Simultaneous Detection of Seven Human Coronaviruses

Subjects: Virology

Contributor: Wenwen Xin

Human coronaviruses (HCoVs) are associated with a range of respiratory symptoms. The discovery of severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome, and SARS-CoV-2 pose a significant threat to human health. The HCoV-MS method is a sensitive assay that combines multiplex PCR with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), to detect and differentiate seven HCoVs simultaneously.

human coronavirus MALDI-TOF MS RT-PCR high throughput

1. Introduction

Coronaviruses (CoVs) are large, enveloped, positive-sense RNA viruses that cause respiratory diseases in a range of animals, including humans [1]. CoVs are divided into four genera, namely δ-CoVs, γ-CoVs, β-CoVs, and α-CoVs, among which β-CoVs and α-CoVs can infect mammals [2]. Seven human CoVs (HCoVs) have been identified, including HCoV-NL63, HCoV-229E, HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2 [1][3]. Respiratory diseases caused by HCoV infection range from mild to severe. Approximately 15–30% of respiratory tract infections worldwide each year are caused by HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. They are mild and self-healing diseases that do not pose a major threat to public health [4].

Studies have shown that HCoV most probably originated in wild animal hosts such as bats. A rich gene pool of SARS-related CoVs was found in bats in a cave in Yunnan, China [5]. Related viruses may reappear at any time and possibly mutate to produce more pathogenic CoV variants. Effective treatment methods are lacking for SARS-CoV, MERS-CoV, and SARS-CoV-2. Moreover, the initial symptoms of HCoVs infection are similar, but the treatment methods are different. The rapid, accurate detection and diagnosis of HCoV will help treat and block the spread, minimizing the loss of life caused by an epidemic. Therefore, it is important to develop a sensitive, high-throughput detection method for HCoV.

The traditional method for detecting HCoVs involves cell culture isolating the virus from clinical specimens. However, this approach is time-consuming, and most of the common cell lines are not suitable for the growth of HCoV. In addition to being time-consuming, these contributing factors do not constitute a conventional diagnostic method [6]. Next-generation sequencing technology can obtain whole-genome information of HCoVs, which contributes to our knowledge of HCoVs and helps the discovery of unknown HCoVs. However, this technology requires sophisticated bioinformatic analysis and is expensive and time-consuming; hence, it is unsuitable for

large-scale population screening [7]. In recent years, real-time multiplex PCR (RT-PCR) and mass spectrometry technologies have gradually been developed into established pathogen detection methods, which are widely used today [8][9][10]. Multiplex RT-PCR is highly specific and sensitive and short detection time, making it a rapid and reliable diagnostic tool. Unfortunately, this methodology has some drawbacks. The types of fluorescence and light sources are limited, and although it is highly sensitive, a correspondingly larger sample size is required [11]. Multiplex RT-PCR theoretically detects more than a dozen viruses [12]. RT-PCR has become the gold standard for HCoV detection [13]. However, when multiple RT-PCRs are run, the sensitivity decreases as the detection factor increases [12][14].

Nucleic acid mass spectrometry analysis, where multiplex PCR (mPCR) is combined with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), designs sequence targets based on single nucleotide polymorphism (SNP) sites to achieve multiple detections [15][16]. Nucleic acid mass spectrometry technology allows multi-target high-throughput screening and can, in principle, reach about 40 detections. This is not possible with other multiplex detection methods. Moreover, MALDI-TOF MS is known for its strong specificity and high sensitivity. At present, nucleic acid mass spectrometry has been widely used for multiple detection and typing of bacteria and viruses. For example, it has been used for the simultaneous detection of 10 duck viruses [17], multi-site typing of Mycoplasma pneumoniae, simultaneous detection of drug resistance [18], and simultaneous detection of 21 common respiratory viruses [19]. There are many detection methods for respiratory viruses, each with different detection specificities, sensitivities, and detection limits [20][21].

2. Performance of the Hcov-MS Method

The initial concentration of the mPCR primers for all HCoVs target genes was 0.5 μM. Mixing of the mass probes extension (MPE) primers was based on equalizing the mass spectrum signal intensity of each primer; hence, the amount added was slightly different according to the molecular weight. None of the primers and probes participated in an extension reaction when deionized water was used as the template for detection. When the mixed plasmid (10^2 copies/μL) was used as the template for detection, target genes of other plasmids could be detected to form specific product peaks by MPE, except NL63-RdRp, which had low amplification efficiency. In this case, the concentration of the NL63-RdRp mPCR primer in the mixed primer (reaction concentration was 4 μM) was used to optimize the system. After optimization, each primer was specifically amplified at 45 cycles.

3. Specificity of the HCoV-MS Method

Nine high-concentration (10^5 copies/μL) plasmids containing target genes were used to verify the specificity of the system, with the number of mPCR cycles set at 30, 35, 40, or 45. The results showed that high-concentration plasmids amplified well in the detection system and that all target genes could be specifically amplified in 45 cycles. Respiratory samples containing high concentrations of ADV7, InfB, H1N1, and H3N2 viruses were used to evaluate the specificity of the HCoV-MS method. The results showed no cross-reactivity, suggesting that the specificity of the HCoV-MS method is good.

4. Sensitivity of the HCoV-MS Method

Serial plasmid dilutions were used to evaluate the sensitivity of the HCoV-MS method. The detection limits of part of the target genes are shown in **Figure 1**, and listed in **Table 1**. The detection limit of the HCoV-MS method was found to be 1–5 copies/reaction.

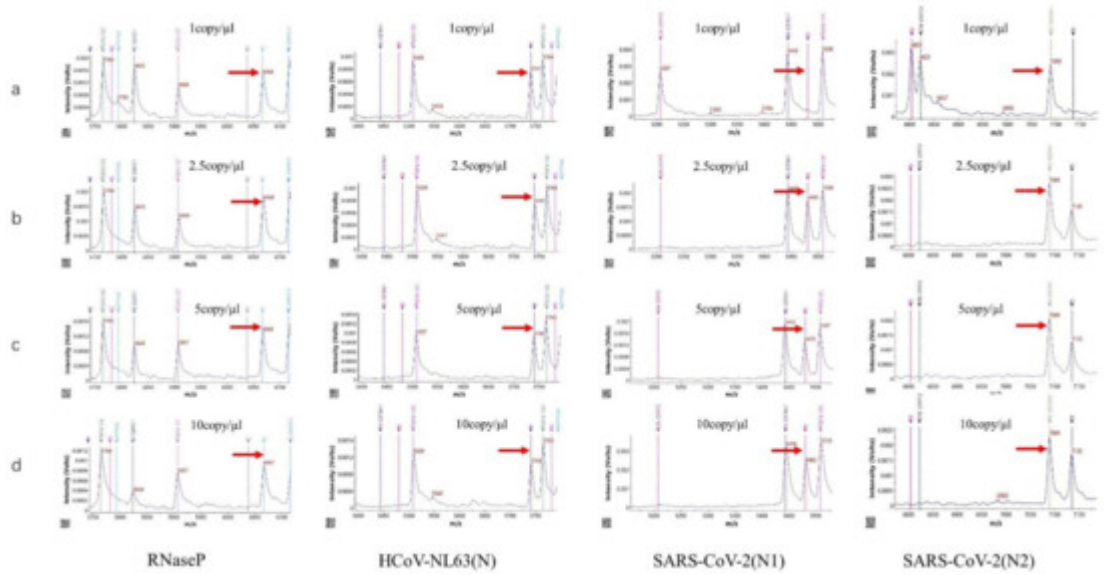

Figure 1. The detection limit of part of target genes: (**a**) 1 copy/reaction, (**b**) 2.5 copies/reaction, (**c**) 5 copies/reaction, and (**d**) 10 copies/reaction. The red arrow in each figure refers to extended or unextended primer.

Table 1. Detection limit of the human coronavirus-mass spectrometry (HCoV-MS) method.

Assays	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

Assays	Target	Detection Limit (Copies/Reaction)
	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
	ORF1b	2.5

A total of 26 SARS-CoV-2 clinical samples were serially diluted, and the HCoV-MS and RT-PCR methods were used for simultaneous detection to compare their detection sensitivities. The detection limit gradient of the 26 SARS-CoV-2 clinical samples is shown in **Figure 2** and **Table 2**. Results for the majority of the clinical samples were the same for the two methods or differed by only 1–2 gradients. Furthermore, only a few samples showed a significantly different detection gradient in the experiment. Evidently, the detection sensitivity of the HCoV-MS method is almost the same as that of RT-PCR.

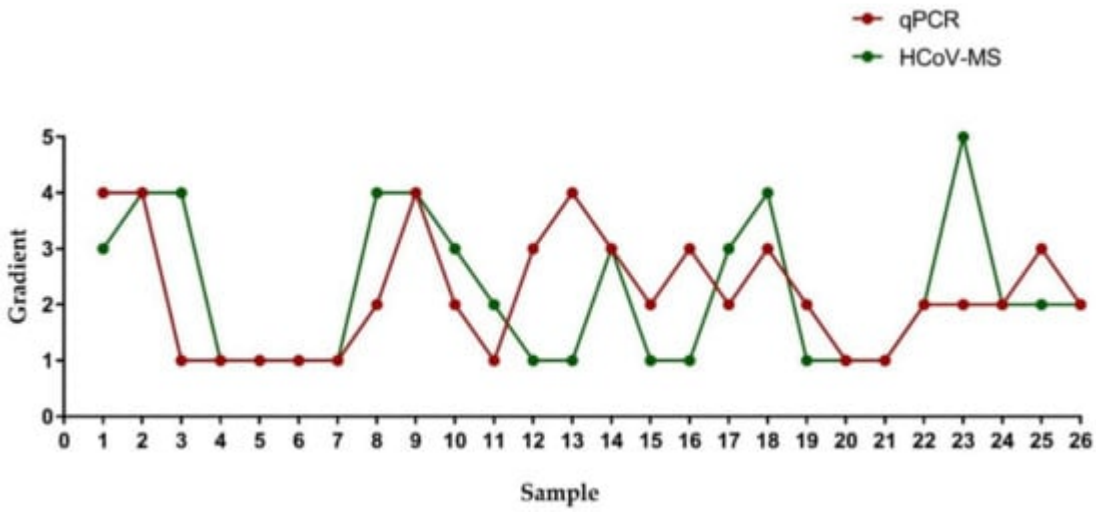

Figure 2. Comparison of the gradient of detection limit of 26 severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) clinical samples. The concentration of the gradient 1–5 is gradually increasing.

Table 2. The detection limit gradient of 26 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) clinical samples.

Sample	Gradient									
	1		2		3		4		5	
	RT-PCR *	HCoV-MS *	RT-PCR	HCoV-MS	RT-PCR	HCoV-MS	RT-PCR	HCoV-MS	RT-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	–	No	–	No	–	42.18/38.98	+	40.41/36.18	+

Sample	Gradient									
	1		2		3		4		5	
	RT-PCR *	HCoV-MS *	RT-PCR	HCoV-MS	RT-PCR	HCoV-MS	RT-PCR	HCoV-MS	RT-PCR	HCoV-MS
	Ct		Ct/40.08		Ct/39.48					
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	+

detection
except for
d with 1
[20][21][22][23][24]
d 100%,
mples of
human/animal throat swabs or cell cultures were obtained in cooperation with UN-CoV-2020. Seven samples were positive, including four SARS-CoV-2 samples, two SARS-CoV samples, and one HCoV-NL63 sample. Moreover, the concentration of individual positive samples was low, but could still be accurately identified, which further highlights the detection ability of the HCoV-MS method.

The first RT-PCR value represents RT-PCR-ROX-N, while the second represents RT-PCR-FAM-ORF1ab. The concentrations of the gradient 1–5 is gradually increasing. [1] "−" represents a negative HCoV-MS result. [2] "+" represents a positive HCoV-MS result. * RT-PCR: real-time PCR. * HCoV-MS: human coronavirus mass spectrometry.

The HCoV-MS method is high-throughput, as reflected in the detection of multiple target genes and the requirement of small sample sizes. The HCoV-MS method could simultaneously detect 384 targets in one run spanning 30 min, with results automatically determined by the relevant software. Moreover, the reagent cost of the HCoV-MS method is relatively low, making this method ideal for large-scale population screening.

The HCoV-MS also has some limitations. This method is difficult to identify new HCoV because it is based on comparing and analyzing known HCoV sequences, selecting gene fragments with conserved intraspecies specificity. In conclusion, the HCoV-MS method has the characteristics of high throughput, speed, and sensitivity, only requiring a small number of samples. Therefore, it is expected to be a supplement to real-time PCR technology.

References

1. Xiu, L.; Zhang, C.; Wu, Z.; Peng, J. Establishment and Application of a Universal Coronavirus Screening Method Using MALDI-TOF Mass Spectrometry. Front. Microbiol. 2017, 8, 1510.

2. Ezhilan, M.; Suresh, I.; Nesakumar, N. SARS-CoV, MERS-CoV and SARS-CoV-2: A Diagnostic Challenge. Measurement 2021, 168, 108335.
3. Fung, T.S.; Liu, D.X. Human Coronavirus: Host-Pathogen Interaction. Annu. Rev. Microbiol. 2019, 73, 529–557.
4. Miller, K.; McGrath, M.E.; Hu, Z.; Ariannejad, S.; Weston, S.; Frieman, M.; Jackson, W.T. Coronavirus interactions with the cellular autophagy machinery. Autophagy 2020, 16, 2131–2139.
5. Xiao-Shuang, Z.; Zeng, L.-P.; Yang, X.-L.; Ge, X.-Y.; Zhang, W.; Lin-Fa, W.; Xie, J.-Z.; Dong-Sheng, L.; Zhang, Y.-Z.; Wang, N.; et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog. 2017, 13, e1006698.
6. Loeffelholz, M.J.; Tang, Y.-W. Laboratory diagnosis of emerging human coronavirus infections–the state of the art. Emerg. Microbes Infect. 2020, 9, 747–756.
7. Gu, W.; Miller, S.; Chiu, C.Y. Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. Annu. Rev. Pathol. Mech. Dis. 2019, 14, 319–338.
8. Gao, J.; Quan, L. Current Status of Diagnostic Testing for SARS-CoV-2 Infection and Future Developments: A Review. Med. Sci. Monit. 2020, 26.
9. Appak, Ö.; Duman, M.; Belet, N.; Sayiner, A.A. Viral respiratory infections diagnosed by multiplex polymerase chain reaction in pediatric patients. J. Med. Virol. 2019, 91, 731–737.
10. Gilsenan-Reed, C.; Higgins, G.; Langlois, N. Determining a sampling regime for PCR detection of respiratory tract viral infection at coronial post-mortem examinations. Forensic Sci. Med. Pathol. 2020, 16, 457–462.
11. Pabbaraju, K.; Wong, A.A.; Ma, R.; Zelyas, N.; Tipples, G.A. Development and validation of a multiplex reverse transcriptase-PCR assay for simultaneous testing of influenza A, influenza B and SARS-CoV-2. J. Virol. Methods 2021, 293, 114151.
12. Li, J.; Mao, N.-Y.; Zhang, C.; Yang, M.-J.; Wang, M.; Xu, W.-B.; Ma, X.-J. The development of a GeXP-based multiplex reverse transcription-PCR assay for simultaneous detection of sixteen human respiratory virus types/subtypes. BMC Infect. Dis. 2012, 12, 189.
13. Vogels, C.B.F.; Brito, A.F.; Wyllie, A.L.; Fauver, J.R.; Ott, I.M.; Kalinich, C.C.; Petrone, M.E.; Casanovas-Massana, A.; Muenker, M.C.; Moore, A.J.; et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. Nat. Microbiol. 2020, 5, 1299–1305.
14. Nörz, D.; Hoffmann, A.; Aepfelbacher, M.; Pfefferle, S.; Lütgehetmann, M. Clinical evaluation of a fully automated, laboratory-developed multiplex RT-PCR assay integrating dual-target SARS-CoV-2 and influenza A/B detection on a high-throughput platform. J. Med. Microbiol. 2021, 70, 001295.

15. Settanni, L.; Corsetti, A. The use of multiplex PCR to detect and differentiate food- and beverage-associated microorganisms: A review. J. Microbiol. Methods 2007, 69, 1–22.
16. Tsuchida, S.; Umemura, H.; Nakayama, T. Current Status of Matrix-Assisted Laser Desorption/Ionization–Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in Clinical Diagnostic Microbiology. Molecules 2020, 25, 4775.
17. Liu, N.; Wang, L.; Cai, G.; Zhang, D.; Lin, J. Establishment of a simultaneous detection method for ten duck viruses using MALDI-TOF mass spectrometry. J. Virol. Methods 2019, 273, 113723.
18. Zhao, F.; Zhang, J.; Wang, X.; Liu, L.; Gong, J.; Zhai, Z.; He, L.; Meng, F.; Xiao, D. A multisite SNP genotyping and macrolide susceptibility gene method for Mycoplasma pneumoniae based on MALDI-TOF MS. iScience 2021, 24, 102447.
19. Zhang, C.; Xiao, Y.; Du, J.; Ren, L.; Wang, J.; Peng, J.; Jin, Q. Application of Multiplex PCR Coupled with Matrix-Assisted Laser Desorption Ionization–Time of Flight Analysis for Simultaneous Detection of 21 Common Respiratory Viruses. J. Clin. Microbiol. 2015, 53, 2549–2554.
20. Leung, E.C.-M.; Chow, V.C.-Y.; Lee, M.K.-P.; Tang, K.P.-S.; Li, D.K.-C.; Lai, R.W.-M. Evaluation of the Xpert Xpress SARS-CoV-2/Flu/RSV Assay for Simultaneous Detection of SARS-CoV-2, Influenza A and B Viruses, and Respiratory Syncytial Virus in Nasopharyngeal Specimens. J. Clin. Microbiol. 2021, 59, e02965-20.
21. Paradis, S.; Lockamy, E.; Cooper, C.K.; Young, S. Clinical evaluation of the molecular-based BD SARS-CoV-2/flu for the BD MAX™ System. J. Clin. Virol. 2021, 143, 104946.
22. Xi, Y.; Xu, C.-Z.; Xie, Z.-Z.; Zhu, D.-L.; Dong, J.-M.; Xiao, G. Development of a reverse transcription recombinase polymerase amplification assay for rapid detection of human respiratory syncytial virus. Mol. Cell. Probes 2019, 45, 8–13.
23. Zhang, W.S.; Pan, J.; Li, F.; Zhu, M.; Xu, M.; Zhu, H.; Yu, Y.; Su, G. Reverse Transcription Recombinase Polymerase Amplification Coupled with CRISPR-Cas12a for Facile and Highly Sensitive Colorimetric SARS-CoV-2 Detection. Anal. Chem. 2021, 93, 4126–4133.
24. Lin, Y.; Fu, Y.; Xu, M.; Su, L.; Cao, L.; Xu, J.; Cheng, X. Evaluation of a PCR/ESI-MS platform to identify respiratory viruses from nasopharyngeal aspirates. J. Med Virol. 2015, 87, 1867–1871.
25. Aoki, A.; Mori, Y.; Okamoto, Y.; Jinno, H. Development of a genotyping platform for SARS-CoV-2 variants using high-resolution melting analysis. J. Infect. Chemother. 2021, 27, 1336–1341.
26. Lope, P.; Maribel, H.; Egma, M.; Henri, B.; Carlos, P. Characterization of influenza A(H1N1)pdm09 isolates of Peru using HRM, a post PCR molecular biology method. Bioinformation 2019, 15, 640–645.

27. Zhou, H.; Zhao, M.; Li, X.; Zhang, D.; Chen, C.; Feng, Z.; Ma, X. Clinical evaluation of the isothermal amplification assays for the detection of four common respiratory viruses in children with pneumonia. Arch. Virol. 2017, 162, 1311–1318.