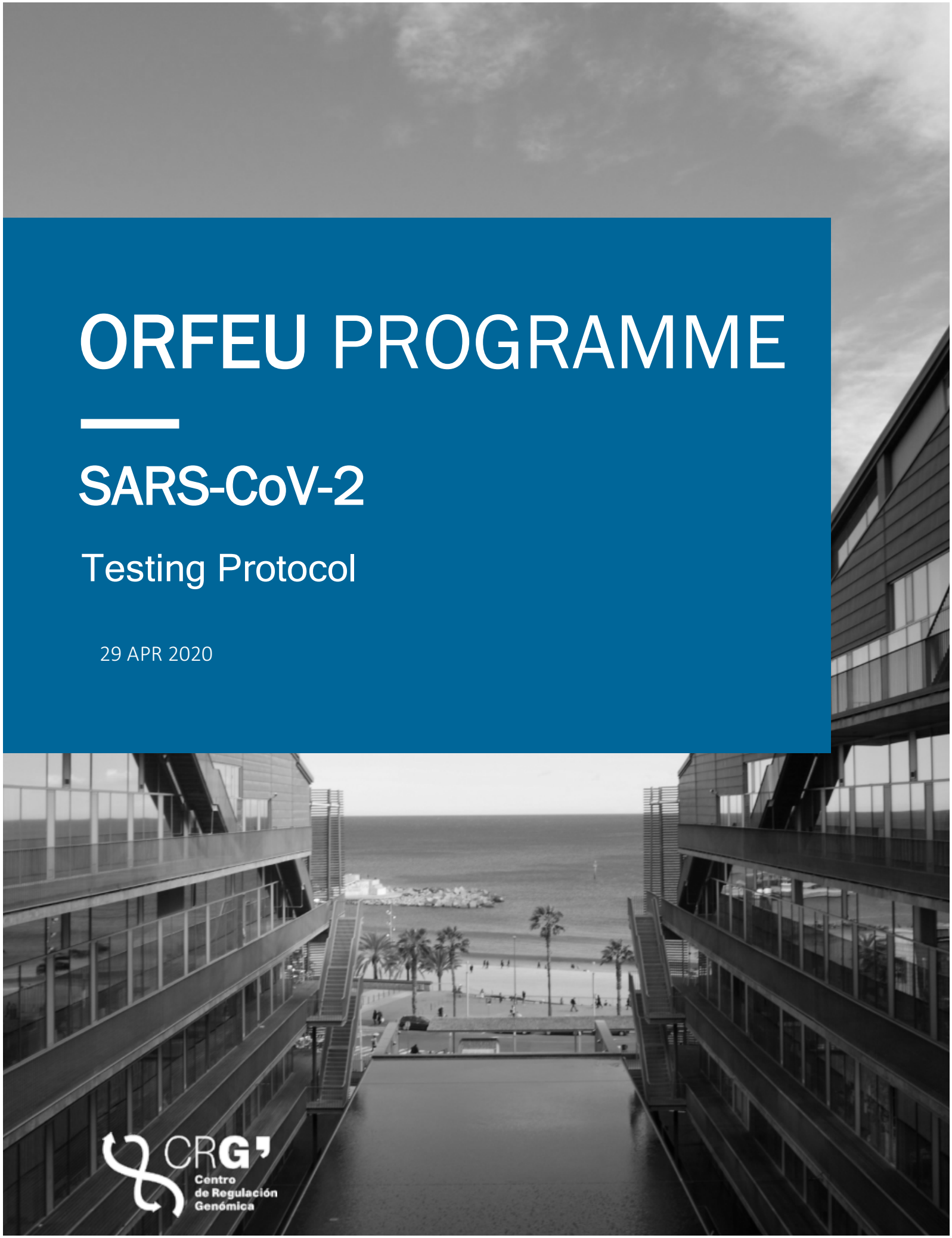
ORFEU PROGRAMME

SARS-CoV-2

Testing Protocol

29 APR 2020



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BACKGROUND

Due to the situation generated by the evolution and the contagion of the COVID-19 and with the aim of meeting the needs arising from the protection of people and other measures adopted by the Government to deal with the COVID-19, the Generalitat de Catalunya commissions research centres to collaborate in a COVID-19 mass detection program, including the CRG, through the ORFEU Program approved by Government Agreement on April 7, 2020. The Department of Health of the Generalitat de Catalunya validates the protocols of the CRG of mass detection of COVID-19 with the technique of extraction of RNA and qPCR.

This document describes the protocol for mass detection of Covid-19 using RT-qPCR at the CRG, including Quality Controls and Biosafety aspects:

1 Production of Test Kits

The Kit production team is in charge of automatized filling of Micronic tubes with 600 microliters of Zymo DNA/RNA Shield Lysis Buffer, using a Tecan Evo 200 automated liquid handling system. These are shipped along cryogenic 81-well racks and printed 2D barcode stickers (Brady Printer i7100). These kits are produced at the Protein Expression Facility (room 512).

KIT PRODUCTION TEAM

- ✓ **Professional profile_**Lab Technician with knowledge of robotic automation
- ✓ **Number needed_** 1 person/ day
- ✓ **IPEs needed_** FFP2 masks, double gloves and lab coats.
- ✓ **Requirements_**Tested coronavirus negative. No contact with PCR disposal team/environment.

IMPORTANT NOTE: The procedure for swab-mediated sample collection has been agreed with CatSalut after two rounds of testing and comparison of procedures (Results available upon request). Swabs compatible with our tube/buffer set up include MSC-96000 (preferred), Roche Ref. 07958030190 or Deltalab Ref. 304281. It has been verified that 10 seconds of swirling of the swab head against the tube walls is sufficient for recovery of biological material directly in Lysis Buffer.

2 Reception of samples at PRBB

Samples will be received at the PRBB cargo deck (moll de càrrega) @ the -1 floor by members of the reception and transport team who will

I. Issue a receipt certificate and communicate reception to sender.

II. Register the shipment in the Inventory of samples received (Origin, Project, Number of samples, date and time).

III. Store the samples in the 4oC room (safely locked and under control by the building security service) located at the PRBB cargo deck until they enter in the processing chain.

RECEPTION & TRANSPORT TEAM

- ✓ **Professional profile_** Technician / scientist with SL2 training
- ✓ **Number needed_** 1 person/shift (2 in total/day), max. 2 shifts/day for reception of samples
- ✓ **IPEs needed_** FFP2 masks, double gloves and lab coats.

3 Transport of samples to the site of sample processing

Racks of tubes with samples are placed in a biological safety "B category eco-container" by members of the "reception and transport team" and moved via the cargo elevator (montacargas) of the Hospital side to the Bacterial Room (room 473.08.07) in the 4th floor Hotel side, which is a SL2 facility.

4 Disinfection of samples

Container with samples arriving to the Bacterial Room (473.08.07) is first disassembled (first layer) in the pre-room by the disinfection team and moved to the hood in the inside room, where the racks are taken out, opened and the Micronic tubes containing the samples disinfected by cleaning the outside with Ethanol 70% (either by spraying on individual tubes or by immersing the rack in an ethanol-filled container).

DISINFECTION TEAM

- ✓ **Professional profile_** Technician/Scientist with BSL2 training.
- ✓ **Number needed_** 1 person/shift (up to 3/day).
- ✓ **IPEs needed_** FFP2 masks, double gloves, glasses, disposable caps, lab coats, footwear and "pijamas".

5 Sample registration

The registration of samples includes the following steps:

I. Racks with disinfected tubes are moved to Aula (4th floor) for sample registration by the "RNA extraction team".

II. Tubes are taken out of the rack individually and 2D barcodes doubly scanned by the sample registration team.

III. Tubes are placed in barcoded Micronic plates and the barcodes below the tubes are scanned with a Micronic plate reader to assign them a plate location.

IV. Records of scans are transferred to LIMS system. LIMS configuration and data organization has been agreed with CatSalut.

SAMPLE REGISTRATION TEAM

- ✓ **Professional profile_** Technician/Scientist familiar with barcode reading and computer data input.
- ✓ **Number needed_** 2-3 people /shift (up to 9 in total / day).
- ✓ **IPEs needed_** FFP2 masks, double gloves, lab coat.

6 RNA extraction

Racks of tubes with scanned barcodes are brought back to Bacterial room (room 473.08.07). The RNA extraction includes the following steps:

I. RNA extracted using the TECAN Dreamprep robot (or Zephyr robot as a backup) by the RNA extraction team.

II. Automatized opening/closing, extraction of aliquot (250 microliters), RNA isolation and transfer to 96 well plates using the TECAN Dreamprep robot (#).

III. For Zephyr robot, a manual spinning step is required and chemical waste segregated.

IV. Leftovers from lysis buffer are stored in -20oC freezer and after one week of storage negative samples are discarded and positives stored in -80 oC freezer and eventually moved to a BioBank.

RNA EXTRACTION TEAM

- ✓ **Professional profile_** Professional profile: technician / scientist with SL2 training and experience with robotic automation
- ✓ **Number needed_** 2 people /shift (up to 6 in total / day)
- ✓ **IPEs needed_** FFP2 masks, double gloves, glasses, disposable caps, footwear and "pijamas".
- ✓ **Requirements_**Tested coronavirus negative. No contact with PCR disposal team/environment

(#) DETAILED PROTOCOL FOR AUTOMATED RNA EXTRACTION:

RNA is extracted using the Quick-DNA/RNA Viral MagBead kit (Zymo; #R2140) which has been fully automated by Tecan on a Tecan DreamPrep NAP Workstation. The robot uses up to 400 ul of sample in DNA/RNA Shield buffer and automates all the subsequent steps of magnetic bead purification of the samples until the elution step, which is performed in 50 ul of RNase-free water.

7 RT-qPCR assays

The RT-PCR assays are conducted according to CDC-006-00019 CDC/DDID/NCIRD/ Division of Viral Diseases protocol released 3/30/2020.

- Sealed 96-well plates with RNA samples are moved via cargo elevator (montacargas) to room 520.04 (Genomics facility) by the "RNA extraction team".
- Samples are transferred to the "RT-qPCR team"* for set up of RT-PCR assays using the TECAN Evo robot which transfers a 5 microliter aliquot of RNA solution to labeled 384 well plates preloaded with 15 microliters of RT-PCR master mix (Luna Universal Probe One-Step RT-qPCR Kit; New England Biolabs; E3006).
- Aliquots of each sample are loaded in three wells containing either the CDC-approved probes for SARS-CoV-2 N1 or N2 amplicons or the RNaseP amplicon as control. Probes and primers for qPCR are purchased from IDT integrated technologies (qPCR probes - 2019-nCoV CDC EUA Kit, 500 rxn, ref. 10006606).

- Labeled covered plates are moved to room 531 for running the RT-PCR assays in APPLIED BIOSYSTEMS 7900HT or in THERMO FISCHER ViiA7, using 384 well blocks.
- Reverse transcription is carried out for 10 minutes at 55 ºC as recommended by the manufacturer, followed by 45 cycles of quantitative PCR with 95 ºC denaturation for 3 seconds and 30 seconds annealing/elongation at 64 ºC.
- Results of PCR amplification are evaluated by members of the Quality Control Team to verify correct amplification of controls and adjust thresholds before exporting the results to the LIMS system agreed with CatSalut. The system provides information about the sample (Barcodes and tracing through the pipeline), Ct values for N1, N2 and RNAse P amplicons and remote access to the amplification curves of the samples, including controls in the same plate, to virologists from CatSalut who will validate the results and produce the diagnosis. Routine checks for errors and inconsistencies are performed automatically upon upload. An example of the display in the LIMS system is provided at the end of the document.
- PCR plates are disposed in cytostatic waste containers located in the same room (531) by personnel not involved in the previous processing steps.
- Storage of leftover RNAs in -80oC freezer and eventually to a BioBank.

RT-qPCR TEAM

- ✓ **Professional profile_** Technician / scientist with experience with robotic automation and real time PCR
- ✓ **Number needed_** 2 people /shift (up to 6 in total / day)
- ✓ **IPEs needed_** FFP2 masks, double gloves, lab coat.
- ✓ **Requirements_** Need to be tested coronavirus negative. No contact with PCR disposal team/environment.

8 QUALITY CONTROLS

8.1 The integral performance of the procedure (accuracy, sensitivity and specificity) has been benchmarked against a set of negative and positive controls (with different levels of viral load) provided by the a) Laboratori de Referència de Catalunya, S.A. (contact: Dr. Mireia Canal, Direcció Tècnica i d'Operacions, email: mcanala@lrc.cat) and Hospital Parc Taulí (contact: Dr. Mateu Espasa, Coordinador Microbiologia, UDIAT-CD, email: mespasa@tauli.cat). Results available upon request.

8.2 Each batch of RNA extractions and RT-qPCR reactions will include positive and negative controls to ensure robust performance of the assay over the whole testing period. Commercial controls include:

- EURM-019 single stranded RNA (ssRNA) fragments of SARS-CoV-2 (provided by the European Commission Joint Research Centre).
- 2019-nCoV_N_Positive Control (IDT integrated technologies, ref. 10006625).
- Hs_RPP30 Positive Control (IDT integrated technologies, ref. 10006626).

Additionally, a weekly control will be run and analysed throughout all PCR instruments to ensure homogenous detection of viral load.

8.3 Technical triplicates of RNA extraction and RT-qPCR are carried out in a subset of samples to assess reproducibility.

8.4 Initial and periodic testing of RNA extraction and RT-qPCR reagents to rule out contaminations of biological material or PCR amplification products.

8.5 Every week a set of control samples provided by CatSalut will be tested in parallel with the rest of the tests to assess uniform performance of the pipeline during the whole duration of the tests.

8.6 Racks of samples with high viral load are reprocessed from the RNA extraction step after removing high viral load samples, to assess potential aerosol contamination of neighboring samples.

9 BIOSAFETY TRAINING OF PERSONNEL INVOLVED

Personnel involved in the tests (technicians, PhD students and postdocs) are selected from the pool volunteers using the following criteria:

I. Highest level of previous biosafety training (lentivirus, mycoplasma). Every CRG member using SL2 facilities has already received biosafety training by a SL3 level specialist.
II. Experience with the technical procedures involved in the tests
III. Medical records (carried out by the Company's Labor Medicine service, to exclude people with pre-existing conditions that may constitute risk factors for potential viral infection)
IV. Time availability and flexibility as reported in the volunteer's forms.
V. Further training includes videoconference training by CRG's Biosafety Advisory extrenal company plus in situ training on Biosafety procedures and gear.

In situ training on technical procedures and the pipeline. This includes safety measures, exact protocols, schedule of work and rest, food and drinks available, etc.

* ANNEX_Biological Safe Handling of SARS-CoV-2 Samples Procedure

10 Approval

This Testing Protocol has been described and approved by:

Owner	Head of Core Facilities
Title	ORFEU Programme_SARS-CoV-2 Testing Protocol at CRG
Version	V1
Version Date	29 APR 2020
Track Changes	V1_Original Protocol
Approved by	Director
Related doc.	ANNEX_Biological Safe Handling of SARS-CoV-2 Samples Procedure  Biological Safe Handling of COVID19

Example of the display of RT-qPCR results in LIMS system.