Online Supplement

Multiplex Molecular Detection of Respiratory Pathogens in Children with Asthma Exacerbation

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Table 1a Primers used in the first round of multiplex nested PCR

Organism	Primer name	Sequence (5' to 3')*	Product length (bp)
Group 1			
Influenza A virus	FluA-OF3	TYGAGGCTCTCATGGARTGGCTAAAG	412
	FluA-OR3	GCTGGCCARMACCATTCTGTTYTCAT	
Influenza A virus H1	H1-OF1	CCCAGGRTATTTCKCCGAYTATGAGG	760
	H1-OR1	TACCATTCCAGTCCACCCCCCTTCA	
Influenza A virus H3	H3-OF1	ATGGGACCTTTTTRTYGAACGCAGCA	519
	H3-OR1	CCCCKAGGAGCAATTAGATTCCCTGT	
Influenza A virus H5	H5N1-VIET-1B	ATCAAACAGATTAGTCCTTGCG	265
	H5N1-VIET-2B	GGCCTCAAACTGAGTGTTCATT	
Influenza B virus	FluB-OF3	AGGAAGRGCAATGGCAGAYAGAGG	883
	FluB-OR2	TGCTGTGTCCCTCCCAAAGAAGAAA	
Group 2			
PIV-1	PIV-1-OF1	TCTGGATCCACCACAATTTCAG	848
	PIV-1-OR1	WACCAGTTGCAGTCTKGGTTTC	
PIV-2	PIV2-F1	CTTGCAGCATTTTCTGGGGAACTCC	716
	PIV2-OR1	GCATCATCATCCTGGGAGCCTCTGT	
PIV-3	PIV-3-OF1	GATTTTTGGAGATGCACGTCTG	1118
	PIV-3-OR1	GAGAGTGTTYTGTTTCGGATGG	
PIV-4	PIV-4AB-IF1	AYGGATGCATTCGAATTCCATCATTC	432
	PIV-4AB-OR1	TCCRTRAGRCCYCCATACAARGG	
Group 3			
RSV A	RSV-A-OF2	CAGCTCCGTTATCACATCTCTAGGAGCC	576
	RSV-A-OR2	TGGGTTGTCTATGAGCAGATAKKAAAC CA	
RSV B	RSV-B-OF2	CGGGCCAGAAGAGAAGCACCACAGTA	673
	RSV-B-OR2	TGATCCTTCTTTGATGTTGGTGGTGC	
HRV	OL26-MOD-RV	CACTTCTGTTTCCCCGGAGCGAG	388
	RV-OR2	GAAACACGGACACCCAAAGTAGTCGGT	
EV **	EV-OF3	CTGCGYTGGCGGCCYMCC	481
	EV-OR2	CCGGATGGCCAATCCAATAACTATATGG T	
Group 4			
HCoV-OC43	HCoVOC43-OF3	CGGTTACACTGTTCAGCCAATYGCA	793

	HCoVOC43-OR3	CCAACCCAAAAATGCTTGTGGTYG	
SARS-CoV	COR1	CACCGTTTCTACAGGTTAGCTAACGA	310
	COR2	AAATGTTTACGCAGGTAAGCGTAAAA	
HCoV-229E	COR229E-IF2	TCACCCATTTGAAGAATTGGAATTTTGG	566
	COR229E-IR2	TCGTACGTAGAAAACCCAGCCTGTGC	
HMPV	Meta-M-OF2	CAATATGGTTCCCTTTGTTTCAGGCCA	462
	Meta-M-OR2	TGGTCTGCTTCACTGCTTATWGCAGCTT	
Group 5			
M. pneumoniae	Mpneumoniae-OF2	GACCATTCCACCCAGCCCCAGC	343
	Mpneumoniae-OR2	GTTCAGCGAGTGGGGTGCGTACAATA	
C. pneumoniae	Chlamy-pneum-OF4	TGCGCTACTTGGTGCGACGCTA	571
	Chlamy-pneum-OR4	CGCCTTTATAGCCCTTGGGTTTRTTT	
HBoV	BocaVP-OF2	GGGACGCACCACAAAACACCT	422
	BocaVP-OR2	GCCAGCTGTGAGGTCATTGTTGT	
Adenovirus 1, 2 & 3	ADVAtoF-OF3	TACATGCACATCKCSGGVCAGGA	983
	ADVAtoF-OR3	CCRGCCARHACHCCCATRTTDCCHGT	

EV: enterovirus; HBoV: human bocavirus; HCoV: human coronavirus; HMPV: human metapneumovirus; HRV: human rhinovirus; PIV: parainfluenza; RSV: respiratory syncytial virus.

* Degenerate primer abbreviations are as follows: M, A/C; R, A/G; W, A/T; S, C/G; Y, C/T; K, G/T; V, A/C/G; H, A/C/T; D, A/G/T; N, A/C/G/T.

** Included coxsackie A9, B1, B2, B3 and B5; Echo 7, 11 and 30; EV71; and polio 1.

Table 1b Primers used in the second round of multiplex nested PCR

Organism	Primer name	Sequence (5' to 3')*	Product length (bp)
Group 1			
Influenza A virus	FluARe-F1	AAGACCAATCCTGTCACCTCTGA	104
	FluARe-R1	CAAAGCGTCTACGCTGCAGTCC	
Influenza A virus H1	H1-IF2	TCGCCGACTATGAGGAACTGAGGGA	431
	H1-IR2	TTGTATCCCCGGGTTCCAGCAGAGT	
Influenza A virus H3	H3-IF2	CCCTTATGATGTGCCGGATTATGCC	259
	H3-IR2	GGTGGTGAACCCCCCAAATGTACAA	
Influenza A virus H5	H5N1-VIET-1A	TGCGACTGGRCTCAGAAATA	172
	H5N1-VIET-4B	GGATTCTTTGTCTGCAGCGT	
Influenza B virus	FLUB-IF3	AAAACAARTGCTCTGCRCCYCAAC	516
	FLUB-IR3	CRTCTCCACCTACTTCRTTYCCCCC	
Group 2			
PIV-1	PIV-1-IF1	AATTGGTGATGCAATATATGCKTATTC	600
	PIV-1-IR1	TCGACAACAATYTTTGGCCTATC	
PIV-2	PIV2-F2	AGGACAGCAGAGGACCTCGGCATG	343
	PIV2-R2	ACCTGATGTTCTTTGCGGTATGGGG	
PIV-3	PIV-3-IF1	CAACTGTGTTCRACTCCCAAAG	717
	PIV-3-IR1	TGGGTTYACTCTCGATTTTTGY	
PIV-4	PIV-4AB-IF2	GACGGATGYYTRCKGWATTGTGT	231
	PIV-4AB-IR2	CCRTRAGRCCYCCATACAARGGAA	
Group 3			
RSV A	RSVA-IF2	TGACCCATTAGTGTTCCCCTCTGATGAAT	228
	RSVA-IR2	CTTCTGGCCTTRCAGTATARGAGCAGT	
RSV B	RSV-B-IF1	GTCGCATCTCAACATTGRAAC	336
	RSV-B-IR1	TGGTGCATAGAGGTGATGTGTG	
HRV	RV-OF2	CACTTCTGTTTCCCCGGAGCGAGG	283
	JWA-1B-MOD-RV	CCGCATTCAGGGGCCGGAG	
EV	EV-IF3	CCTCCGGCCCCTGAATGCG	106
	EV-IR3	CCAAAGTAGTCGGTTCCGCYRCRGA	
Group 4			
HCoV-OC43	HCoVOC43-IF2	CKGTGCCCTCTCCATTAAATTGGG	635
	HCoVOC43-IR2	GACCCGAACAGTGCTCACCTATGCC	

SARS-CoV	COR3	AGTGAGATGGTCATGTGTGG	210
	COR4	CACTCATAGAGCCTGTGTTG	
HCoV-229E	COR229E-IF3	TTGGGATTCTAATTGGGCCTTTGTTGC	361
	COR229E-IR3	GCTCGGCACGGCAACTGTCATGTAT	
HMPV	Meta-M-IF2	CCCTTTGTTTCAGGCCAAYACACCACC	431
	Meta-M-IR2	GCAGCTTCAACAGTRGCTGATTCACTC TC	
Group 5			
M. pneumoniae	Mpneumoniae-OF1	AGGGGGTTCTTCAGGCTCAGGTCAA	160
	Mpneumoniae-OR1	CCCCACACATCATTCCCCGTATTA	
C. pneumoniae	Chlamy-pneum-IF6	RCCTACWGGATCCGCTRCTGCRAA	317
	Chlamy-pneum-IR6	GCRCCTACGCTCCAAGMRAAAGWRG	
HBoV	BocaVP-IF4	TGGGGATTTCCACTGGAGGG	176
	BocaVP-IR3	CCATGGAGTTGTGACGCAGCG	
Adenovirus 1, 2 & 3	ADVAtoF-IF3	TGGCYWSCACNTWCTTTGACATYMG	463
	ADVAtoF-IR3	GCRWAWGAHCCRTARCAKGGYTDCAT	

EV: enterovirus; HBoV: human bocavirus; HCoV: human coronavirus; HMPV: human metapneumovirus; HRV: human rhinovirus; PIV: parainfluenza; RSV: respiratory syncytial virus.

* Degenerate primer abbreviations are as follows: M, A/C; R, A/G; W, A/T; S, C/G; Y, C/T; K, G/T; V, A/C/G; H, A/C/T; D, A/G/T; N, A/C/G/T.

Table 1c Thermal cycling parameters for multiplex nested PCR

Cycling conditions for Group 1 (1st and 2nd round)

94˚C	5 min	
94˚C	36 sec	40 cycles
64˚C	36 sec	
72˚C	1 min	
72˚C	10 min	
15˚C	20 min	

Cycling conditions for Group 2 (1st and 2nd round)

94˚C	5 min	
94˚C	36 sec	40 cycles
55˚C	36 sec	
72˚C	1 min	
72˚C	10 min	
15˚C	20 min	

Cycling conditions for Group 3 (1st and 2nd round)

94˚C	5 min	
94˚C	30 sec	35 cycles
64˚C	30 sec	
72˚C	30 sec	
72˚C	10 min	
15˚C	20 min	

Cycling conditions for Group 4 (1st and 2nd round)

94˚C	5 min	
94˚C	36 sec	40 cycles
64˚C	36 sec	
72˚C	1 min	
72˚C	10 min	
15˚C	20 min	

Cycling conditions for Group 5 (1st round)

94˚C	5 min	
94˚C	40 sec	}
62˚C	40 sec	} 40 cycles
72˚C	1 min	
72˚C	10 min	
15˚C	20 min	

Cycling conditions for Group 5 (2nd round)

94˚C	5 min	
94˚C	40 sec	}
58˚C	40 sec	} 40 cycles
72˚C	1 min	
72˚C	10 min	
15˚C	20 min	

Electrophorese the PCR products on 1.5 % agarose for 1 hour at 120V, with 11 cm x 14 cm gel size and visualize PCR products using SYBR Safe pre-staining.