

Pathogen	fluorescent channel	Melting temperature $T_m$ (°C)	MPA tube NO.
IFA		52.0±1.0	
PIV-3	FAM	42.0±1.0	
IFB		33.5±1.0	
PIV-1		54.5±1.0	
CP	VIC	48.0±1.0	Reaction tube 1
MP		41.0±1.0	
PIV-2		35.0±1.0	
RSV		53.0±1.0	
PIV-4	ROX	46.0±1.0	
HRV		39.0±1.0	
HADV		31.0±1.0	
SARS-CoV-2-N		35.0±1.0	
SARS-CoV-2-ORF1ab	FAM	44.5±1.0	
SARS-CoV-2-N & SARS-CoV-21ab		35.0±1.0&44.5±1.0	
COV-HKU1		35.5±1.0	Reaction tube 2
COV-NL63	VIC	46.0±1.0	
HBOV		56.0±1.0	
HMPV		36.5±1.0	
COV-OC43	ROX	47.5±1.0	
COV-229E		56.5±1.0	

**Supplementary Table S1.** Fluorescent channel and Melting temperature of each respiratory pathogen probe

pathogens	Viral load ( copies/mL )	Result (+/-)
H1N1 ( 2009 )、HADV-1	10 <sup>3</sup>	+
H1N1、HADV-3	10 <sup>3</sup>	+
H3N2、HADV-7	10 <sup>3</sup>	+
H5N1	10 <sup>3</sup>	+
H7N9	10 <sup>3</sup>	+
PIV1、229E	10 <sup>3</sup>	+
PIV2、HKU1	10 <sup>3</sup>	+
PIV3、OC43	10 <sup>3</sup>	+
PIV4、NL63	10 <sup>3</sup>	+
IFB-Victoria、RSV-A	10 <sup>3</sup>	+
IFB-Yamagat、RSV-B	10 <sup>3</sup>	+
HBOV、HMPV	10 <sup>3</sup>	+
MP、CP	10 <sup>3</sup>	+
HRV	10 <sup>3</sup>	+

**Supplementary Table S2:** Determination of the limit of detection of the MPA assay

Infection type	Pathogens	Number	Percentage (%)
Mono virus	IFA	11	11.58
	HADV	5	5.26
	HMPV	1	1.05
	229E	4	4.21
	PIV3	2	2.11
	OC43	1	1.05
	IFB	4	4.21
	RSV	4	4.21
	HRV	5	5.26
	CP	1	1.05
	MP	7	7.37
	COVID-19	26	27.37
Two viruses	COVID-19+HRV	2	2.11
	HADV+HRV	1	1.05
	HADV+MP	2	2.11
	229E+HRV	1	1.05
	PIV2+RSV	1	1.05
	RSV+MP	13	13.68
Triple viruses	HADV+HRV+MP	1	1.05
	HADV+RSV+MP	1	1.05
	PIV2+RSV+MP	2	2.11

**Supplementary Table S3:** Positive samples with respiratory pathogens confirmed by both the MPA assay and NGS.