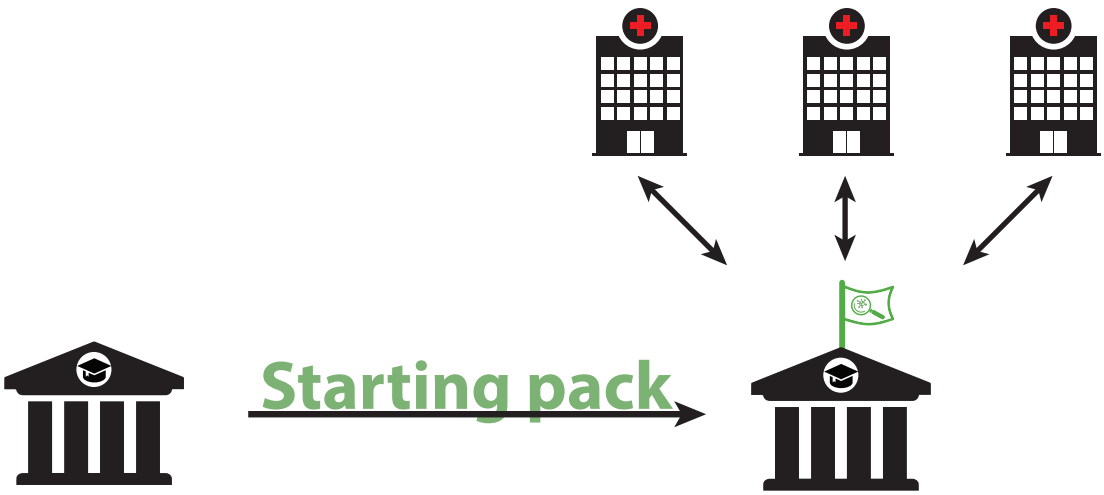

All over the world the lack of screening capacity is a major issue in the SARS-Cov2 pandemy containment. Hereby we share protocols and procedures to help academic institutions and any molecular biology laboratory to provide support to population health care system.

In order to guide every laboratory with expertise in molecular biology to develop SARS-CoV2 screening plateform, this starting pack will fasten the changes from basic requirement needed to run RTqPCR test, to build a robust structure.

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Starting pack

Requirement

The following protocol requires essential Institution infrastucture, researcher skills and material to ensure efficiency and safe screening procedure

Institution

- ☐ Biological and chemical waste elimination flow

Researcher skills

- ☐ Molecular biology manipulation
- ☐ Aseptic experimentation in Biosafety Level 2+ (BL2+)
- ☐ Reverse transcription and quantitative PCR analysis

Material

- ☐ Class II Biological Safety Cabinet
- ☐ Chemical hood
- ☐ High speed 1,5ml tube refrigerated centrifuges
- ☐ Quantitative PCR machines
- ☐ Micropipettes p1000/p100/p10
- ☐ Centrifuges for 15 mL falcons tubes and qPCR plate
- ☐ -80°C freezer and 4°C fridge

Starting pack
Protocol

VIRUS INACTIVATION

1. Sample processing

100µl sample → + 1ml of Guanidinium Iso-thiocyanate Solution + 5µl of Internal Control RNA (IC)

REDO with PBS sample 5x dilution

100µl sample → + 400 µL PBS → 100µl sample → 1ml of Guanidinium Thiocyanate Solution + 5µl of Internal Control RNA (IC)

RNA EXTRACTION

1. Homogeneisation — *Vortex sample 10 sec + Incubate 5 minutes at RT*

2. Addition of Chloroform — + 200µl Chloroform

Vortex sample 10 sec + Incubate 5 minutes at RT

Centrifuge 12.000g
10 minutes
4°C

During centrifugation time

3. Preparation 22 labeled tubes
+ LABEL
+ 500µl Iso/Glyco. X 22

4. Transfer RNA aqueous phase — 500µl aqueous phase in new labeled eppendorf + Iso/Glyco

5. Add Isoprop + Glycoblue

If there is no aqueous phase up → + 100µl RNAse free water

Vortex sample 10 sec + Incubate 5 minutes at RT

Centrifuge 12.000g
10 minutes
4°C

6. Wash the RNA blue pellet — Discard supernatant

Even without visible pellet continue the extraction

+ 900µl Ethanol 75%

Gently mix by inverting tube

Centrifuge 12.000g
10 minutes
4°C

7. Dry the blue pellet — Discard supernatant with 1ml pipette and narrow tips + leave the tube open under chemical hood
(min 5 min - max 10 min)

8. RNA resuspension — + 30µl RNAse free water
Incubate at room temperature until complete resuspension of the blue pellet

one step Resverse Transcript - Qantitative PCR (RTqPCR)

1. Primers and probe mix for SARS and IC

Primers and Probe Mix for SARS
+ 10 µl of E_Sarbeco_Probe [100µM]
+ 20 µl of E_Sarbeco_Fw [100µM]
+ 20 µl of E_Sarbeco_Rev [100µM]
+ 950 µl of RNAse free water

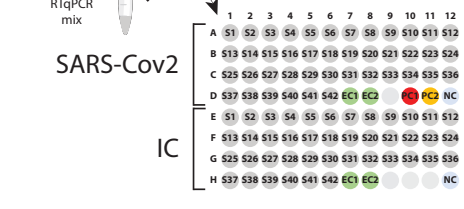
Primers and Probe Mix for IC (SBV)
+ 10 µl of IC_Probe [100µM]
+ 20 µl of IC_Fw [100µM]
+ 20 µl of IC_Rev [100µM]
+ 950 µl of RNAse free water

2. RTqCPR mix for SARS and IC

Volume in µ	Per reaction	1 plate	2 plates	3 plates	4 plates	5 plates
5X Master Mix	4	200	400	600	800	1000
EuroscriptII (RT+RNAse i)	0.2	10	20	30	40	50
Primers and Probes Mix	4	200	400	600	800	1000
RT Additive	0.2	10	20	30	40	50
RNAse free water	7.6	380	760	1140	1520	1900

3. Plate preparation

RTqPCR mix + 16µL /well



Tested RNA | NC: water | PC1: RNA of SARS2 | PC2: | EC: PBS control

+ 4µL/well

4. Run

Temperature PCR cycle
- 48 °C 10 min
- 95 °C 3 min
- 45 cycles: 95°C 15 sec
  58°C 30 sec

Eurogentec Takyon
One-Step RTqPCR

Starting pack

Validation and Quality procedure

qPCR result validation

Plate

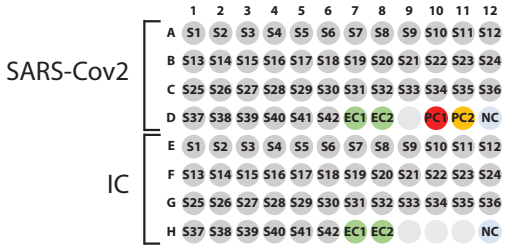
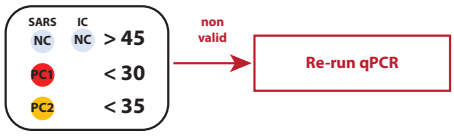
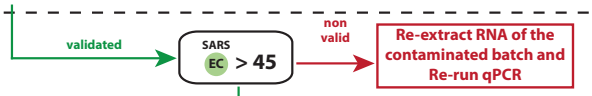


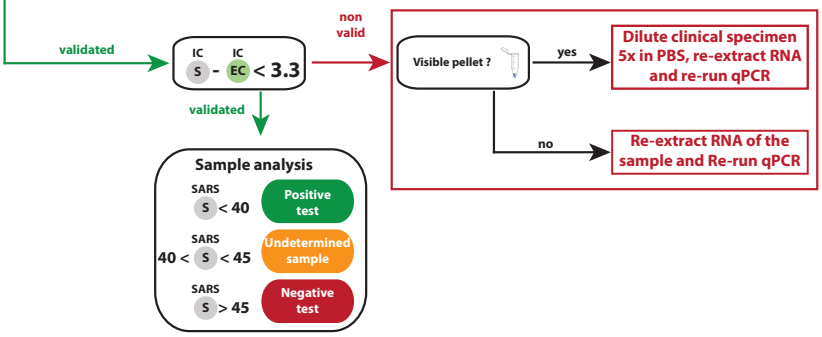
Plate validation



Extraction batch validation

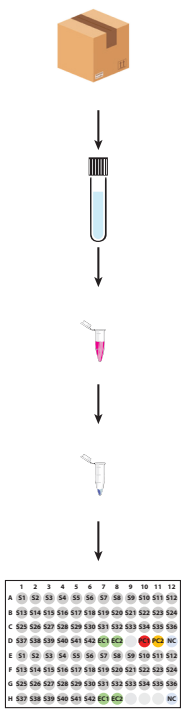


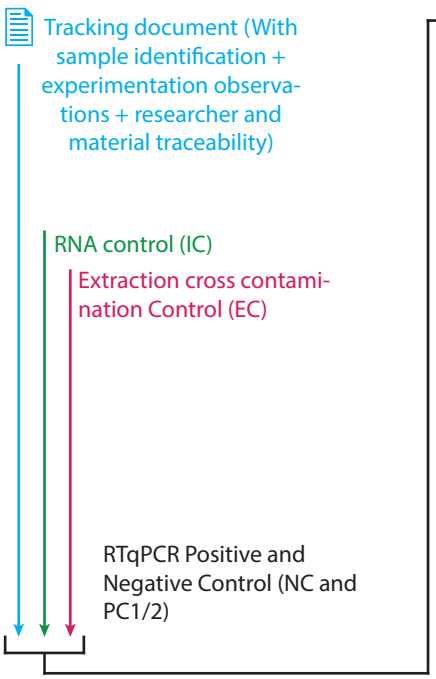
Sample validation

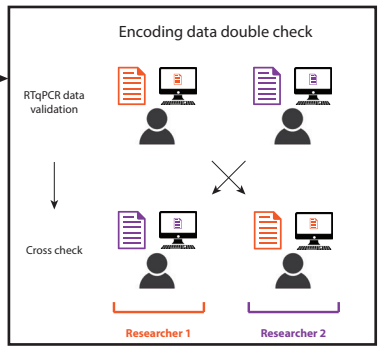


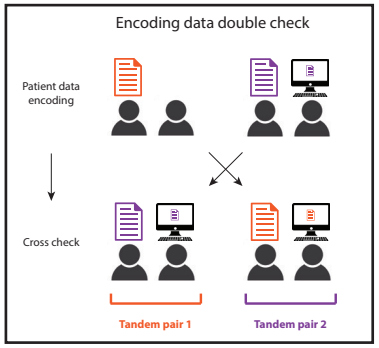
Quality check

Process steps





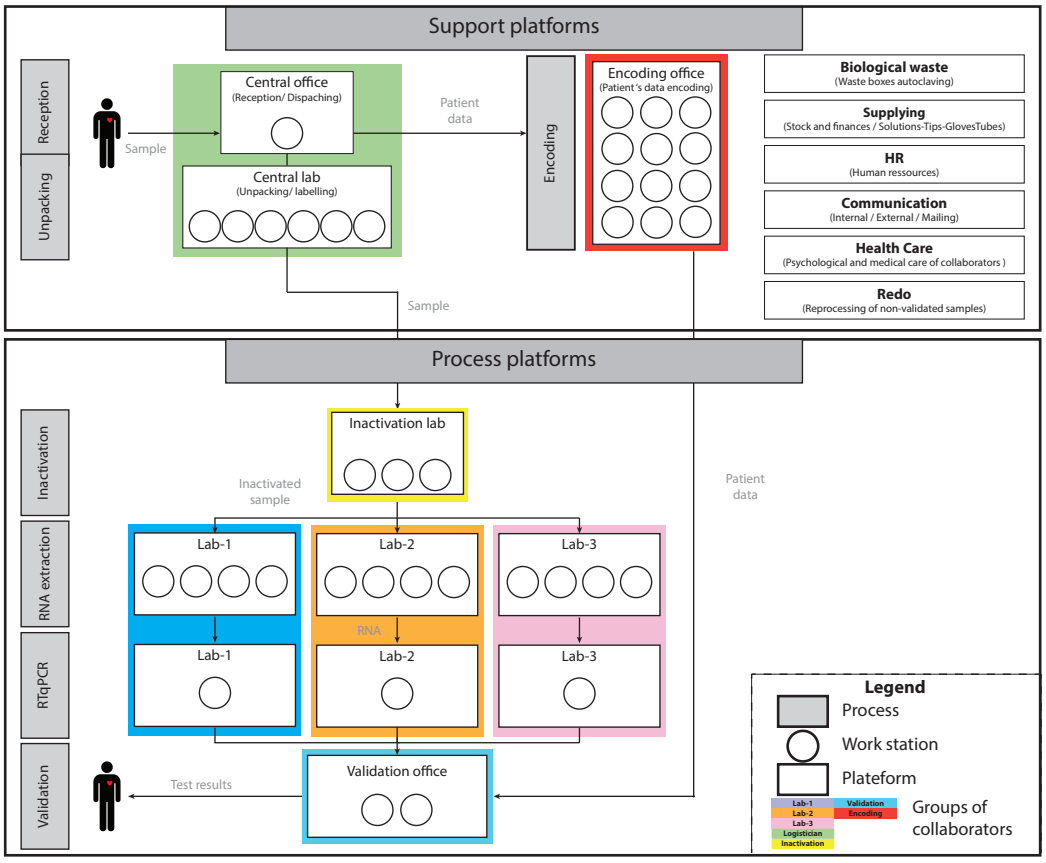


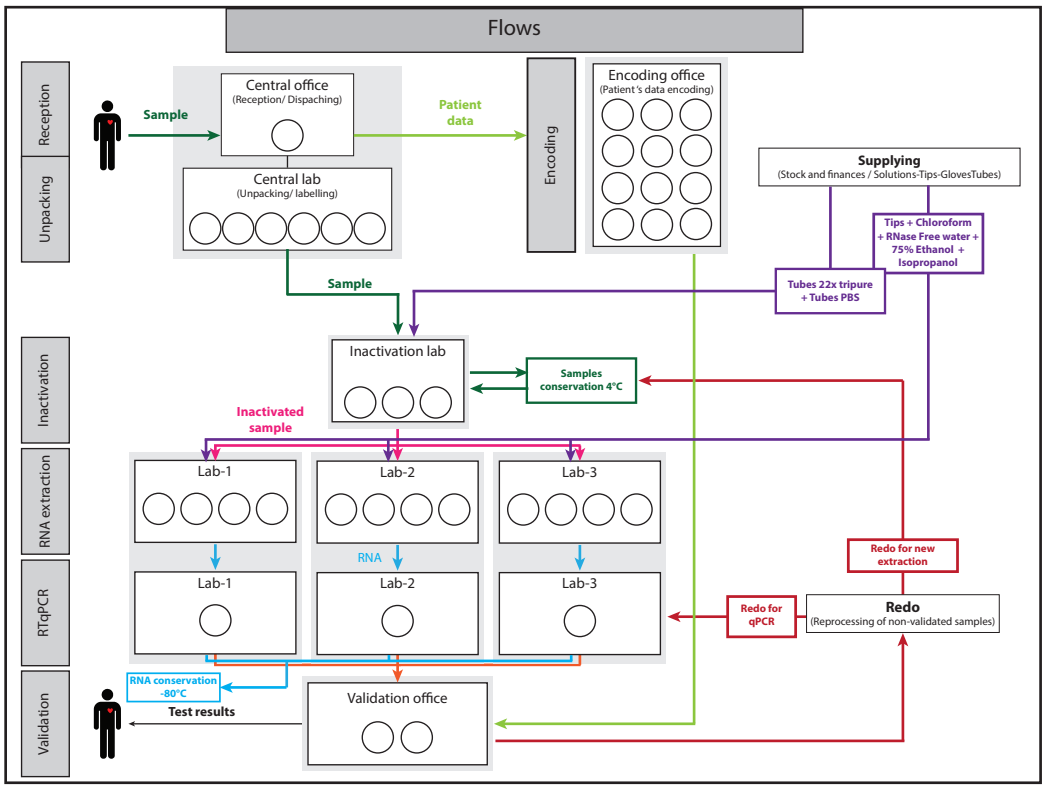


Starting pack

Project structure

The following structure has been established at the UNAMUR university to analyze 500 samples / day. This process encompasses sample reception and result encoding in the belgian public health platform. It is presented here as an example of the process. Each circle corresponds to one workbench inside a platform. Each platform is dedicated to a defined process. RNA extraction and RTqPCR steps need several platforms due to the human and material resource requirement.





Reception

Ensure tracking of intrance samples by intern ID assignation and edit tracability document

Unpacking

Remove patient sample from its bag in safe condition

Inactivation

Open sample tubes in safe condition and add 100µL in Guanidinium Thiocyanate Solution to ensure virus inactivation.

RNA extraction

Extract RNA by guanidinium thiocya-nate-phenol-chloroform method. This step requires numerous of molecular biology researchers.

RTqPCR

Preparation of RTqPCR mix and plate preparation. RTqPCR assays. To prevent any cross contamination the mix is prepared in a fully isolated room.

Encoding

Ensure patient data file implementation and link between patient and intern ID number.

Validation

Analyze RTqPCR data. Results are validated based on double check.

Starting pack

Safety advices and sample preservation

Process steps

Protective equipment

Sample preservation

Unpacking — CLASS II Biological Safety Cabinet

Inactivation — CLASS II Biological Safety Cabinet

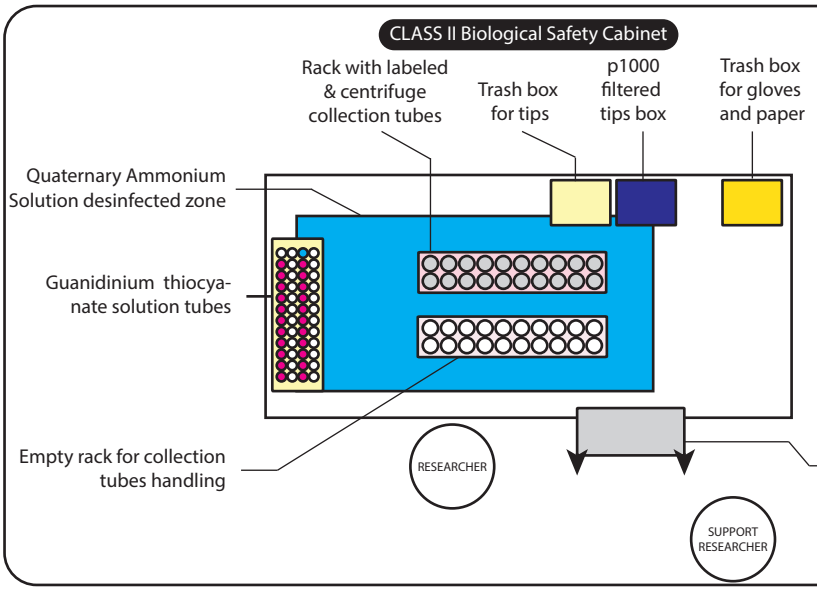
RNA extraction — Chemical Hood

RTqPCR

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
C	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36
D	S37	S38	S39	S40	S41	S42	EC1	EC2		PC1	PC2	NC
E	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
F	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
G	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36
H	S37	S38	S39	S40	S41	S42	EC1	EC2				NC

- Keep patient sample as much as possible at 4°C
- Inactivated samples can be kept at -80°C freezer
- Perform RNA extraction in RNAse free conditions
- Store extracted RNA at 4°C before RTqPCR and next at -80°C for long time conservation

CLASS II Biological Safety Cabinet



CLASS II BSF working dress