**Table 1.** The Primers of mPCR and MPE

|  |  |  |  |
| --- | --- | --- | --- |
| **Target gene** | **mPCR primer (F)****1** | **mPCR primer (R) 1** | **MPE primer** |
| Human *RNase P* | AGATTTGGACCTGCGAGCG | GAGCGGCTGTCTCCACAAGT | CAGTAGCTGTTTCTGAACT |
| HCoV-229E-*N* | ACGGTGTTAGGCGCAAGAAT | AGGAGCACGGGAGTCAGGTT | CCAACCAGAGATACCACACTTCAA |
| HCoV-229E-*RdRp* | GGACCACGAGCAGTCCATGT | GTTCTGCCCTCATGCCAAGT | CCCCATGTATAACTTACTTAAAGG |
| HCoV-NL63-*N* | GTTGCTGCTGTTACTTTGGCT | CCCTGGGTTGAGAAAGAGGCT | TACCAGTCGAAGTCACCT |
| HCoV-NL63-*RdRp* | CACTTGTTACAACTGCTGGTT | TGTCAACCTAACTGARTGTGT | GAAAGCAATTAGGTTTGGT |
| HCoV-OC43-*N* | AGCAACCAGGCTGATGTCAA | GGCGGAAACCTAGTCGGAAT | ATTCGCTACTTGGGTCCCGAT |
| HCoV-OC43-*RdRp* | CCCAGGATGTGGTGTTGCTAT | CGCAATCCAATGCATGACACA | CGCATGACACATGGTCAG |
| HCoV-HKU1-*N* | ACTCCCGGTCATYATGCTGG | TTCGYTCAGATTGGTCARCC | GGAGAAGTTTTCTTGAGGATT |
| HCoV-HKU1-*RdRp* | ACACACCGYTATCGTTTGTCT | CAAGCAGAGCACTAGCAGATG | GGGTGCATAGCAGGATCTGCTGCATA |
| MERS-CoV-*N* | TTGGCGGAGACAGGACAGAA | GGAATGGGAGTGCTGCTTCG | CCAAAATTAATACCGGGAATGGA |
| MERS-CoV-*E* | ACACTCTTGGTGTGTATGGCT | GCGGGCTGAACTAACAGGGTA | CCGGCTACTAGATTATGTGTGCAAT |
| MERS-CoV-*RdRp* | GGAGAACGTGTACGCCAAGC | AGCACACCGACTAAACCAGC | AGCCAAGCTATCTTAAACA |
| MERS-CoV-*ORF1b* | GCTGCTCTTCTTGCCGGTTC | TGGTCAAGGGCTGTGCATCA | CCCCACAGGGTCATCAACAAT |
| SARS-CoV-*N* | TGATGAAGCTCAGCCTTTGCC | AATCATCCATGTCAGCCGCAG | GGGCAGAGACAAAAGAAGCAGCCC |
| SARS-CoV-*E* | GCCATCCTTACTGCGCTTCG | ACGCGAGTAGACGTAAACCG | TCTTGTTAACGTGAGTTTA |
| SARS-CoV-*ORF1b* | AGAAACGCCCGTAATGGTGT | CTAGCTTGTGCTGGTCCCTT | GGGTTTTAATAACAGAAGGTTCAGT |
| SARS-CoV2-*ORF1b* | TGCTGTAGATGCTGCTAAAGC | GCCTGACCAGTACCAGTGTG | CCCATCTTAACACAATTAGTGATTGG |
| SARS-CoV2-*N1* | AGAATGGAGAACGCAGTGGG | CGGTGAACCAAGACGCAGTA | CGACGTTGTTTTGATCG |
| SARS-CoV2-*N2* | CAACTCCAGGCAGCAGTAGG | TGTCAAGCAGCAGCAAAGCA | TCTGGCTGGCAATGGCGGTGAT |
| SARS-CoV2-*S* | ACAGGCACAGGTGTTCTTACT | TGGATCACGGACAGCATCAGT | CTCAATTTGGCAGAGACATT |
| SARS-CoV2-*S*（D614G） | ACTTCTAACCAGGTTGCTGTTCTT | CACGCCAAGTAGGAGTAAGTTGAT | CCAGGGACTTCTGTGCAGTTAACA |

1 The specific primers used in this study contained a 5’ 10-base extension (ACGTTGGATG)

**Table 2.** Sample Information and Identification Results of UN-CoV-2020

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample**  **set ID** | **Sample ID** | **Sample Type** | **Sample description** | **Result** |
| A | RKI-SW-1.1 | Human throat swab | possibly contaminated  (CoV pos1 or neg2) | CoV pos: *SARS-CoV-2* |
| RKI-SW-1.2 | Human throat swab | possibly contaminated  (CoV pos or neg) | CoV pos: *HCoV-NL63* |
| RKI-SW-1.3 | Human throat swab | Uncontaminated  (neg control) | CoV neg |
| B | RKI-SW-2.1 | Human throat swab | possibly contaminated  (CoV pos or neg) | CoV pos: *SARS-CoV-2* |
| RKI-SW-2.2 | Human throat swab | possibly contaminated  (CoV pos or neg) | CoV neg |
| RKI-SW-2.2 | Human throat swab | Uncontaminated  (neg control) | CoV neg |
| C | RKI-SW-3.1 | Livestock throat swab | possibly contaminated  (CoV pos or neg) | CoV pos: *SARS-CoV-2* |
| RKI-SW-3.2 | Livestock throat swab | possibly contaminated  (CoV pos or neg) | CoV pos: *SARS-CoV* |
| RKI-SW-3.3 | Livestock throat swab | Uncontaminated  (neg control) | CoV neg |
| D | RKI-SW-4.1 | Cell culture supernatant | possibly contaminated  (CoV pos or neg) | CoV pos: *SARS-CoV-2* |
| RKI-SW-4.2 | Cell culture supernatant | possibly contaminated  (CoV pos or neg) | CoV pos: *SARS-CoV* |
| RKI-SW-4.3 | Cell culture supernatant | Uncontaminated  (neg control) | CoV neg |

1. pos: positive.

2. neg: negative.

**Table 3** Detection limit of HCoV-MS method

|  |  |  |
| --- | --- | --- |
| **Name** | **Target** | **Detection limit (copies/reaction)** |
| Human RNase P | Human *Rnase P* | 1 |
| HCoV-NL63 | *N* | 1 |
| *RdRp* | 1 |
| HCoV-229E | *N* | 2.5 |
| *RdRp* | 2.5 |
| HCoV-OC43 | *N* | 2.5 |
| *RdRp* | 2.5 |
| HCoV-HKU1 | *N* | 1 |
| *RdRp* | 2.5 |
| MERS-CoV | *N* | 1 |
| *RdRp* | 2.5 |
| *E* | 2.5 |
| *ORF1b* | 2.5 |
| SARS-CoV | *E* | 5 |
| *N* | 5 |
| *ORF1b* | 5 |
| SARS-CoV-2 | *N1* | 2.5 |
| *N2* | 2.5 |
| *S* | 2.5 |
| *S（D614G）* | 2.5 |
| *ORF1b* | 2.5 |

**Table 4** The detection results of *SARS-CoV-2* simulated samples by two detection methods

|  |  |  |
| --- | --- | --- |
| **Gradient** | **Result of QuanSNP** | **Result of RT-PCR (mean±SD)** |
| 1 | neg1 | neg (43.61±0.33) |
| 2 | neg | neg (40.86±0.15) |
| 3 | pos2 | pos (39.45±0.25) |
| 4 | pos | pos (37.50±0.40) |
| 5 | pos | pos (36.31±0.35) |
| 6 | pos | pos (35.06±0.11) |
| 7 | pos | pos (32.79±0.32) |
| 8 | pos | pos (31.06±0.08) |
| 9 | pos | pos (29.37±0.09) |
| 10 | pos | pos (28.91±0.55) |

The concentration of gradient 1-10 is gradually increasing. 1. neg: negative. 2.pos: positive.

**Table 5** The gradient of detection limit of 26 *SARS-CoV-2* clinical samples

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Gradient** | | | | | | | | | |
| **1** | | **2** | | **3** | | **4** | | **5** | |
| **real-time PCR** | **HCoV-MS** | **real-time PCR** | **HCoV-MS** | **real-time PCR** | **HCoV-MS** | **real-time PCR** | **HCoV-MS** | **real-time PCR** | **HCoV-MS** |
| 1 | No Ct/No Ct | -1 | No Ct/No Ct | - | 40.74/No Ct | +2 | 36.87/No Ct | + | 34.17/No Ct | + |
| 2 | No Ct/No Ct | - | No Ct/No Ct | - | 40.65/No Ct | - | 36.30/No Ct | + | 34.36/No Ct | + |
| 3 | 35.94/No Ct | - | 32.84/No Ct | - | 33.35/No Ct | - | 32.56/No Ct | + | 30.16/No Ct | + |
| 4 | 36.88/34.01 | + | 35.96/32.03 | + | 32.92/29.77 | + | 31.24/27.63 | + | 28.52/25.92 | + |
| 5 | 39.64/33.68 | + | 37.01/32.01 | + | 34.79/30.05 | + | 33.03/27.92 | + | 30.6/25.83 | + |
| 6 | 37.33/35.04 | + | 36.67/32.81 | + | 35.45/31.17 | + | 34.28/29.21 | + | 32.9/27.14 | + |
| 7 | 40.63/34.79 | + | 40.11/32.60 | + | 38.66/30.97 | + | 35.83/28.46 | + | 33.83/26.86 | + |
| 8 | No Ct/No Ct | - | No Ct/39.02 | - | 39.35/36.45 | - | 36.3/34.71 | + | 35.15/32.30 | + |
| 9 | No Ct/No Ct | - | No Ct/No Ct | - | No Ct/No Ct | - | 39.33/36.50 | + | 36.32/34.60 | + |
| 10 | No Ct/No Ct | - | 37.23/37.43 | - | 37.06/34.76 | + | 35.16/32.90 | + | 33.24/30.99 | + |
| 11 | 39.02/36.78 | - | 36.35/33.84 | + | 34.35/31.44 | + | 32.36/30.16 | + | 30.66/28.16 | + |
| 12 | No Ct/No Ct | + | No Ct/No Ct | + | No Ct/38.39 | + | 35.95/35.15 | + | 34.61/32.06 | + |
| 13 | No Ct/No Ct | + | No Ct/No Ct | + | No Ct/No Ct | + | 34.99/No Ct | + | 33.94/38.01 | + |
| 14 | No Ct/No Ct | - | No Ct/No Ct | - | No Ct/38.16 | + | 37.12/35.66 | + | 34.52/33.22 | + |
| 15 | No Ct/No Ct | + | 37.15/36.10 | + | 37.04/33.67 | + | 35.26/31.76 | + | 33.01/30.57 | + |
| 16 | No Ct/No Ct | + | 40.81/No Ct | + | No Ct/38.45 | + | 39.89/36.62 | + | 37.84/35.04 | + |
| 17 | No Ct/No Ct | - | No Ct/39.73 | - | 39.31/37.82 | + | 37.87/36.36 | + | 36.43/34.64 | + |
| 18 | No Ct/No Ct | - | No Ct/40.08 | - | No Ct/39.48 | - | 42.18/38.98 | + | 40.41/36.18 | + |
| 19 | No Ct/No Ct | + | 37.22/36.00 | + | 36.90/34.46 | + | 34.81/33.69 | + | 32.92/31.26 | + |
| 20 | 37.80/32.63 | + | 36.22/30.56 | + | 33.91/28.28 | + | 32.24/26.46 | + | 29.53/23.98 | + |
| 21 | 39.93/34.69 | + | 36.10/32.38 | + | 32.28/30.01 | + | 30.51/27.60 | + | 27.69/25.37 | + |
| 22 | No Ct/No Ct | - | No Ct/38.56 | + | 38.56/39.32 | + | 37.10/34.75 | + | 36.13/33.54 | + |
| 23 | No Ct/No Ct | - | 35.15/No Ct | - | 34.93/No Ct | - | 34.15/38.72 | - | 30.79/35.09 | + |
| 24 | No Ct/No Ct | - | 39.17/36.95 | + | 36.61/34.35 | + | 35.95/31.70 | + | 33.32/29.73 | + |
| 25 | No Ct/No Ct | - | No Ct/No Ct | + | No Ct/38.88 | + | 36.66/37.20 | + | 33.94/35.70 | + |
| 26 | No Ct/No Ct | - | 36.46/No Ct | + | 34.97/38.94 | + | 35.39/35.67 | + | 33.65/34.16 | + |

The first value of real-time PCR results represents real-time PCR-ROX-*N* and the second value represents real-time PCR-FAM-*ORF1ab*. The determination of real-time PCR results was based on the 2019-nCoV nucleic acid detection kit (Shengxiang Biotechnology, Hunan, China.). The concentration of gradient 1-5 is gradually increasing.

1. “-” represents the result of HCoV-MS method is negative.

2. “+” represents the result of HCoV-MS method is positive.

**Table 6** Comparison of the results of HCoV-MS method and real-time PCR

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Name | | HCoV-MS | | |  |  | real-time PCR | |
|  | pos1 | F-pos2 | neg3 | F-neg4 |  | pos | | neg |
| *HCoV-229E* | 1 | 0 | 0 | 0 |  | 1 | | 0 |
| *SARS-CoV-2* | 91 | 0 | 59 | 0 |  | 91 | | 59 |

1. pos: positive. 2. F-pos: False positive. 3. neg: negative. 4. F-neg: False negative.