Supplementary Note 2 | Hypothetical deployment protocol for large-scale testing using LAMP-Seq

All steps below are performed by one sequencing center or one testing center per one day. In this hypothetical deployment scenario, a total of 96 testing centers are served by one sequencing center, which processes up to 90,240 samples per day. No compressed barcoding is employed in this simple scenario. As we are not experts in the field of clinical testing, we are putting forth this hypothetical deployment scenario as a starting point to engage with experts.

Step 1: Preparing reagent plates (at sequencing center)

- Using an automated de-capper and a *MultiDrop* reagent dispenser, 495μl of RT-LAMP master mix lacking template and FIP primer are dispensed into 960 96-matrix-tube plates ("reagent plates"). Plates are stored in a cold room.
- Using a *Beckmann Biomek* robot, for each of 10 barcode source plates (A-J):
    - An operator places the barcode source plate on the robot deck and unseals it.
    - An operator places 96 reagent plates in the robot's hotel.
    - An operator places 8 96-well tip boxes in the robot's hotel.
    - In sets of 12 reagent plates (to be performed 8 times per source plate):
        - The robot retrieves 12 reagent plates from the hotel and places them on the deck,
        - using the 96-well head, the robot aspirates 65 μl of primer solution per well from the source plate,

        - the robot de-caps one reagent plate at a time, and without changing tips, dispenses 5 μl of primer solution into each of 12 reagent plates present on the deck,
        - the robot discards the tips,
        - the robot returns the 12 reagent plates to the hotel.
    - The operator removes the 96 reagent plates from the hotel, and stores them for shipping in a cold room.
- A courier delivers ten reagent plates with orthogonal barcodes A-J to each testing center. A total of 96 testing centers are served by each sequencing center.

<u>Step 2: Processing swab samples (at testing center)</u>

- For each of 10 reagent plates:
    - The plate is placed in a sterile hood on a cooling device.
    - Swab samples are taken from individuals by a medical worker using personal protective equipment.
    - Swabs are inserted in successive order into individual matrix tubes from the reagent plate; the shaft of the swab is removed, and the tube is sealed.
    - A unique barcode printed on the tube is scanned using a mobile device, allowing to link reaction IDs to sample IDs through a database. A solution for privacy-aware or anonymous delivery of testing results has to be designed by experts in the field.
    - The last two tubes of every plate are left empty or spiked with a positive control RNA.

    - When the plate is complete, it is incubated in the sterile hood in two ovens (30 minutes 65 °C, 10 minutes 95 °C),
    - The plate is stored at room temperature.
- At the end of the day or when all plates have been processed, an operator unseals all plates in a sterile hood, and pours them into a plastic container.
- The combined liquid in the container is mixed, and a small sample of the pool is transferred to a 2-ml plastic screw cap tube, which is used in Step 3.
- A courier delivers the tube to the serving sequencing center.

Step 3: Processing and sequencing pooled samples (at sequencing center)

- An operator gathers 96 pools from testing centers in a rack, and transfers 1 μl of each pool into a 96-well plate pre-filled with 99 μl of water, with well positions being determined by the testing center IDs. Dilutions are mixed with a 12-channel pipet.
- The operator prepares a PCR plate with 90 μl master mix per well (water, 2x NEBNext High-Fidelity PCR master mix, two primers).
- The operator stamps over 10 μl of each template dilution into the PCR plate using a 12-channel pipet.
- The operator runs the PCR plate in a 96-well temperature cycler according to the protocol.
- The operator unseals the plate.
- The operator prepares a secondary PCR plate with 90 μl master mix per well (water, 2x NEBNext High-Fidelity PCR master mix).
- The operator stamps over 5 μl from a plate of unique primer combinations from a stock plate using a 12-channel pipet.

- The operator stamps over 5 μl from the first PCR plate into the secondary PCR plate using a 12-channel pipet.
- The operator runs the PCR plate in a 96-well temperature cycler according to the protocol.
- On ice, the operator pools 20 μl of each of 96 secondary PCR reactions in a reservoir.
- The operator runs the pool on a 2% EX E-Gel (10 wells x 10 μl pool plus 10 μl water per well).
- The operator cuts the bands of appropriate size from the gel, and purifies the DNA using a Qiagen agarose gel purification kit according to the manufacturer's instructions.
- The operator re-purifies the DNA using a Qiagen PCR purification kit according to the manufacturer's instructions.
- The operator quantifies the DNA using a *NanoDrop* photospectrometer.
- The operator inserts the DNA solution into a *NextSeq High-Output 75-cycle* kit (Illumina), and initiates a run, which takes 14 hours.

Step 4: Data analysis

- An operator transfers the *NextSeq* data to a server computer.
- The operator initiates barcode deconvolution using a predefined *SampleSheet* and the *bcl2fastq* tool (Illumina), which takes 2 hours.
- The operator inflates the compressed output files on the server, resulting in FASTQ files of about 50 GB in total size, which takes 1 hour.
- The operator executes a software program that analyzes 96 FASTQ files and saves a list of positive / negative / unresolved sample barcodes to a file, which takes 4 hours.

- The operator copies the results file to a data distribution server, and executes a software program that stores the results in a database.
- Mechanisms of privacy-aware or anonymous retrieval of personal testing results have to be designed by experts in the field.