Supplemental material

Table S1 Reference strains used in the CRV-MS method design

Virus species	Virus types	Genogroup	Serotype	Strains	GenBank accession No.
human rhinovirus		A		HRV-A28_p1292_s6470_2000	JQ747751
		B		HRV-B06_p1159_sR2069_2009	JQ837717
		C		HRV-C22_p1268_s3839_1999	JN621242
Human enterovirus	EV71	B		2258-CVA-79	AF135880
		C		SHZH98	AF302996
	Coxsackievirus A16	A		G-10	U05876
		B		shzh00-2	AY895127
Human adenovirus		A			AC_000005
		B		human/ARG/ak38_AdV7h/2003/7[P7H7F7]	JX423386
		B1		GB	NC_011203
		B2		Slobitski	NC_011202
		C			NC_001405
		D		Hicks; NIAID V-209-003-014	NC_010956
		E			NC_003266
Influenza virus		A	H1N1	Beijing/132/2010(H1N1)	KF918706
			H1N1	Uberlandia/444/2006(H1N1)	KF918355
			H1N1	California/04/2009	FJ966082
			H3N2	Ontario/001/2013	KC526207
		B		B/Christchurch/54/2007	CY150665.1

Human coronavirus 229E			NC_002645
Human coronavirus OC43		ATCC VR-759	NC_005147
Human coronavirus NL63		Amsterdam I	NC_005831
Human coronavirus HKU1			NC_006577
Human metapneumovirus	A	CAN97-83	NC_004148
	B	HMPV/USA/C2-175/2005/B	KC562219
Respiratory syncytial virus	A	RSVA/GN435/11	JX627336
	B	B1	NC_001781
Human parainfluenza virus	1	HPIV1/USA/32193A/2010	KF687315
	2		AB176531
	3	HPIV3/MEX/1110/2004	KF687321
	4a	HPIV-4a_QPID08-0015	KF878965
	4b	04-13	JQ241176
Human bocavirus	1	ZJ92	JX887482
	2	W298	FJ948860
	3	IM10	GQ867667
	4	HBoV4-NI-385	NC_012729

Table S2 Sequences and molecular weights of unextended (UEP) and extended primers (EP) to detect Primers used in the CRV-MS method

ID*	Target region	Sense Primer#	Anti-sense Prime#	UEP	Amplicon size(bp)	UEP,Da	Extension	EP, Da
HRV	5'UTR	ACGTTGGATGTGTTCTAGCCTGCGTYGCTG	ACGTTGGATGAACACGGACACCCAAYGTAG	CTGAATGCCGGCCC	244	4224.8	C	4471.9
HEV	5'UTR	ACGTTGGATGGTGTGTCGTAAYGGGRAACT	ACGTTGGATGCTCAATTGTCACCATASGCAG	TGCAGCGGAACCGAC	119	4587.0	T	4914.1
AdV	Hexon	ACGTTGGATGCTTGTAIACGTAGGGYCAGG	ACGTTGGATGTTACYACCGTCAGTGYAAAC	TTGTGGTCCCGTGATC	146	4879.2	T	5150.4
Flu-A	MP	ACGTTGGATGTCTACGCTGKAGTCCTCGCT	ACGTTGGATGGACCAATCTTGTCAYCTCTG	AGGAGCTGGGCACGGT	106	4987.2	G	5234.4
Flu-A H1N1	HA	ACGTTGGATGCTGAGRGAGCAATTGAGTIC	ACGTTGGATGTCCCGTTATGGGAGCYTGAT	CCTTTCCAGAGGTTCGAAATATTC	141	7302.8	C	7549.9
Flu-A H1N1 pdm09	HA	ACGTTGGATGTGAATCGTGGTAGTCCAAAG	ACGTTGGATGGGTAAAGAGTTCYACCACCT	TGTCGCATTGTAAGTCCAAAT	161	6420.2	G	6667.4
Flu-A H3N2	HA	ACGTTGGATGAGTGCTTTTGAGATCTGCTG	ACGTTGGATGGCGGGTTTCATAGAYAATGG	ATACCAACCCTGAAACCG	129	5421.6	T	5692.8
Flu-B	MP	ACGTTGGATGAGAAGGCCATGAYAGCTCAG	ACGTTGGATGAGCCCTGTGTGYATGTGATG	GTAGAGGTCAATGCAAGTAAAACT	161	7433.9	A	7705.1
HCoV-229E	N	ACGTTGGATGACCTTCCAAGYTTGTTCACT	ACGTTGGATGATGGGCTGATGKATCTGAAC	CCCTACAAAGGACTATCAACAAG	119	6994.6	C	7281.8
HCoV-NL63	N	ACGTTGGATGGGAARCTTGTCCCTATTGG	ACGTTGGATGGCATACGCCAACGCTCTTGA	ATTGATGAGCAGATTGGTT	98	5897.9	G	6185.1
HCoV-HKU1	N	ACGTTGGATGACAGAGTCTTCTACARAAGG	ACGTTGGATGGCTGRTACTCAGCACATTTC	AGGATCCGTAGCAAGTAAACTATG	110	7409.8	A	7736.9

HCoV-OC43	N	ACGTTGGATGTCTTAT GACCACYCTGACGC	ACGTTGGATGGCTATA ACGGCGCAATTAGG	TAGGCCCATCTTGT TGTTGA	167	6114.0	T	6385.2
RSV-A	N	ACGTTGGATGCCTRTG GTAGAAGYTTGTGC	ACGTTGGATGCCCAAG GATATAGCRAACAG	CAAAATAGAAGATT GTGCTATAC	122	7079.6	C	7326.8
RSV-B	N	ACGTTGGATGGCACAT CATAATTGYGAGTG	ACGTTGGATGAATAAG GATCAGCTGCTITC	ACGCAATATTATCT CCTGTACT	106	6644.3	A	6971.4
PIV1	HN	ACGTTGGATGGCAAAG RAGAGATCTCACAC	ACGTTGGATGCAATYG TGTTCTCGCATATC	AAACAGAAGTCAT GCAAC	100	5509.6	A	5780.8
PIV2	HN	ACGTTGGATGCAGCTT TTGCGATTGATTCC	ACGTTGGATGGGGATA ATACAYCAATCTGC	CTTAGCGATTGATT CCATCA	111	6067	C	6354.2
PIV3	HN	ACGTTGGATGCTTGTT GTTGAGATTGYGCC	ACGTTGGATGTGCATC ATCAGGRATAGAAG	TTAAGCCATCATAA TTGACAATATC	89	7608.0	A	7935.1
PIV4	HN	ACGTTGGATGATATAR TCGACCRTCTGCAC	ACGTTGGATGGGGAG ACTTATACAGAGTGC	CCCAGAAAGTTAAA ATTTCACAATCTT	141	8210.4	T	8481.6
HMPV-A	N	ACGTTGGATGACCCTC ATCATTYCAACAAG	ACGTTGGATGTCTCTG ARCCTAYAGCTGTG	AGGCGAAATATTAC AAATATGCTGCA	146	8011.3	G	8298.5
HMPV-B	N	ACGTTGGATGTCTCYC CACACAAAYGTGTT	ACGTTGGATGAAAAG AGATGTAGGCACCAC	GTTGCAATGATGAA GGT	97	5289.5	G	5536.6
HBoV1	NP1	ACGTTGGATGACAGAA ATATGTTCTYGCAC	ACGTTGGATGGTCTGC TTCTGTCRGTGAGG	GGTGTGAAAGATGT AATTACT	135	6524.3	G	6811.5
HBoV2	NP1	ACGTTGGATGGGCAGC TACTTRACTGACTC	ACGTTGGATGTTTTCC TCCWGCYTTGCTTG	AACAAAATGAAGTT CTTAGTTAAAATA	141	8322.5	C	8569.7
HBB		ACGTTGGATGAAAGCA GCACTTGACTAGAG	ACGTTGGATGGCTAAA ATGTCTCACTTGGG	ACCTAGAGTATTTT TATACATGCTCT	97	7910.2	A	8237.3
RNaseP		ACGTTGGATGTGAATA GCCAAGGTGAGCGG	ACGTTGGATGCGGTGT TTGCAGATTTGGAC	CAAGTCCCTGTCTC CA	109	4777.1	C	5064.3

*Flu-A: Influenza virus A; Flu-B: Influenza virus B; PIV: parainfluenza virus; HCoV: human coronavirus; HEV: human enterovirus; HRV: human rhinovirus; AdV: adenovirus; HMPV: human metapneumovirus; RSV: respiratory syncytial virus; HBoV: human bocavirus

[#]10 mer tag=ACGTTGGATG placed at 5'-end of each primer.

Table S3. The experimental procedure of CRV-MS method

Experimental procedure	Cycle conditions/instrument
Primary PCR **Mastermix:** Total Reaction volume: 5 μL, Containing: • 2 μL DNA template • 500 nM of each primer • 500 μM of each dNTP (dATP, dCTP, dGTP, dUTP) • 0.2 U Sequenom PCR Enzyme • 10x PCR Buffer • 4 mM $MgCl_2$ • 0.2U Uracil-DNA Glycosylase	45°C for 2 min 95°C for 4 min 95°C for 30 s 56°C for 30 s 72°C for 1 min 45 cycles 72°C for 5 min
SAP Treatment (2μL) • 10x PCR Buffer • SAP	37°C for 40 min 85°C for 5 min 1 cycle
iPLEX Pro Extend Reaction (2μL) • 10x PCR Buffer • Thermosequenase Termination Mix • Extension Primer Mix • 1.35U ThermoSequenase	95°C for 30s 95°C for 5s 52°C for 5s 80°C for 5s 5cycles 40cycles 72°C for 30s
Desalting • 6 mg Clean Resin to each 384-well plate	

Sample dispensing • 10 nl of each iPLEX product onto a 384-spot SpectroChip II	MassARRAY Nanodispenser RS 1000 instrument (Sequenom Inc.).
Data acquisition	MassARRAY MALDI-TOF MS SpectroAcquire software MassARRAY Typer software, version 4.0.3 (Sequenom Inc.).

Figure S1

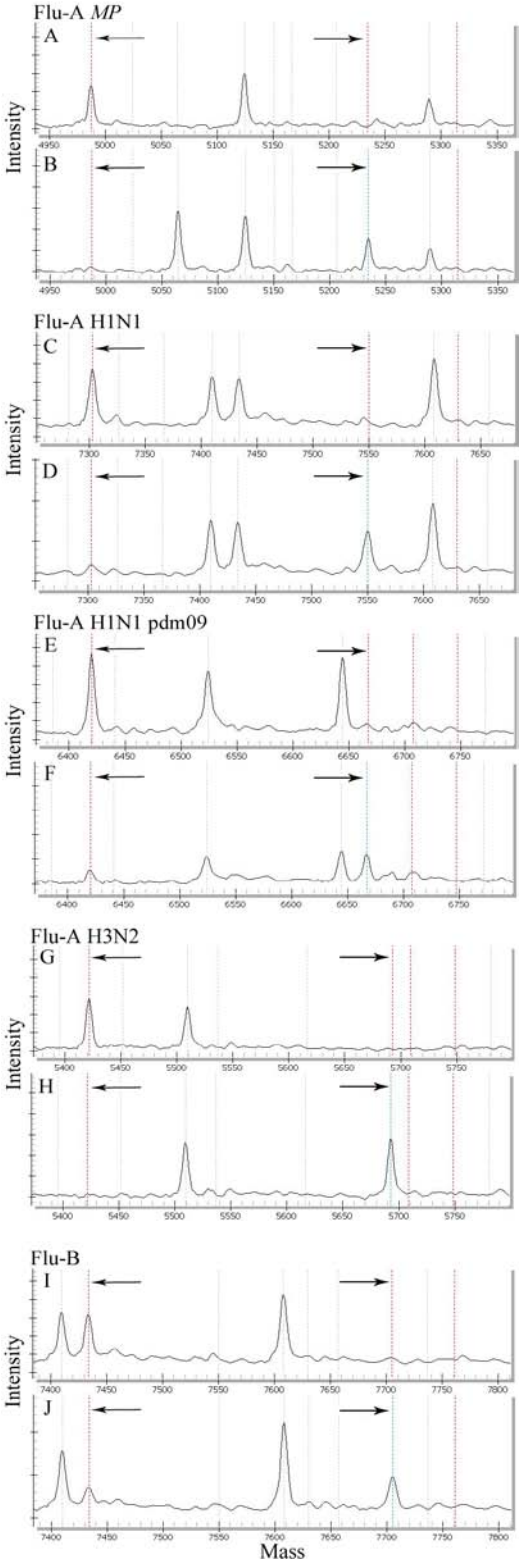

Figure S1. Detection of influenza virus A (Flu-A) and influenza virus B (Flu-B) using the CRV-MS method. In the mass spectrometry mass spectra, the dotted lines in the left and the dotted lines in the right represent the unextended primers and the extended primers of the assay, respectively. A: Flu-A *MP* negative; B: Flu-A *MP* positive; C: Flu-A H1N1 negative; D: Flu-A H1N1 positive; E: Flu-A H1N1 pdm09 negative; F: Flu-A H1N1 pdm09 positive; G: Flu-A H3N2 negative; H: Flu-A H3N2 positive; I: Flu-B negative; J: Flu-B positive.

Figure S2

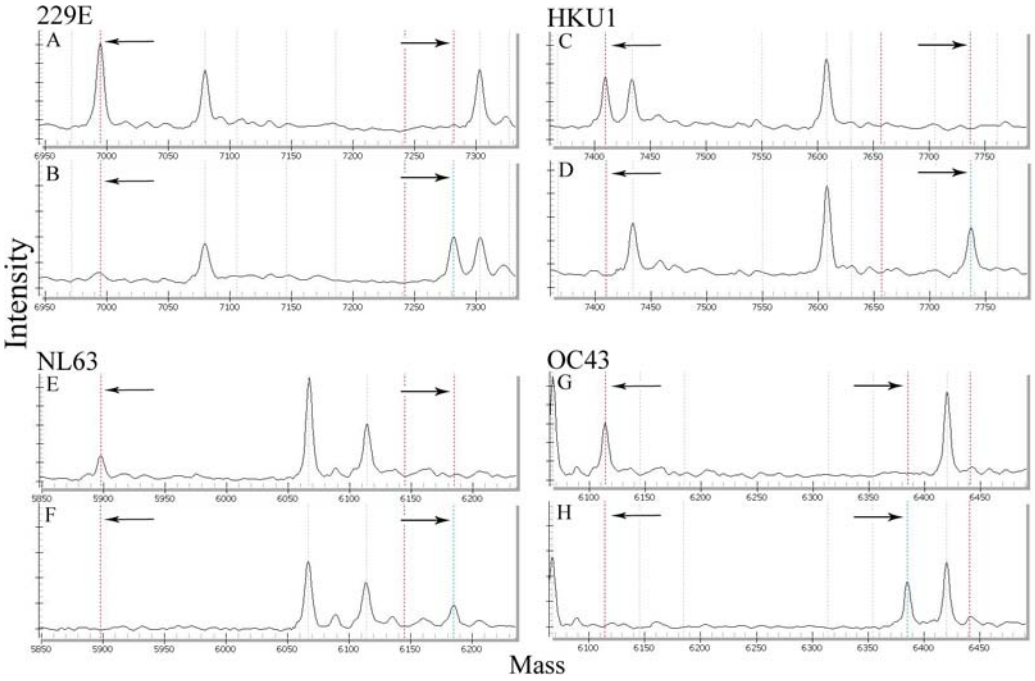

Figure S2. Detection of human coronavirus (HCoV) using the CRV-MS method. In the mass spectrometry mass spectra, the dotted lines in the left and the dotted lines in the right represent the unextended primers and the extended primers of the assay, respectively. A: HCoV-229E negative; B: HCoV-229E positive; C: HCoV-HKU1 negative; D: HCoV-HKU1 positive; E: HCoV-NL63 negative; F: HCoV-NL63 positive; G: HCoV-OC43 negative; H: HCoV-OC43 positive.

Figure S3

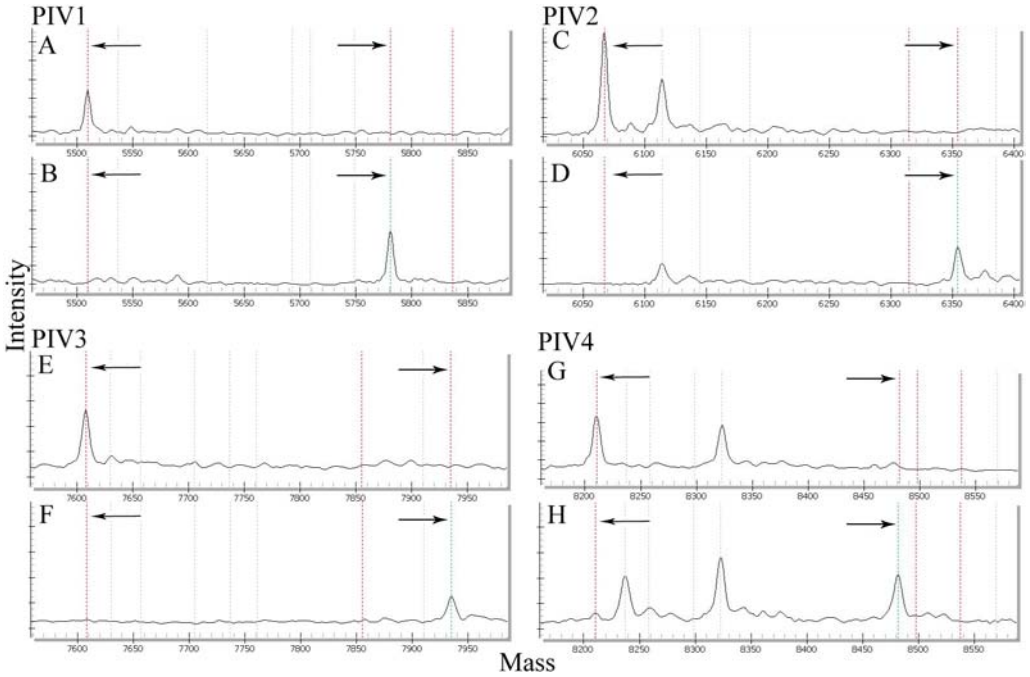

Figure S3. Detection of parainfluenza virus (PIV) using the CRV-MS method. In the mass spectrometry mass spectra, the dotted lines in the left and the dotted lines in the right represent the unextended primers and the extended primers of the assay, respectively. A: PIV1 negative; B: PIV1 positive; C: PIV2 negative; D: PIV2 positive; E: PIV3 negative; F: PIV3 positive; G: PIV4 negative; H: PIV4 positive.

Figure S4

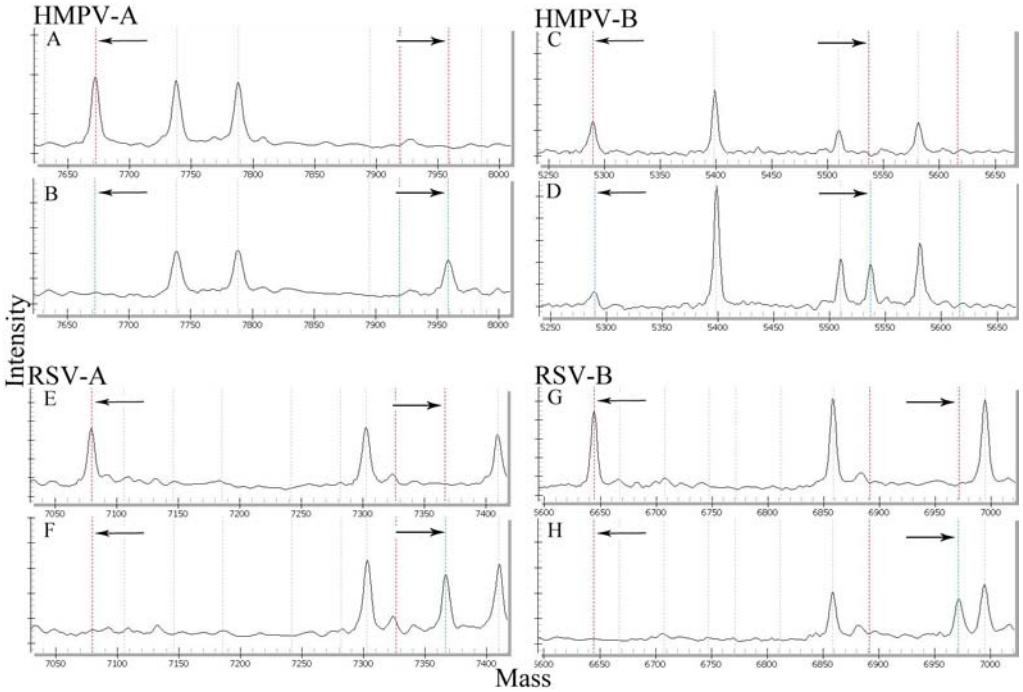

Figure S4. Detection of human metapneumovirus (HMPV) and respiratory syncytial virus (RSV) using the CRV-MS method. In the mass spectrometry mass spectra, the dotted lines in the left and the dotted lines in the right represent the unextended primers and the extended primers of the assay, respectively. A: HMPV-A negative; B: HMPV-A positive; C: HMPV-B negative; D: HMPV-B positive; E: RSV-A negative; F: RSV-A positive; G: RSV-B negative; H: RSV-B positive.

Figure S5

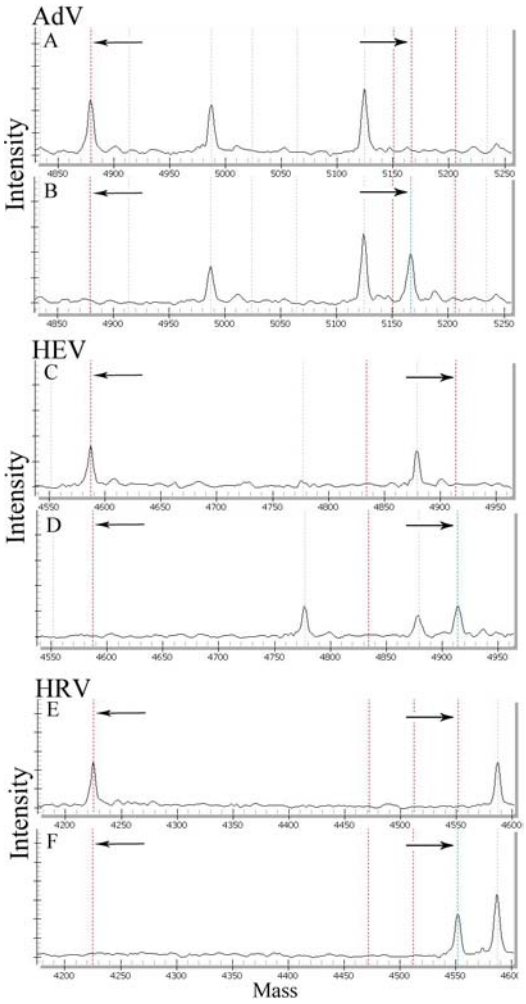

Figure S5. Detection of adenovirus (AdV), human enterovirus (HEV) and human rhinovirus (HRV) using the CRV-MS method. In the mass spectrometry mass spectra, the dotted lines in the left and the dotted lines in the right represent the unextended primers and the extended primers of the assay, respectively. A: AdV negative; B: AdV positive; C: HEV negative; D: HEV positive; E: HRV negative; F: HRV positive.