Protocol for Real-Time RT-PCR SARS-CoV-2 (2019-nCoV) (RdRp IP4/Institut Pasteur) with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-QSY

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit , ThermoFischer ref 11732-088
- Elution Buffer AE (1000 ml) Macherey-Nagel™ ref 740917.1 (This component is not provided with the OneStep RT-PCR kit); usually provided as elution buffer in the extraction kit, (10mM TrisHCl 0.5mM EDTA, pH=9).

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening.
- Lyoph-P&P is respudended as described:
  - Add 182 µl of AE Elution buffer
  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 50µL-volume
  - Rehydrated P&P must be incubated at room temperature for 10 min after which
  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Table 1. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 14 days

Number of tests/vial	24
AE Elution buffer (µL)	182*

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. Preparation of the reaction MIX

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12.5
Rehydrated Primers and probe P&P**	7.0
SSIII/Taq EnzymeMix*	0.5
	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase
**, as indicated in Table 1.

3. Cycling program and RT-PCR reaction

1: 50°C for 15 min
2: 95°C for 2 min
3: 95°C for 15 sec
4: 58°C for 45 sec
Plate Read
5: GOTO 3, 44 more times

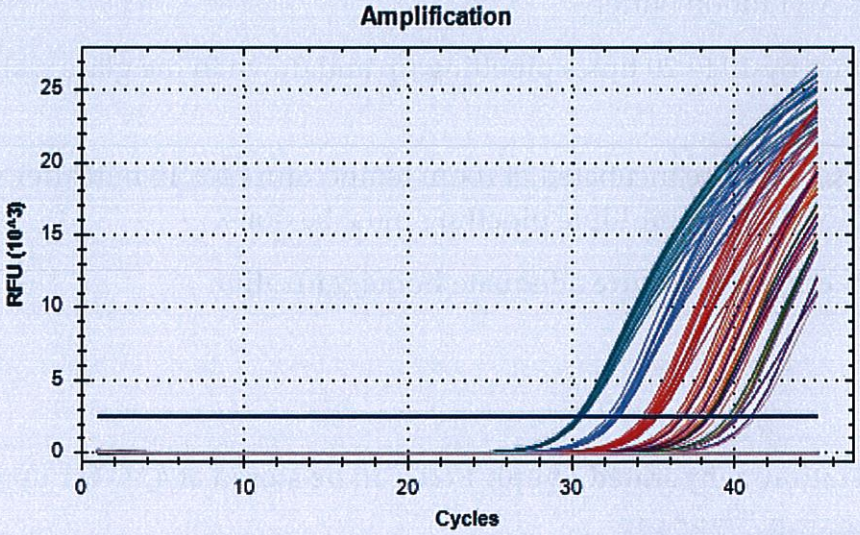

Figure. Results obtained with 5-fold serial dilutions of positive control.

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CERTIFICATE OF PARTICIPATION

awarded to

Unité des virus émergents, Aix-Marseille University, France

to attest completion of ERLI-Net & EVD-LabNet

EXTERNAL QUALITY ASSESSMENT SCHEME FOR

MOLECULAR DIAGNOSTICS OF SARS-COV-2, JULY 2020

Covering the following:

- Accurate detection of SARS-CoV-2 in 12 coded and inactivated samples

Organised by:
European Centre for Disease Prevention and Control
Mike Catchpole, Chief Scientist

Performed under ECDC/2017/002 specific contract 3 by:
National Institute for Public Health and the Environment – The Netherlands (RIVM)
Chantal Reusken, coordinator EQA and EVD-LabNet

Charité – Berlin (CUB)
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