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**Supplementary Data**

**Similar aerosol emission rates and viral loads in upper respiratory tracts for COVID-19 patients with Delta and Omicron variant infection**

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**Methods**

**1. Initial screening of SARS-CoV-2 in the exhaled breath condensates**

SARS-CoV-2 in the exhaled breath condensate (EBC) was initially detected with a HONGSHI SLAN-96S Real-Time PCR Detection System (Shanghai Hongshi Medical Technology Co., LTD, Shanghai, China) by using a commercial kit (Liferiver, Shanghai ZJ Bio-Tech Co., Ltd, Shanghai, China). The cycling program was performed as followed: 45 °C for 10 min, 95 °C for 3 min, and 45 cycles at 95 °C for 15 s and 58 °C for 30 s.

**2. Quantitative method by using SARS-CoV-2 RNA reference material**

The viral load in all samples were further confirmed by using standard curve based on SARS-CoV-2 RNA reference material containing *ORF1ab*, *N* and *E* genes (provided by China National Institute of Metrology.

Each absolute quantitative experiment requires SARS-CoV-2 RNA reference material with concentrations of 103 copies/mL, 104 copies/mL, 105 copies/mL, 106 copies/mL, 107 copies/mL, 108 copies/mL. A standard curve containing the linear relationship between virus concentration and Ct values was drawn in each round of experiment. At the same time, the Ct value of EBC obtained by each nucleic acid amplification was substituted into the standard curve to calculate the viral concentration of EBCs. In this study, the corresponding relationship between the concentration of standard substance and Ct value (Supplementary Table S2), the standard curve (Supplementary Fig. S1), and the original Ct value and the calculation results (Supplementary Table S3 and Table S4) are described as followed.

**3 Calculation of** **the breath emission rate**

The exhaled breath in 5 min was collected and 800 µL exhaled breath condensate (EBC) was collected form each patient. Then 200 µL EBC was used for RNA extraction and finally got 50 µL RNA. A total of 4 µL RNA was used for reverse transcription reaction and got 20 µL of reverse transcription product (cDNA). Then the cDNA was further quantified by using standard plasmid containing spike gene of SARS-CoV-2. The breath emission rate of each patient was calculated by the following equation:

Breath emission rate (viruses/hour) = CcDNA×VcDNA×(VRNA/VRNA for reverse)×(VEBC/VEBC for RNA Extraction)×(60 min/TEBC)

Concentration in exhaled air (copies/m3) = Breath emission rate/Breathing rate

CcDNA: the concentration of cDNA (copies/µL); VcDNA: the overall volume of cDNA; VRNA: the overall volume of RNA; VRNA for reverse: the volume of RNA used for reverse transcription reaction; VEBC: the overall volume of EBC; VEBC for RNA Extraction: the volume of EBC used for RNA extraction; TEBC: the time for EBC collection.



Figure S1. Standard curve in the absolute quantitative experiment for EBCs from the patients.

Table S1. The characteristics of patients with COVID-19.

Table S2. Relationship between standard substance concentration and Ct value in the absolute quantitative experiment for EBCs from the patients.

|  |  |  |
| --- | --- | --- |
| *N* gene reference material (Copies/mL) | *N* gene Ct values of reference material | Average *N* gene Ct values of reference material |
| 103 | 39.11 | 38.32 | 38.86 | 38.76 |
| 104 | 35.47 | 34.68 | 36.44 | 35.53 |
| 105 | 38.33 | 31.53 | 31.42 | 33.76 |
| 106 | 28.21 | 28.46 | 35.35 | 30.67 |
| 107 | 32.07 | 24.38 | 26.78 | 27.74 |
| 108 | 28.63 | 20.79 | 28.3 | 25.9 |

Table S3. Original Ct value and the calculation results in the absolute quantitative experiment for EBCs from patient with Delta infection.

|  |  |  |  |
| --- | --- | --- | --- |
| Patient ID | Ct values of EBCs for *N* gene | Absolute quantitative value (Copies/μL) | Average absolute quantitative value (Copies/μL) |
| 3 | 36.09 | 9.05 | 617.89 |
| 11 | 39.4 | 0.49 |
| 18 | 33.53 | 86.75 |
| 19 | 39.68 | 0.38 |
| 25 | 29.52 | 2992.82 |

Table S4. Original Ct value and the calculation results in the absolute quantitative experiment for EBCs from patient with Omicron infection.

|  |  |  |  |
| --- | --- | --- | --- |
| Patient ID | Ct values of EBCs for *N* gene | Absolute quantitative value (Copies/μL) | Average absolute quantitative value (Copies/μL) |
| 1 | 35.72 | 12.54 | 58.52 |
| 3 | 34.92 | 25.42 |
| 6 | 33.32 | 104.42 |
| 7 | 33.14 | 122.41 |
| 8 | 37.51 | 2.58 |
| 12 | 40.46 | 0.19 |
| 34 | 40.43 | 0.20 |
| 36 | 33.57 | 83.73 |
| 37 | 40.3 | 0.22 |
| 40 | 40.61 | 0.17 |