Supporting Information

for

An evaluation of RT-qPCR primer-probe sets to inform public health interventions based on COVID-19 sewage tests

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References
Supplementary Methods

RT-qPCR primer-probe sets.
All sequences of primers and probes were synthesized by BGI (Hong Kong) using PAGE and HPLC purification, respectively. The specific sequences of primer-probe sets were listed in Table S2, respectively. Four out of the seven sets target at the N gene, two sets target at the ORF1 gene, and the other one targets at the E gene. All synthesized oligonucleotides were dissolved in DEPC-treated water prior to use.

One-step RT-qPCR conditions.
We used the same RT-qPCR reagents and recommended annealing temperatures in the present study. For RT-qPCR, TaqMan Fast Virus 1-step Master Mix (ThermoFisher, USA) was used with 4 µl of RNA template and standardized primers and probes with concentrations of 500 nM and 250 nM, respectively. PCR conditions were reverse transcribed for 5 mins at 50°C and initial denaturation for 20 s at 95°C, followed by 45 cycles of 5 s at 95°C and 30 s at specific annealing temperature (Table S2) on the Applied Biosystems ViiA7 qPCR machine (ThermoFisher). For every batch of RT-qPCR detection assays, a non-template control (NTC) was included.

We also tested the performance of iTaq one-step RT-qPCR kit (Bio-Rad) by N1 set using conditions recommended by the manufacturer as follows: reverse transcribed for 10 mins at 50°C and initial denaturation for 20 s at 95°C, followed by 45 cycles of 5 s at 95°C and 30 s at the 55°C.

Detective efficiency using different primer-probe sets for stored samples.
To evaluate the stability and persistence of SARS-CoV-2 virus RNA in sewage samples stored at ambient temperature (25°C), 250 µL heat-inactivated SARS-CoV-2 virus obtained from cell culture was spiked into 750 mL sewage collected from a local sewage treatment plant. The decay experiment was conducted in triplicate with a starting viral concentration of $10^7$ copies per L. Triplicate sub-samples of 30 mL taken on specific time points (Day 0, 1, 2, 12, 18, 25, 47 and 69) were concentrated and extracted RNA using same methods as above for determination of the detective efficiency using different primer-probe sets.

Diagnostic evaluation using SARS-CoV-2 virus spiked sewage samples.
For diagnostic evaluation, we also used two RNA extracted from SARS-CoV-2 virus spiked sewage samples. The SARS-CoV-2 virus isolation was performed as previously described and heat-inactivated at 56°C for 30 mins. Two SARS-CoV-2 virus spiked sewage samples were obtained by spiking 100 µL inactivated virus with lower concentration (~$10^6$ and $10^7$ copies per µL, respectively) with 900 µL negative sewage samples. RNA samples were respectively extracted from 100 µL SARS-CoV-2 virus spiked sewage samples using Trizol Plus RNA purification kit.
Table S1. Summary of primer-probe sets used in the references.

| Primer-probe Sets          | The Number of Reference |
|----------------------------|-------------------------|
| N1                         | 76                      |
| N2                         | 66                      |
| E                          | 30                      |
| RdRp                       | 16                      |
| N3                         | 12                      |
| ORF1ab                     | 7                       |
| NIID_2019-nCoV_N           | 5                       |
| CCDC-N                     | 4                       |
| N_Sarbeco                  | 3                       |
| HKU-N                      | 2                       |
| HKU-ORF1b                  | 1                       |
| ORF1a                      | 1                       |
| RBD2                       | 1                       |

Note: 1. Total reference number is 112; 2. Multiple sets may be used in one reference.

We retrieved 112 (68 journal papers and 44 preprints) published experimental studies on sewage testing for SARS-CoV-2 from the PubMed database using the combinations of a few keywords, including "sewage + COVID-19", "sewage + SARS-CoV-2", "wastewater + COVID-19", "wastewater + SARS-CoV-2", "waste water + COVID-19", and "waste water + SARS-CoV-2", from the first publication on April 9, 2020 to the most recent one on July 2, 2021. We found that 105 out of these 112 studies (94%) used at least one out of the seven primers we evaluated in our study. Data used to make this table can be found in Source Data Table S1.
Table S2. Primer-probe sets used in this study.

| Target | Country/Region | Name          | Anneling temperature (℃) | Sequence (5’-3’)                                      | Position       | Organization |
|--------|----------------|---------------|--------------------------|-------------------------------------------------------|----------------|--------------|
| N      | USA            | N1-F          | 55                       | GACCCCCAAAATCAGCGAAAT                                 | 28,287-28,306  | US CDC       |
|        |                | N1-R          |                          | TCTGGTACTGCAATTTGAAATCTG                              | 28,335-28,358  |              |
|        |                | N1-P          |                          | FAM-ACCCCGCATATACGGTGTGGACC-BHQ1                      | 28,309-28,332  |              |
|        |                | N2-F          | 55                       | TTACAAACATTGACGCCCAA                                 | 29,164-29,183  |              |
|        |                | N2-R          |                          | GCGCGACATTGCAAGAA                                     | 29,213-29,230  |              |
|        |                | N2-P          |                          | FAM-ACAATTGGCAATTTGACGCTTCAG-BHQ1                     | 29,188-29,210  |              |
|        | Hong Kong      | N3-F          |                          | GGGAGCTTGAATACCAAAAA                                  | 28,681-28,702  |              |
|        |                | N3-R          | 55                       | TGTAGCAGGATTGACGATTG                                  | 28,732-28,752  |              |
|        |                | N3-P          |                          | FAM-AYCACATTGGAACACCCGCAATCCTG-BHQ1                   | 28,704-28,727  |              |
|        |                | HKU-N-F       | 58                       | TAATCAGACAAAGGAACTGATA                                | 29,145-29,166  | HKU          |
|        |                | HKU-N-R       |                          | CGAAGGTGTCACCTTCAATG                                  | 29,235-29,254  |              |
|        |                | HKU-N-P       |                          | FAM-GCAATTGGAACATTTGACGCGG-BHQ1                       | 29,177-29,196  |              |
| E      | Germany        | E-F           |                          | ACAGGTACGTTAAATAGTTAATACGCT                         | 26,266-26,291  | Charité       |
|        |                | E-R           | 55                       | ATATTGAGCAGATAGCACACA                                  | 26,357-26,378  |              |
|        |                | E-P           |                          | FAM-AACATGACCCATCCTACTGCACGCTCC-BHQ1                  | 26,329-26,354  |              |
| ORF1   | China          | ORF1ab-F      | 58                       | CCGTCTGGGTTTTTACACCTA                                 | 13,342-13,362  | China CDC    |
|        |                | ORF1ab-R      |                          | ACGATTTGCTCAGCTAGCTGA                                 | 13,442-13,460  |              |
|        |                | ORF1ab-P      |                          | FAM-CCTGTGCTGGTGAAGTGGTGCTGATGGGTTACGCTGCCT          | 13,377-13,404  |              |
|        | Hong Kong      | HKU-ORF1b-F   | 58                       | TGGGGTTTTAACGTTACCT                                   | 18,778-18,797  | HKU          |
|        |                | HKU-ORF1b-R   |                          | AACRCGGCTAACAACAGCCTC                                  | 18,889-18,909  |              |
|        |                | HKU-ORF1b-P   | 58                       | FAM-TAGTTGTGATGCWATCATGACTAG-BHQ1                      | 18,849-18,872  |              |
### Table S3. Matrix effect (%) in small-volume samples.

| Viral load (Log 10 copy/µL) | N1 | N2 | N3 | E | HKU-N | ORF1ab | HKU-ORF1b |
|----------------------------|----|----|----|---|-------|--------|-----------|
| 4.8                        | -11| 27 | 17 | 13| 20    | 24     | -3        |
| 3.8                        | 5  | 42 | 10 | -7| 26    | 34     | 1         |
| 2.8                        | -1 | 36 | 6  | 24| 19    | 27     | 2         |
| 1.8                        | 4  | 27 | -3 | -4| 13    | 41     | -15       |
| 0.8                        | 19 | 35 | -15| -10|4     | 31     | 31        |

### Table S4. Matrix effect (%) in large-volume samples.

| Viral load (Log 10 copy/µL) | N1 | N2 | N3 | E | HKU-N | ORF1ab | HKU-ORF1b |
|----------------------------|----|----|----|---|-------|--------|-----------|
| 5.3                        | -79| -94| -87| -88| -85   | -81    | -91       |
| 4.3                        | -78| -94| -87| -89| -83   | -77    | -88       |
| 3.3                        | -72| -93| -83| -87| -81   | -64    | -88       |
| 2.3                        | -70| -92| -82| -88| -72   | -20    | -82       |
| 1.3                        | -69| -91| -91| -100| -75  | -45    | -89       |
| 0.3                        | -45| -9 | -96| /  | /     | /      | /         |

### Table S5. Decay rate constants measured using seven primer-probe sets.

|          | \( K_{\text{mean}} \) (day\(^{-1}\)) | 95% CI                  | \( R^2 \)   |
|----------|----------------------------------------|-------------------------|-------------|
| N1       | 1.28344                                | 1.27991 to 1.28698      | 1.0000      |
| N2       | 1.43151                                | 1.35628 to 1.50674      | 0.9995      |
| N3       | 1.228                                  | 1.215 to 1.240          | 1.0000      |
| E        | 1.008                                  | 0.9747 to 1.042         | 0.9997      |
| HKU-N    | 1.304                                  | 1.259 to 1.352          | 0.9997      |
| ORF1ab   | 1.01907                                | /                       | 0.9952      |
| HKU-ORF1b| 1.012                                  | 1.011 to 1.013          | 1.0000      |

\( K_{\text{mean}} \) (day\(^{-1}\)): decay rate was estimated by one-phase decay model
95% CI: 95% confidence intervals
| Positive pool sample No. | Sample name | Sampling date | Population size | Total population size | Case number | Total correlated case number |
|--------------------------|-------------|---------------|-----------------|-----------------------|-------------|----------------------------|
| Sample 1                | YT-1        | 2021/1/20     | 357             | 1071                  | 10          | 30                         |
| Sample 1                | YT-2        | 2021/1/19     | 357             | 1071                  | 10          |                            |
| Sample 1                | YT-3        | 2021/1/21     | 357             | 1071                  | 10          |                            |
| Sample 2                | YT-4        | 2021/1/19     | 228             | 586                   | 4           | 20                         |
| Sample 2                | YT-5        | 2021/1/21     | 227             | 586                   | 2           |                            |
| Sample 2                | RS5-4       | 2021/1/19     | 131             | 586                   | 14          |                            |
| Sample 3                | YT 010      | 2021/1/20     | 337             | 1981                  | 1           | 4                          |
| Sample 3                | YT 018      | 2021/1/21     | 358             | 1981                  | 1           |                            |
| Sample 3                | YT 001      | 2021/1/19     | 1286            | 1981                  | 2           |                            |
| Sample 4                | RS5-1       | 2021/1/16     | 131             | 423                   | 10          | 25                         |
| Sample 4                | RS4-1       | 2021/1/18     | 292             | 423                   | 15          |                            |
| Sample 5                | RS6-1       | 2021/1/18     | 329             | 958                   | 15          | 32                         |
| Sample 5                | RS4-2       | 2021/1/17     | 292             | 958                   | 14          |                            |
| Sample 5                | YT 010      | 2021/1/21     | 337             | 958                   | 3           |                            |
| Sample 6                | KTS2        | 2020/12/21    | 207836          | 465448                | 16          | 76                         |
| Sample 6                | C&W site 1A | 2021/3/12     | 49776           | 465448                | 16          |                            |
| Sample 7                | KTS1        | 2020/12/22    | 207836          |                       | 30          |                            |
| Sample 7                | RS6-2       | 2021/1/19     | 329             | 752                   | 14          | 42                         |
| Sample 7                | RS4-3       | 2021/1/19     | 292             | 752                   | 14          |                            |
| Sample 7                | RS5-2       | 2021/1/17     | 131             | 752                   | 14          |                            |
| Sample 8                | YT 006-1    | 2021/1/20     | 961             | 2883                  | 3           | 9                          |
| Sample 8                | YT 006-2    | 2021/1/19     | 961             | 2883                  | 3           |                            |
| Sample 8                | YT 006-3    | 2021/1/21     | 961             | 2883                  | 3           |                            |
| Sample 9                | YT008       | 2021/1/19     | 616             | 1221                  | 4           | 9                          |
| Sample 9                | YT007       | 2021/1/20     | 228             | 1221                  | 3           |                            |
| Sample 9                | YT020       | 2021/1/19     | 377             | 1221                  | 2           |                            |
| Sample 10               | YTM 048-1   | 2021/1/24     | 3494            | 9620                  | 7           | 15                         |
| Sample 10               | YTM 048-2   | 2021/1/25     | 3494            | 9620                  | 7           |                            |
| Sample 10               | YTM 049     | 2021/1/24     | 2632            |                       | 1           |                            |
| Sample 10               | KLC2        | 2020/12/22    | 149017          | 341580                | 28          | 34                         |
| Sample 11 | KT site 5A | 2021/3/1 | 57861 | 5 |
|-----------|------------|----------|-------|---|
| WTSS1     | 2021/2/15  | 134702   | 1     |   |
| Sample 12 |            |          |       |   |
| SPW1      | 2021/1/12  | 416      | 2     |   |
| SW        | 2021/1/2   | 2421     | 2     |   |
| TT1       | 2021/1/1   | 1436     | 2     |   |
| Sample 13 |            |          |       |   |
| TMP       | 2020/12/24 | 115643   | 7     |   |
| WTSS2     | 2021/3/1   | 134702   | 1     |   |
| KC site 2A| 2021/3/11  | 57756    | 1     |   |
| Sample 14 |            |          |       |   |
| YTM 065   | 2021/1/29  | 718      | 2     |   |
| YTM 070   | 2021/1/29  | 799      | 1     |   |
| YTM 016   | 2021/1/21  | 175      | 1     |   |
| Sample 15 | RS6-3      | 2021/1/21| 329   | 19|
| YTM 110   | 2021/2/12  | 239      | 947   | 1 |
| CW2       | 2021/1/12  | 379      | 1     |   |
| Sample 16 | PS7        | 2021/1/12| 2234  | 3 |
| PS2       | 2021/1/12  | 4460     | 50529 | 3 |
| KT5       | 2021/2/15  | 43835    |       |   |
| Sample 17 | YTM 047    | 2021/1/25| 3231  | 4 |
| CW 004    | 2021/2/13  | 301      | 5358  | 4 |
| KC 006    | 2021/1/25  | 1826     |       |   |
| Sample 21 | KC 002     | 2021/1/24| 590   | 1530|
| YT 004    | 2021/1/19  | 940      |       |   |
| Sample 22 | YT 018     | 2021/1/19| 358   | 735 |
| YT 020    | 2021/1/20  | 377      |       |   |
| Sample 23 | Virus spiked sewage-1 | | | |
| Sample 24 | Virus spiked sewage-2 | | | |
| Sample 25 | RS5-3      | 2021/1/18| 131   | 423 |
| RS4-4     | 2021/1/16  | 292      |       |   |
| Negative pool sample No. | Sample name | Sampling date | Population size | Total population size | Case number | Total correlated case number |
|-------------------------|-------------|---------------|-----------------|-----------------------|-------------|-----------------------------|
| Sample 1                | C&W-005     | 2021/2/16     | 380             | 1374                  | 0           | 0                           |
|                         | C&W-006     | 2021/2/16     | 428             |                       | 0           |                             |
|                         | C&W-007     | 2021/2/16     | 566             |                       | 0           |                             |
| Sample 2                | C&W-008     | 2021/2/16     | 391             | 0                     | 0           |                             |
|                         | C&W-009     | 2021/2/28     | 851             | 1601                  | 0           | 0                           |
|                         | C&W-012     | 2021/2/28     | 359             |                       | 0           |                             |
| Sample 3                | E-003       | 2021/2/2      | 9860            | 14463                 | 0           | 0                           |
|                         | E-004       | 2021/2/2      | 1610            |                       | 0           |                             |
|                         | E-007       | 2021/2/2      | 2993            |                       | 0           |                             |
| Sample 4                | E-008       | 2021/2/2      | 3552            | 0                     | 0           |                             |
|                         | E-009       | 2021/2/2      | 5485            |                       | 0           |                             |
|                         | E-011       | 2021/2/4      | 211             |                       | 0           |                             |
| Sample 5                | E-012       | 2021/2/4      | 1136            | 3770                  | 0           | 0                           |
|                         | E-013       | 2021/2/4      | 666             |                       | 0           |                             |
|                         | E-014       | 2021/2/4      | 1968            |                       | 0           |                             |
| Sample 6                | E-020       | 2021/2/10     | 496             | 5226                  | 0           | 0                           |
|                         | E-021       | 2021/2/10     | 825             |                       | 0           |                             |
|                         | E-022       | 2021/2/10     | 3905            |                       | 0           |                             |
| Sample 7                | E-023       | 2021/2/10     | 605             | 4759                  | 0           | 0                           |
|                         | E-024       | 2021/2/10     | 3347            |                       | 0           |                             |
|                         | E-026       | 2021/2/12     | 807             |                       | 0           |                             |
| Sample 8                | E-027       | 2021/2/12     | 1162            | 2798                  | 0           | 0                           |
|                         | E-028       | 2021/2/12     | 859             |                       | 0           |                             |
|                         | E-029       | 2021/2/12     | 777             |                       | 0           |                             |
| Sample 9                | E-005       | 2021/2/1      | 488             | 1846                  | 0           | 0                           |
|                         | E-034       | 2021/2/15     | 340             |                       | 0           |                             |
|                         | FTE8        | 2021/2/17     | 1018            |                       | 0           |                             |
| Sample 10               | KC-007      | 2021/1/26     | 962             | 2545                  | 0           | 0                           |
|                         | KC-013      | 2021/1/26     | 983             |                       | 0           |                             |
|                         | KC-015      | 2021/1/26     | 600             |                       | 0           |                             |
| Sample 11               | WTS-020     | 2021/2/26     | 32310           | 33453                 | 0           | 0                           |
|                         | YT-021      | 2021/1/21     | 551             |                       | 0           |                             |
| Sample 12 | YTM-030 | 2021/1/22 | 592 | 0 | 0 |
|-----------|---------|-----------|-----|---|---|
| KT-010    | 2021/2/1| 955       |     |   | 0 |
| SSP-009   | 2021/1/24| 415      |     |   | 0 |
| TC5C      | 2021/2/22| 2601     |     |   | 0 |
| Sample 13 | YL-001  | 2021/1/29| 145 |   | 0 |
| YL-008    | 2021/2/14| 114      |     |   | 0 |
| YTM       | 2021/2/24| 795      |     |   | 0 |
### Table S7. Comparative analysis of Ct value for positive pooled samples

| No. | Population | Correlated case number | N1 Ct_1 | N2 Ct_1 | N3 Ct_1 | E Ct_1 | HKU-N Ct_1 | HKU-ORF1ab Ct_1 | ORF1b Ct_1 | N1 Ct_2 | N2 Ct_2 | N3 Ct_2 | E Ct_2 | HKU-N Ct_2 | HKU-ORF1ab Ct_2 | ORF1b Ct_2 |
|-----|------------|------------------------|---------|---------|---------|--------|-----------|----------------|-----------|---------|---------|---------|--------|-----------|----------------|-----------|
| 1   | 1,071      | 30                     | 37.1    |       | 37.1    |        |           | 40.1 | 41.4    |        |         |         |        |          |                          |           |
| 2   | 586        | 20                     |       |       | 35.6    | 37.1   |           |        |         |        |         |         |        |          |                          |           |
| 3   | 1,981      | 4                      | 36.8    |       |         | 39.9    |           | 36.5 | 37.0    |        |         |         |        |          |                          |           |
| 4   | 423        | 25                     | 30.8    | 30.8   | 33.3    | 33.9   | 31.6    | 31.7   | 31.8   | 31.8   | 33.6   | 36.5   | 37.0    | 31.3 | 31.5    |                          |           |
| 5   | 621        | 32                     |       |       |         | 39.9    |           |        |         |        |         |         |        |          |                          |           |
| 6   | 465,448    | 76                     | 32.0    | 32.8   | 34.7    | 34.8   | 32.6    | 32.8   | 33.2   | 33.7   | 36.3   | 38.6   |        | 33.4 | 34.3    |                          |           |
| 7   | 752        | 42                     |       |       |         | 38.5    |           |        |         |        |         |         |        |          |                          |           |
| 8   | 2,883      | 9                      |       |       |         | 38.9    |           |        |         |        |         |         |        |          |                          |           |
| 9   | 1,221      | 9                      |       |       |         | 39.2    |           |        |         |        |         |         |        |          |                          |           |
| 10  | 9,620      | 15                     | 32.9    | 33.4   | 35.6    | 36.6   | 33.8    | 34.0   | 34.8   |        | 37.9   | 39.2   |        | 33.5 | 35.4    |                          |           |
| 11  | 341,580    | 34                     |       |       | 38.7    |         |           |        |         |        |         |         |        |          |                          |           |
| 12  | 1,852      | 6                      |       |       | 34.3    | 35.5   | 39.3    |        | 36.2   | 36.3   |        | 40.5   |        | 38.2 |         |                          |           |
| 13  | 308,101    | 2                      |       |       |         | 39.6    |           |        | 39.8   |        |        |        |        |          |                          |           |
| 14  | 1,692      | 4                      | 36.9    |       |         | 38.4    |           |        |         |        |        |        |        |          |                          |           |
| 15  | 947        | 21                     |       |       | 35.9    | 36.5   | 39.8    |        | 37.5   |        |        |        |        |          |                          |           |
| 16  | 50,529     | 7                      |       |       | 36.4    | 38.9   | 39.1    | 37.5   |        |        |        |        |        |          |                          |           |
| 17  | 4,745      | 3                      | 33.9    | 35.8   | 36.8    | 36.8   | 34.8    | 35.1   |        |        |        |        |        |          |                          |           |
| 18  | 57,756     | 28                     | 32.0    | 32.5   | 34.5    | 34.7   | 32.9    | 32.9   | 33.2   | 33.3   | 36.0   | 36.1   |        |        |          |                          |           |
| 19  | 43,835     | 2                      | 34.9    | 35.8   | 36.8    | 38.7   | 36.1    | 37.2   | 37.9   |        |        |        |        |          |                          |           |
| 20  | \\        | \ Diluted virus RNA    | 32.5    | 32.6   | 33.1    | 33.3   | 31.8    | 32.7   | 36.8   |        | 37.9   | 40.2   |        |        |          |                          |           |
| 21  | \\        | \ Diluted virus RNA    | 29.0    | 29.2   | 31.6    | 31.9   | 29.6    | 29.6   | 29.0   | 29.0   | 33.4   | 33.8   | 30.4 | 30.5 |        |                          |           |
| 22  | 423        | 25                     |       |       | 37.1    | 37.1   | 37.1    |        | 37.1   |        | 37.1   |        | 37.1   |        | 37.1 | 37.1    |                          |           |

**Number (Positive pool/Total):**
- 15/22
- 12/22
- 15/22
- 11/22
- 8/22
- 2/22
- 9/22

**Positivity rate (%):**
- 68
- 55
- 68
- 50
- 36
- 9
- 41
### Table S8. Comparative analysis of Ct value for negative pooled samples

| No. | Population (case number) | Correlated case number | N1 Ct_1 | N1 Ct_2 | N2 Ct_1 | N2 Ct_2 | N3 Ct_1 | N3 Ct_2 | E Ct_1 | E Ct_2 | HKU-N Ct_1 | HKU-N Ct_2 | ORF1ab Ct_1 | ORF1ab Ct_2 | ORF1b Ct_1 | ORF1b Ct_2 |
|-----|--------------------------|------------------------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|-------------|-------------|-------------|-------------|-------------|
| 1   | 1,374 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 2   | 1,601 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 3   | 14,463 0                 |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 4   | 9,248 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 5   | 3,770 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 6   | 5,226 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 7   | 4,759 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 8   | 2,798 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 9   | 1,846 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 10  | 2,545 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 11  | 33,453 0                 |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 12  | 3,971 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |
| 13  | 1,054 0                  |                        |         |         |         |         |         |         |         |         |             |             |             |             |             |             |

|       | Number (Negative pool/Total) | 13/13 | 13/13 | 11/13 | 13/13 | 13/13 | 13/13 | 13/13 | 12/13 |
|-------|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Specificity rate (%) | 100 | 100 | 85 | 100 | 100 | 100 | 100 | 92 |
| Analysis tests (N=35, P: 22, N: 13) | SELECTION |
|-----------------------------------|-----------|
|                                   | N1 | N3 | N1&N3 | (N1&N3)+E | (N1&N3) +N2 | N1+N2 | N1+N3 | N1+E |
| % Sensitivity                     | 68 | 68 | 86    | 64        | 55          | 50    | 50    | 41    |
| % Specificity                     | 100| 85 | 85    | 100       | 100         | 100   | 100   | 100   |
| % Positive Predictive Value (PPV) | 100| 88 | 90    | 100       | 100         | 100   | 100   | 100   |
| % Negative Predictive Value (NPV) | 65 | 61 | 79    | 62        | 57          | 54    | 54    | 50    |
| % False Positive Rate             | 0  | 15 | 15    | 0         | 0           | 0     | 0     | 0     |
| % False Negative Rate             | 32 | 32 | 14    | 36        | 45          | 50    | 50    | 59    |

“&” represents the union of results from two primer-probe sets. “+” represents the intersection of results from different primer-probe set.
Table S10. MIQE checklist.
The MIQE checklist was provided in a separate Excel.
Figure S1. **Common primer check for high quality genomes.** All seven primer-probe sets are targeting the conserved genetic regions. (credit: https://www.gisaid.org/)
Figure S2. Decay of SARS-CoV-2 revealed using different primer-probe sets. a, b, c, d, e, f, and g Decay curves detected by N1 (a), N2 (b), N3 (c), E (d), HKU-N (e), ORF1ab (f), and HKU-ORF1b (g). And the Ct value was shown in (h). Colours indicate the seven tested primer-probe sets. Y-axis represents the concentration of detected SARS-CoV-2 at time T. Error bar amplitude matches mean ± standard deviation of triplicates.
Figure S3. Performance comparison of two RT-qPCR reagents.
*: NC_045512.2 is the GenBank accession of the reference genome.

**Figure S4. The genome sequence from one positive pool sample.**
The accession number of the Pool sample_22 sequence is MZ578003.
References
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2. Chin, A. W.; Chu, J. T.; Perera, M. R.; Hui, K. P.; Yen, H.-L.; Chan, M. C.; Peiris, M.; Poon, L. L., Stability of SARS-CoV-2 in different environmental conditions. *The Lancet Microbe* **2020**, *1*, (1), e10.