste channel through Waste port 1.
    As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



> **!** It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

15. Follow one of the two options described in the next steps of the protocol.

20.2 To run a second library on the flow cell straight away

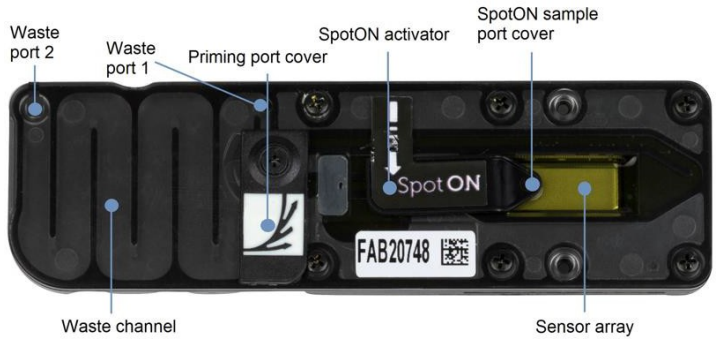
1. Thaw one tube of Storage Buffer (S) at room temperature.
2. Mix contents thoroughly by pipetting and spin down briefly.
3. Rotate the flow cell priming port cover clockwise so that the priming port is visible.
4. Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few microlitres):
    a. Set a P1000 pipette to 200 µl
    b. Insert the tip into the priming port
    c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip.

d. Visually check that there is continuous buffer from the priming port across the sensor array.

5. Slowly add 500 µl of Storage Buffer (S) through the priming port of the flow cell.

6. Close the priming port.

7. Using a P1000, remove all fluid from the waste channel through Waste port 1.
   As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



> ! It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

8. Re-run the flow cell check procedure described earlier in this protocol

9. Prepare a second library using a minimum of 24 samples and a different combination of LAMP primers and Rapid Barcodes to the ones used for the first library.

10. Load the recalibrated flow cell with the second library, following the instructions in the "Priming and loading the flow cell" section of this IFU. Once the flow cell has been primed and loaded, start a new sequencing experiment in MinKNOW.

We recommend storing libraries in Eppendorf DNA LoBind tubes at 4°C for short term storage or repeated use, for example, re-loading flow cells after recalibrations. For single use and long-term storage of more than 3 months, we recommend storing libraries at -80°C in Eppendorf DNA LoBind tubes.

20.3 To store the flow cell for later use

1. Thaw one tube of Storage Buffer (S) at room temperature.

2. Mix contents thoroughly by pipetting and spin down briefly.

3. Rotate the flow cell priming port cover clockwise so that the priming port is visible.

4. Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few microlitres):

5. Set a P1000 pipette to 200 µl

6. Insert the tip into the priming port

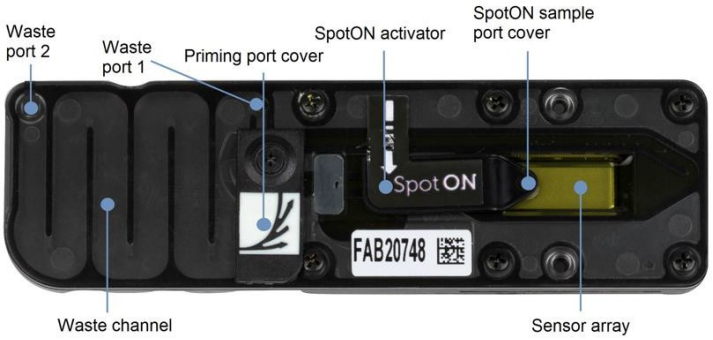
7. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip.

8. Visually check that there is continuous buffer from the priming port across the sensor array.

9. Slowly add 500 µl of Storage Buffer (S) through the priming port of the flow cell.

10. Close the priming port.

11. Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



> **!** It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

12. The flow cell can now be stored at 4-8°C.

13. When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to room temperature for ~5 minutes.

14. Follow the protocol for running fewer than 96 samples at a time.

21. Quality Control

It is necessary to include at least two wells of No Template Control (NTC) and at least two wells of Positive Control (CTL) per plate of full 96 or per subset of these when running fewer than 96.

Controls run in duplicate, plates should be invalidated if any of the four controls come back with unexpected results.

When preparing your samples, make a note of the positions of the CTL and NTC wells in each plate. Check the results in the analysis reports (found in the `lamPORE_results` TSV file and `lamPORE_report` PDF file) for these positions:

Control	Result in analysis report	Recommendation
NTC	Positive	All tests in this plate are invalid and need to be re-run
	Negative	All tests in this plate are invalid and need to be re-run
	Invalid	**Correct result**
	Inconclusive	All tests in this plate are invalid and need to be re-run
CTL	**Positive**	**Correct result**
	Negative	All tests in this plate are invalid and need to be re-run
	Invalid	All tests in this plate are invalid and need to be re-run
	Inconclusive	All tests in this plate are invalid and need to be re-run

22. Analysis

The analysis pipeline:

- Basecalls raw data from the LamPORE device, producing FASTQ files
- Demultiplexes the reads by Rapid Barcode during basecalling
- Demultiplexes the reads by the FIP barcodes
- Aligns the reads against the SARS-CoV-2 genomic targets and human β-actin
- Generates a PDF and TSV report with the barcode ID and the test result
- Generates a TSV file describing the number of reads for each barcode which align to the targets should a more in-depth analysis be required

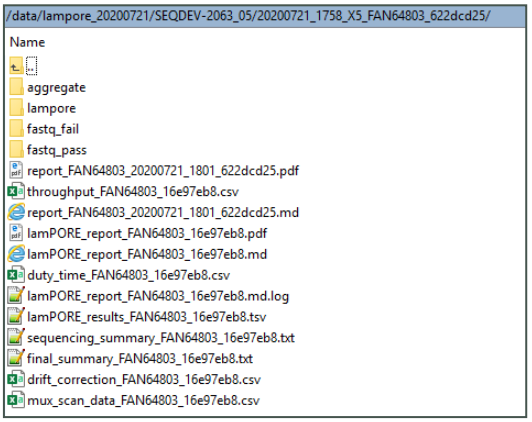
Only reads that have had barcodes identified will then be aligned against the SARS-CoV-2 genome or human β-actin.

Output of the sequencing and analysis pipeline

The files with test results are placed in the /data folder, with the following structure/file naming convention:

```
{data}/
{experiment_id}/
{sample_id}/
{date_time}_{device_id}_{flowcell_id}_{protocol_unique_identifier}/
```

e.g. `/data/lampore_20200617/lampore/20200617_1636_X1_FAO39500_e29f6fcd`
The experiment results can be found in the `lamPORE_report` PDF and the `lamPORE_results` TSV file.



Analysis report description

The report is a TSV file describing number of reads which align to given targets and have given barcodes, as well as metadata for run tracking. A PDF report is also generated, containing results for every barcoded sample.

The fields in the TSV file are as follows:

Column	Meaning
Barcode	RB and FIP barcode combination
target_human_ACTB	Number of reads aligning to the β-actin target
target_nCoV2019_AS1	Number of reads aligning to the ORF1a target in the SARS-CoV-2 genome
target_nCoV2019_E1	Number of reads aligning to the E1 target in the SARS-CoV-2 genome
target_nCoV2019_N2	Number of reads aligning to the N2 target in the SARS-CoV-2 genome
target_unclassified	Number of unclassified reads (reads with barcodes that do not align with the correct specificity)
acquisition_run_id	A unique alphanumeric number to identify the run
protocol_group_id	Experiment name assigned when setting up the run in MinKNOW
sample_id	Name assigned to the flow cell when setting up the run in MinKNOW
flow_cell_id	Unique identification code of the flow cell
started	Experiment start date and time
call	**Positive:** SARS-CoV-2 detected (≥50 SARS-CoV-2 reads) **Inconclusive:** low number of reads against SARS-CoV-2 (SARS-CoV-2 reads between 20 and 49) **Negative:** SARS-CoV-2 not detected (<20 SARS-CoV-2 reads) **Invalid:** insufficient number (<50) of classified reads from both SARS-CoV-2 and β-actin to make a call

Interpretation of results

Before interpreting sample results, it is important to verify that the run has been successful. This is achieved by reviewing the performance of the controls:

- No Template Control (NTC): Result from the NTC should be “Invalid”
- Positive Control (CTL): Result from CTL should be “Positive”

Assay Validity

Targets	Read counts	Result	Action
SARS-CoV-2 regions (AS1, E1 and N2) and ACTB	< 50	Invalid	Repeat test - if the test remains invalid, consider collecting a new specimen
	≥ 50	Valid	Valid test – continue to asses assay output using Assay result table below

Assay Result

Targets	Read counts	Result	Action
SARS-CoV-2 regions (AS1, E1 and N2)	≥ 50	Positive	The presence of SARS-CoV-2 is detected
	between 20 and 49	Inconclusive	Repeat test. If the test remains inconclusive, consider collecting a new specimen
	<20	Negative	The presence of SARS-CoV-2 is not detected

If all four controls do not yield the above results, the whole plate should be re-tested.

The LamPORE PDF and TSV report will classify each sample as Positive, Negative, Inconclusive and Invalid. See the table in the Analysis Report Description for a more detailed breakdown of the classification.

It is important to note that this result is to be used by a healthcare professional in combination with patient symptoms and healthcare history to inform the next course of treatment.

23. Performance characteristics

23.1. Limit of detection

To determine the LamPORE limit of detection, a set of contrived samples (swab and saliva) were prepared.

An aliquot of each negative sample pool (swab and saliva) was spiked with SARS-CoV-2 virions and serially diluted with the remaining material to create a dilution series of contrived positive samples.

From each contrived sample, a 140 µl aliquot was inactivated by addition to 560 µl buffer AVL (QIAGEN), incubation at ambient temperature for 10 minutes followed by the addition of 560 µl 100% molecular-grade ethanol. The entire inactivated volume was then extracted manually using the QiaAMP Viral RNA Mini Kit according to manufacturer's instructions, with final elution of RNA into 50 µl of nuclease-free water.

The limit of detection (LoD) achieved in this study was 7–10 genome copies/µl of extracted RNA. This equates to 140–200 copies per reaction.

23.2. Analytical sensitivity and specificity

To ascertain the sensitivity and specificity of LamPORE, a retrospective study was conducted at two separate sites testing samples which were established to be positive or negative by qPCR.

Site 1: 150 positive samples were selected from samples collected March to April 2020 determined to be SARS-CoV-2 PCR positive by initial PHE RdRp assay, with Ct values of 16–37 re-verified with the Altona assay that includes E gene as a target. Ct values were ultimately determined using the Ct values of the Altona Assay.

220 negative samples were collected prior to the pandemic period, October to December 2019 and extracted using the same method.

Samples were selected according to previous determination as:

Negative prior pandemic samples:

- 40 positive for influenza A
- 40 positive for RSV
- 40 positive for rhinovirus/enterovirus
- 20 positive for other respiratory pathogens (e.g. adenovius/parainfluenza/mycoplasma)
- 80 with no pathogen detected (majority only tested for influenza/RSV)

Negative pandemic samples:

30 Negative samples determined as positive for non-SARS-CoV-2 coronaviruses were collected

from October 2019 to March 2020.

RNA was extracted from these samples by taking 150–200 µl of specimen using the DSP Virus/Pathogen Kit on the QIAsymphony platform, including carrier RNA and MS2 internal control for PCR. RNA was eluted into 60 µl, and frozen in 10 and 20 µl aliquots.

Site 2: A range of positive samples were selected based on their SARS-CoV-2 E gene cycle threshold (Ct) value, with 50% ct <30 and 50% ct >30. Nose and throat combined swab (NTCS) samples were collected as a first choice of sample type. Throat swabs (TS) only were chosen when NTSW sample types were insufficient.

Samples were extracted using Roche MagNa Pure 96 DNA and Viral NA Small Volume Kit. 200 µl samples were extracted and eluted in 100 µl. 6 µl of extract was used for in-house PCR assays.

The results of the two-site evaluation of 514 samples are summarised below:

PCR result	LamPORE results				
	Positive	**Negative**	**Inconclusive**	**Invalid**	**Total**
Positive	226	2	0	1	**229**
Negative	1	278	3	3	**285**
	227	**280**	**3**	**4**	**514**

Replicate 1 sensitivity: 226/ 228 99.1% (96.9–99.9)
Replicate 1 specificity: 278/ 279 99.6% (98.0–100)

The assay performance is therefore concluded as:

Assay sensitivity	**99.1%**
Assay specificity	**99.6%**

The positive samples used for the study contained the following range of viral loads (as determined by qPCR):

Number of positives by Ct (E gene)		
< 30	30–34.9	≥ 35
190	27	12

23.3 Test reproducibility

The sites performed a replicate of the initial test to determine assay reproducibility. A selection of samples were also exchanged between sites to establish cross site variability for both LamPORE but also the reference assay (qPCR).

	REP2				
REP1	POS	NEG	INC	INVALID	
POS	221	3	3	0	227
NEG	1	255	2	3	261
INC	0	2	0	0	2
INVALID	1	2	0	1	4
	223	262	5	4	477/494

Reproducibility of assay: 477/494 96.6% (94.5–98.0)

23.4 Cross-reactivity

To assess the potential for cross-reactivity with other viruses, we aligned the LAMP primer sequences against sequences of common viruses as well as coronaviruses related to SARS-CoV-2. We determined sequence identity by dividing the sum of aligned primer bases by the sum of primer lengths.

Pathogen	GenBank
Adenovirus A	NC_001460.1
Adenovirus B1	NC_011203.1
Adenovirus B2	NC_011202.1
Adenovirus C	NC_001405.1
Adenovirus D	NC_010956.1
Adenovirus E	NC_003266.2
Adenovirus F	NC_001454.1
Bordetella pertussis (BPP-1)	NC_005357.1
Candida albicans (L757)	NC_018046.1
Chlamydia pneumoniae	NC_005043.1
Coronavirus 229E	NC_002645.1
Coronavirus HKU1	NC_006577.2
Coronavirus NL63	NC_005831.2
Coronavirus OC43	NC_006213.1
Enterovirus D68	KP745766.1
Haemophilus influenzae	NC_017451.1
Human Metapneumovirus	NC_039199.1
Influenza A (H1N1)	FJ966079.1
Influenza A (H3N2)	KT002533.1
Influenza B (Victoria)	MN230203.1
Influenza B (Yamagata)	MK715533.1
Legionella pneumophila	NZ_CP016029.2

Pathogen	GenBank
MERS-CoV (England 1)	NC_038294.1
MERS-CoV (HCoV-EMC)	NC_019843.3
Mycoplasma pneumoniae (C267)	NZ_CP014267.1
Pneumocystis jirovecii	NC_020331.1
Pseudomonas aeruginosa	NZ_CP022001.1
Respiratory syncytial virus	NC_001803.1
Rhinovirus 1	NC_038311.1
Rhinovirus 14	NC_001490.1
Rhinovirus C	NC_009996.1
SARS-CoV-1	NC_004718.3
SARS-CoV-2 (WU)	MN908947.3
Staphylococcus epidermidis (ATCC 12228)	NZ_CP022247.1
Streptococcus pneumoniae (AP200)	NC_014494.1
Streptococcus pyogenes (AP1)	NZ_CP007537.1
Streptococcus salivarius (YMC-2011)	NC_018285.1
Human parainfluenza 1	NC_003461.1
Human parainfluenza 2	NC_003443.1
Human parainfluenza 3	NC_001796.1
Human parainfluenza 4	NC_021928.1
Tuberculosis (H37Rv)	NC_000962.3

Table 1: Organisms assessed *in silico* for potential cross-reactivity to the SARS-CoV-2 LamPORE assay.

SARS-CoV, which is closely related to SARS-CoV-2, was the sole virus to have a match against the total sequence length of the SARS-CoV-2 primers greater than the recommended threshold of 80%. The E-gene primer set has a match >90% with SARS-CoV, but the AS1 and N2 primer sets differ significantly, matching at only 44.5% and 74%, respectively. The likelihood of a false positive is low since SARS-CoV is not known to be in active circulation at present.

23.5 Interference study

LamPORE is designed to work with a number of upstream sample processing methods. As such, an evaluation has been performed on reagent and buffer solutions commonly deployed during sample lysis, deactivation and extraction.

The substances tested were as follows:

- Tween-20, Isopropanol(“IPA”),
- Ethanol(“EtOH”),
- Sodium azide,

- VHB Buffer(Mag-Bind® Viral DNA/RNA 96 Kit, Omega Bio-Tek)*,
- RMP Buffer (Mag-Bind® Viral RNA XPress Kit, Omega Bio-Tek)*,
- Lysis LBF(RNAdvance Viral Kit, Beckman Coulter)†
- Wash WBE(RNAdvance Viral Kit, Beckman Coulter)‡

**Contains guanidine thiocyanate ("GuSCN"). †Contains GuSCN, boric acid, tris hydroxymethyl aminomethane(Tris) buffer, and polyoxyethylated octyl phenol("POP"). ‡Contains GuSCN and POP.*

Interfering substance	Concentrations to be examined			
	A	B	C	
Tween-20	0.002%	0.01%	1%	(w/v)
Isopropanol ("IPA")	1%	2.5%	10%	(v/v)
Ethanol ("EtOH")	1%	2.5%	10%	(v/v)
Sodium azide	0.008%	0.04%	0.2%	(w/v)
VHB Buffer	0.025%	0.125%	0.625%	(v/v)
RMP Buffer	0.004%	0.02%	0.1%	(v/v)
Lysis LBF	0.00002%	0.0001%	0.0005%	(v/v)
Wash WBE	0.025%	0.125%	0.625%	(v/v)

Table 2: Interfering substances chosen for the test and the concentrations used.

The interfering substances (at their respective concentrations) listed in Table 2 were tested in triplicate against the LamPORE kit components' ability to detect 200 copies SARS-CoV-2 RNA, as outlined in Table 2.

Results show that when operating at concentrations expected "A" and above expected "B" of a lab methods, the assay is unaffected. Isopropanol (IPA) and sodium azide at very high "C" concentrations within the RNA sample (10% (v/v) and 0.2% (w/v), respectively) appear to affect the ability to report *Positive* calls.

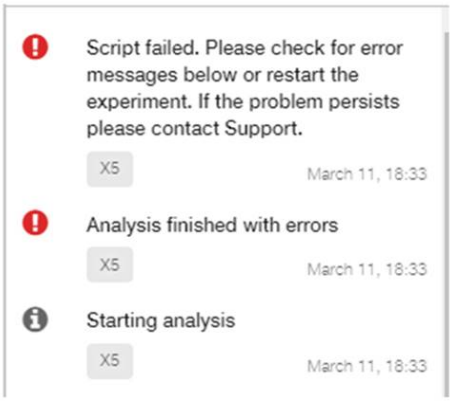
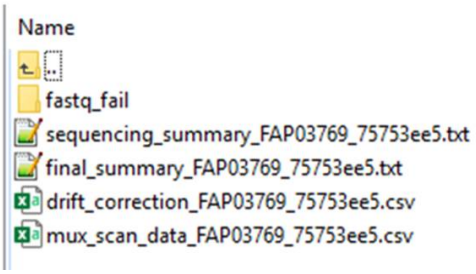
24 Troubleshooting

Hardware and software

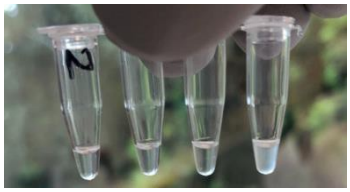
Issue	Solution

Networking issue – an error message appears in the MinKNOW message panel saying **Could not start experiment. Please ensure your computer is connected to the internet, and that you have applied the relevant exceptions to the firewall, as specified in the Computer Requirements document**.	A proxy may be set by editing the `user_conf` file.
	
	Navigate to `/opt/ont/MinKNOW/conf/user_conf`. Then edit this portion of the file:
	
	`"proxy": {`
	`"cereal_class_version": 0,`
	`"use_system_settings": true,`
	`"auto_detect": true,`
	`"auto_config_script": "",`
	`"https_proxy": "",`
	`"proxy_bypass": ""`
	
	`https_proxy` should be in the style of: `scheme://[username:password@]host:port` or `http://domain\\username:password@host:port`, where `scheme` is one of https, socks, socks4 or socks5.
	
	Once the proxy is set up, perform a hardware check to ensure the device is working correctly and connected to the internet.

Run fails to start – an error message appears in the MinKNOW message panel saying **Script failed. Please check for error messages below or restart the experiment. If the problem persists, please contact Support**.	Power the box and off (do not reboot and ensure no other flow cells are running at the same time) and run the hardware check after reseating the flow cell. If the hardware check is successful, restart the experiment as normal. If the hardware check fails, insert the flow cell into a new position and retry the hardware check. If the hardware check is successful, restart the experiment as normal. If the run still fails to start, contact support@oxfordnanoporedx.com with details of your issue. Support will also require the MinKNOW logs from: /var/log/minknow/
Run fails to complete – an error message appears in the MinKNOW message panel saying **Script failed. Please check for error messages below or restart the experiment. If the problem persists, please contact Support.**	Contact support@oxfordnanoporedx.com with details of your issue. Support will also require the MinKNOW logs from: /var/log/minknow/

Run fails to complete and run reports are not generated – two error message appear in the MinKNOW message panel saying **Script failed. Please check for error messages below or restart the experiment. If the problem persists, please contact Support.** and **Analysis finished with errors.** 	This error is generally associated with a lack of adapted library loaded on the flow cell and no useable data at the end of the LamPORE run. To check this, navigate to the data folder corresponding to the run, and confirm the absence of .fastq passed folder and results run reports:  To troubleshoot, follow the instructions below: 1. Check the LAMP plate. If you notice the liquid in the wells is turbid, this indicates a successful LAMP reaction, so most likely there was an issue with the library preparation. Repeat the library preparation and ensure that you follow the exact steps described in the IFU. 2. If the liquid in the LAMP plate is not turbid, this indicates that the LAMP reaction has failed. Repeat the LAMP reaction and library preparation, following the exact steps described in the IFU.
Disk usage alert – an error message appears in the MinKNOW message panel saying **You only have X_GB of space free, which is insufficient for the run. Please free up some space, otherwise your run will stop in Y Mins**	The SSD storage on the device is filling up and will not be able to complete the run. Free up some space on your device according to the details in the GridION User Manual to prevent the run terminating.
Disk usage alert – an error message appears in the MinKNOW message panel saying **Disk too low to continue. Stopping data acquisition. Please clear some space and restart the experiment**.	The SSD storage is insufficient to continue running the test, and MinKNOW has terminated the run early. Free up some space on your device according to the details in the GridION User Manual.

General troubleshooting

Issue	Solution
The report shows a Positive, Negative or Inconclusive call in a well with NTC	The entire plate should be considered as void, and all samples and controls re-run. Follow the guidance at the beginning of the "LAMP and library preparation" step to prevent cross-contamination of samples.
The report shows a Negative, Invalid or Inconclusive call in a well with CTL	The entire plate should be considered as void, and all samples and controls re-run. If the issue persists, resuspend and use a different vial of CTL. Ensure you are storing the CTL tubes at -80°C. Follow the guidance at the beginning of the "LAMP and library preparation" step to prevent cross-contamination of samples.
A high number of Invalid calls in the report	Invalid suggests insufficient data was produced during the assay. There could be a number of causes: i. Product failed to amplify: This may occur due to incorrect storage of reagents, incorrect addition of sample, interfering substances, or incorrect temperature settings in the thermal cycler. A user can visually check if amplification has been successful as the vials containing the positive controls will be turbid in a visual inspection.  If the positive control wells are clear, then amplification has failed. ii. Library preparation failed: If the amplification reaction is shown to be successful, the fault may be from the library preparation step. The Rapid Barcodes may have become compromised during shipping/storage or the user may have lost the library during the SPRI clean-up step. Both these steps would need to be repeated to ascertain the potential cause. iii. Flow cell error: Users are required to check their flow cell before initiating a run. The flow cell can become damaged through the addition of an air bubble into the system. This can be ascertained by looking at the flow cell or the flow cell channel panel during the experiment. iv. Issue with RNA extraction: Repeat the extraction, adhering to the manufacturer's instructions.

A higher number of positives than expected	Should there be a higher than expected level of positives, there is a potential that cross-contamination has occurred. To minimise cross-contamination, follow the instructions in this document. Should users wish to perform a trial, there is sufficient CTL and No Template Control provided in the kit to run a series of tests.

25 Technical support

All protocols, troubleshooting and information can be found on: www.oxfordnanoporedx.com

Please contact Technical Support by e-mail at support@oxfordnanoporedx.com for any product inquiries, complaints and adverse event reporting.

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