

**Table S1** Patient demographics

		Number (%)
NIH style age groups	Neonates or newborns (birth to <1 month)	1 (0.68%)
	Infants (1 month to <1 year)	5 (3.42%)
	Children (1 year to 12 years)	89 (60.96%)
	Adolescents (13 years to 17 years)	7 (4.79%)
	Adults (18 years to 64 years)	25 (17.12%)
	Older adults (65 years or older)	19 (13.01%)
Gender	Male	82 (56.16%)
	Female	64 (43.84%)
Patient type	Inpatients	76 (52.05%)
	Outpatients	70 (47.95%)

**Table S2** Frequency of detection for individual pathogens and discrepant analysis (upper respiratory tract specimens)

Pathogen targets	No. of positive specimens		Discrepant analysis
	RP2.1plus	SPOTFIRE R	
Adenovirus	15	18	Two specimens were positive by RP2.1plus and negative by SPOTFIRE R Panel. The Ct values were 40 by the alternative method.
			Two specimens were negative by RP2.1plus and positive by SPOTFIRE R Panel. The Ct values were 37.8 and 40 by the alternative method.
			Three specimens were negative by RP2.1plus and positive by SPOTFIRE R Panel. The results were negative by the alternative method. The patients had no recent history of adenovirus infection.
			<b>SPOTFIRE R Panel: 2FN, 3FP, 113TN and 15TP</b>
<b>Coronavirus</b>			Reported as 'coronavirus (seasonal)' without subtype by SPOTFIRE R Panel.
229E	3	3	One specimen was negative by RP2.1plus and positive by SPOTFIRE R Panel. The result was negative by the alternative method. The patient had no recent history of coronavirus infection.
HKU1	2	2	
NL63	7	7	
OC43	6	6	
Unclassified	0	1	<b>SPOTFIRE R Panel: 0FN, 1FP, 114TN and 18TP</b>
SARS-CoV-2	17	18	One specimen was positive by RP2.1plus and negative by SPOTFIRE R Panel. The result was negative by the alternative method. The patient had no recent history of SARS-CoV-2 infection.
			Two specimens were negative by RP2.1plus and positive by SPOTFIRE R Panel. The Ct values were 40 and 44.3 by the alternative method.
			<b>SPOTFIRE R Panel: 0FN, 0FP, 118TN and 15TP</b>
Metapneumovirus	6	6	<b>SPOTFIRE R Panel: 0FN, 0FP, 127TN and 6TP</b>
Rhinovirus/ enterovirus	23	34	One specimen was positive by RP2.1plus and negative by SPOTFIRE R Panel. Rhinovirus was detected by the alternative method.
			Eleven specimens were negative by RP2.1plus and positive by SPOTFIRE R Panel. Rhinovirus was detected by the alternative method.
			One specimen was negative by RP2.1plus and positive by SPOTFIRE R Panel. Rhinovirus and enterovirus were not detected by the alternative methods. No clinical history was available.
			<b>SPOTFIRE R Panel: 1FN, 1FP, 98TN and 33TP</b>
<b>Influenza A virus</b>	<b>41</b>	<b>41</b>	<b>SPOTFIRE R Panel: 0FN, 0FP, 92TN and 41TP</b>
No subtype/ equivocal	1	1	H1-2009 was detected by the alternative method, The Ct value was 30.65.
H1-2009	14	13 (+ 1 no subtype)	The result of SPOTFIRE R Panel for the discrepant specimen was 'no subtype'. The Ct value was 36.2 by the alternative method.
			<b>SPOTFIRE R Panel: 1FN, 0FP, 119TN and 13TP</b>
H3	25 (+1 no subtype)	26	The result of RP2.1plus for the discrepant specimen was 'no subtype' in the first run and 'equivocal' in the repeated run. For SPOTFIRE R Panel, the result was 'H3'. The Ct value was 31.6 by the alternative method.
			<b>SPOTFIRE R Panel: 0FN, 0FP, 106TN and 27TP</b>
Influenza B virus	8	8	<b>SPOTFIRE R Panel: 0FN, 0FP, 125TN and 8TP</b>
<b>Parainfluenza virus</b>			Reported as 'parainfluenza virus' without subtype by SPOTFIRE R Panel.
Type 1	7	7	The discrepant specimen was negative by RP2.1plus and positive by SPOTFIRE R Panel. Parainfluenza virus 4 was detected by the alternative method.
Type 2	5	5	
Type 3	7	7	
Type 4	3	4	
			<b>SPOTFIRE R Panel: 0FN, 0FP, 110TN and 23TP</b>
Respiratory syncytial virus	10	10	One specimen was positive by RP2.1plus and negative by SPOTFIRE R Panel. The result was negative by the alternative method, and vice versa.
			<b>SPOTFIRE R Panel: 0FN, 1FP, 123TN and 9TP</b>
<i>B. paraptussis</i>	3	4	One specimen was negative by RP2.1plus and positive by SPOTFIRE R Panel. The result was negative by the alternative method. No clinical history was available.
			<b>SPOTFIRE R Panel: 0FN, 1FP, 129TN and 3TP</b>
<i>B. pertussis</i>	2	2	<b>SPOTFIRE R Panel: 0FN, 0FP, 131TN and 2TP</b>
<i>C. pneumoniae</i>	1	1	<b>SPOTFIRE R Panel: 0FN, 0FP, 132TN and 1TP</b>
<i>M. pneumoniae</i>	13	13	<b>SPOTFIRE R Panel: 0FN, 0FP, 120TN and 13TP</b>

Ct, threshold cycle; FN, false negative; FP, false positive; TN, true negative; TP, true positive

**Table S3** Discrepant analysis of co-detections (upper respiratory tract specimens)

Specimen		Results			Remarks
No.	Type	RP2.1plus	SPOTFIRE R	Discrepant analysis	
1	NPS	Flu A H3	Flu A H3 RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
2	NPS	Flu A H3 RV/EV	Flu A H3 RV/EV BPP	<i>B. paraptussis</i> was not detected. No clinical history was available.	RP2.1plus = SPOTFIRE R
9	NPS	Flu A H3	Flu A H3 ADV PIV	Adenovirus was not detected. No recent history of adenovirus infection. Parainfluenza virus 4 was detected	RP2.1plus < SPOTFIRE R
10	NPS	Flu A H3	Flu A H3 RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
19	NS-TS	SARS-CoV-2 ADV	ADV	SARS-CoV-2 was not detected. No recent history of SARS-CoV-2 infection.	RP2.1plus = SPOTFIRE R
20	NPS	MP	MP RV/EV	Rhinovirus was detected. Rhinovirus was detected by RP2.1plus 5 days ago.	RP2.1plus < SPOTFIRE R
22	NS	PIV4	PIV4 ADV	Adenovirus was not detected. No recent history of adenovirus infection.	RP2.1plus = SPOTFIRE R
28	NPS	ADV RV/EV	RV/EV	Adenovirus was detected (Ct value: 40)	RP2.1plus > SPOTFIRE R
29	NPS-TS	ADV	ADV CoV RV/EV	Seasonal coronavirus was not detected with no recent history of infection Rhinovirus was detected	RP2.1plus < SPOTFIRE R
32	NPS	PIV3 CoV OC43	PIV CoV ADV SARS-CoV-2	Adenovirus was not detected with no recent history of infection SARS-CoV-2 was detected (Ct value: 44.3)	RP2.1plus < SPOTFIRE R
41	NPS	PIV3 RV/EV	PIV	Rhinovirus was detected	RP2.1plus > SPOTFIRE R
45	NPS	RSV CoV NL63	RSV CoV RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
55	NPS	Flu A H1-2009	Flu A H1-2009 RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
70	NPS	RV/EV MP	RV/EV MP ADV SARS-CoV-2 RSV	Adenovirus was detected (Ct value: 40) SARS-CoV-2 was detected (Ct value: 38.2) RSV was not detected. No clinical history was available.	RP2.1plus < SPOTFIRE R
78	NPS	MP	MP ADV	Adenovirus was detected (Ct value: 37.8)	RP2.1plus < SPOTFIRE R
79	NPS	BP	BP RV/EV	Rhinovirus or enterovirus was not detected. No clinical history was available.	RP2.1plus = SPOTFIRE R
84	NPS	Flu A H1-2009	Flu A H1-2009 RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
87	NPS	PIV2	PIV RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
91	NPS	SARS-CoV-2 RSV	SARS-CoV-2 RSV RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
102	NPS	ADV RSV	ADV	Respiratory syncytial virus was not detected	RP2.1plus = SPOTFIRE R
126	NPS	CoV NL63	CoV RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
137	NPS-TS	CoV NL63 PIV2 & 4	CoV PIV RV/EV	Rhinovirus was detected	RP2.1plus < SPOTFIRE R
142	NPS	ADV RV/EV	RV/EV	Adenovirus was detected (Ct value: 40)	RP2.1plus > SPOTFIRE R

ADV, adenovirus; BPP, *Bordetella paraptussis*; CoV, coronavirus; Ct, threshold cycle; Flu, influenza; MP, *Mycoplasma pneumoniae*; NPS, nasopharyngeal swab; NS, nasal swab; TS, throat swab; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV/EV, rhinovirus/enterovirus

**Table S4** Results on lower respiratory tract specimens

Specimen No.	Type	RP2.1 <i>plus</i> results	SPOTFIRE R results	Remarks
63	Sputum	1 <sup>st</sup> run: flu A (no subtype) 2 <sup>nd</sup> run: flu A (no subtype)	Flu A (no subtype)	Alternative method: H1-2009, Ct value: 41
83	BALF	<i>Chlamydia pneumoniae</i>	<i>Chlamydia pneumoniae</i>	
90	Sputum	SARS-CoV-2 Flu A H3	SARS-CoV-2 Flu A H3	
100	Sputum	Negative	Negative	
101	Sputum	Parainfluenza virus 3	Parainfluenza virus	
103	Sputum	Negative	Negative	
114	Sputum	Respiratory syncytial virus	Respiratory syncytial virus	
115	ETA	Negative	Negative	
116	Sputum	Flu A H3	Flu A H3	
117	Sputum	Negative	Negative	
118	Sputum	SARS-CoV-2	SARS-CoV-2	
119	Sputum	SARS-CoV-2	SARS-CoV-2	
120	Sputum	Negative	Negative	

BALF, bronchoalveolar lavage fluid; ETA, endotracheal aspirate; flu A, influenza A virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

**Table S5** Results on EQAP samples

Sample	RP2.1 <i>plus</i> results	SPOTFIRE R results	Expected results
1	Influenza B virus Parainfluenza virus 1	Influenza B virus Parainfluenza virus	Influenza B virus Parainfluenza virus 1
2	Influenza A virus H1-2009	Influenza A virus H1-2009	Influenza A virus H1-2009
3	Influenza B virus Rhinovirus/enterovirus	Influenza B virus Rhinovirus/enterovirus	Influenza B virus Rhinovirus/enterovirus
4	Influenza A virus H3 Parainfluenza virus 4	Influenza A virus H3 Parainfluenza virus	Influenza A virus H3 Parainfluenza virus 4
5	Adenovirus <i>Bordetella pertussis</i>	Adenovirus <i>Bordetella pertussis</i>	Adenovirus <i>Bordetella pertussis</i>