

# THE LANCET

## Respiratory Medicine

### Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed.  
We post it as supplied by the authors.

Supplement to: Ceulemans LJ, Khan M, Yoo S-J, et al. Persistence of SARS-CoV-2 RNA in lung tissue after mild COVID-19. *Lancet Respir Med* 2021; published online June 9. [http://dx.doi.org/10.1016/S2213-2600\(21\)00240-X](http://dx.doi.org/10.1016/S2213-2600(21)00240-X).

## *Appendix*

### **Methodology on single-molecule fluorescence RNA in situ hybridisation.**

Fluorescence RNA in-situ hybridisation was performed using the RNAscope manual assay with the Multiplex Fluorescent Detection Kit v2 (Bio-Techne, Cat# 323110) according to the manufacturer's protocols. Briefly, FFPE sections were deparaffinised in xylene and dehydrated in a series of ethanol steps. Tissue was pretreated with hydrogen peroxide, followed by permeabilisation in target retrieval reagent (Bio-Techne, Cat# 322000) at ~99°C for 15 min in a steamer. Tissue was then digested in Protease Plus (Bio-Techne, Cat# 322330) at 40°C for 20 min. RNAscope probes were V-nCoV-N (Bio-Techne, Cat# 846081), V-nCoV-N-C2 (Bio-Techne, Cat# 846081-C2), V-nCoV2019-S-C2 (Bio-Techne, Cat# 848561-C2), and BSG (Bio-Techne, Cat# 472721). Probes were hybridised at 40°C for 2 hr. Signal amplification was performed accordingly, followed by development of appropriate HRP channels with dyes Opal 520 (Akoya Biosciences, Cat# FP1487001KT), Opal 570 (Akoya Biosciences, Cat# FP1488001KT) and Opal 690 (Akoya Biosciences, Cat# FP1497001KT). Nuclear staining was done with DAPI (Invitrogen, Cat# D1306). Slides were mounted in abberior MOUNT Solid Antifade (abberior GmbH). Confocal images were taken with a Zeiss LSM 800.

## **Clinical case summary of the patient who died with acute COVID-19, 5 days after positive qRT-PCR result from a nasopharyngeal swab.**

An 89-year-old male patient presented to the emergency department after four days of fever, coughing, nausea and vomiting.

Nasopharyngeal swab confirmed the diagnosis of COVID-19. Chest computed tomography revealed bilateral ground-glass opacities and crazy paving pattern. The patient was an active smoker with arterial hypertension, diabetes, coronary artery disease and atrial flutter in his medical history. Due to late onset rheumatoid arthritis the patient was treated with chronic low dose methylprednisolone.

Two days after admission, the patient was transferred to the intensive care unit. Supportive therapy included remdesivir, antibiotics, low-molecular weight heparin, steroids and adequate fluid management. Despite maximal non-invasive ventilatory support and prone positioning, the patient deteriorated quickly due to hypoxemia.

Due to his age and comorbidities, the patient was not intubated. Five days after hospital admission and nine days after onset of symptoms the patient died due to COVID-19 induced respiratory failure.