Appendix

Table I. Primers and probes sequences used for respiratory viral targets.

Target Primers and Probe set	Sequence	Sequence Reference and supplier
FLU A		World Health
11A forward	5'-AAGACCAATCCTGTCACCTCTGA-3'	Organisation, 2007. SA
11B reverse	5'-CAAAGCGTCTACGCTGCAGTCC-3'	
11P probe	5'-FAM-TTTGTGTTCACGCTCACCGTGCC-BHQ-3'	
FLU A H1		Personal
16A forward	5'-GGAATAGCCCCCCTACAATTG-3'	communication. Anon.
16B reverse	5'-AATTCGCATTCTGGGTTTCCTA-3'	HPA, Colindale. SOP
16P probe	5'-FAM-CGTTGCCGGATGGA-MGBNFQ-3'	V-5413. LT
FLU A H3		Personal
17A forward	5'-CCTTTTTGTTGAACGCAGCAA-3'	communication. Anon.
17B reverse	5'-CGGATGAGGCAACTAGTGACCTA-3'	HPA, Colindale. SOP
17P probe	5'-FAM-CCTACAGCAACTGTTACC-MGBNFQ-3	V-5413. LT
FLU A H5		Health Protection
9A forward	5'-GGATGGCAGGGAATGGTAGA-3'	Agency (2009).
9B reverse	5'-TCTATTGCCTTTTGAGTGGATTCT T-3'	LT
9P probe	5'FAM-TGGGTACCACCATAGCAAYGAGCAGG-MGBNFQ-3'	
FLU B		Van Elden, L J R et al,
4A forward	5'-AAATACGGTGGATTAAATAAAAGCAA-3'	2001. SA
4B reverse	5'-CCAGCAATAGCTCCGAAGAAA-3'	
4P probe	FAM-5'-CACCCATATTGGGCAATTTCCTATGGC-3'-TAMRA	
RSV A		Gunson, R N et al,
4A forward	5'-AGATCAACTTCTGTCATCCAGCAA-3'	2005. SA
4B reverse	5'-TTCTGCACATCATAATTAGGAG TATCAAT-3'	
4P probe	5'-FAM-CACCATCCAACGGAGCACAGGAGAT-BHQ-3'	
RSV B		Personal
5A forward	5'-AAGATGCAAATCATAAATTCACAGGA-3'	Communication, Anon.
5B reverse	5'-TGATATCCAGCATCTTTCAGTATCTTTATA-3'	Specialist Virology
5P1 probe	5'-FAM-TTCCCTTCCTAACCTGGACATAGCATATAACATACCT-BHQ-3'	Centre, Gartnavel. SA
MPV 1A		Kuypers, J et al. 2005.
3A forward	5'-GCCGTTAGCTTCAGTCAATTCAA-3'	LT
3B reverse	5'-TCCAGCATTGTCTGAAAATTGC-3'	
3P probe	5'-FAM-CAACATTTAGAAACCTTCT-MGBNFQ-3'	
MPV 2B		Kuypers, J et al. 2005.
3A forward	5'-GCCGTTAGCTTCAGTCAATTCAA-3'	LT
3B reverse	5'-TCCAGCATTGTCTGAAAATTGC-3'	
4P probe	5'-FAM-CGCACAACATTTAGGAATCTTCT-MGBNFQ-3'	
PF1		Personal
2A forward	5'-GTGATTTAAACCCGGTAATTTCTCA-3'	Communication, Anon.
2B reverse	5'-CCTTGTTCCTGCAGCTATTACAAGA-3'	Specialist Virology
2P probe	5'-FAM-ACCTATGACATCAACGAC-MGBNFQ-3'	Centre, Gartnavel. LT
PF2		Personal
2A forward	5'-ATGAAAACCATTTACCTAAGTGATGGA-3'	Communication, Anon.
2B reverse	5'-CCTCCYGGTATRGCAGTGACTGAAC-3'	Specialist Virology
2P probe	5'-FAM-TCAATCGCAAAAGC-MGBNFQ-3'	Centre, Gartnavel. LT

PF3		Gunson, R N et al,
3A forward	5'-CCAGGGATATAYTAYAAAGGCAAAA-3'	2005. SA
3B reverse	5'-CCGGGRCACCCAGTTGTG-3'	
3P probe	5'-FAM-TGGRTGTTCAAGACCTCCATAYCCGAGAAA-BHQ-3'	
COV 229E		Gunson, R N et al,
6A forward	5'-CAGTCAAATGGGCTGATGCA-3'	2005. SA
6B reverse	5'-AAAGGGCTATAAAGAGAATAAGGTATTCT-3'	
6P probe	5'-FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ-3'	
COV OC43		Gunson, R N et al,
7A forward	5'-CGATGAGGCTATTCCGACTAGGT-3'	2005. SA
7B reverse	5'-CCTTCCTGAGCCTTCAATATAGTAACC-3'	
7P probe	5'-FAM-TCCGCCTGGCACGGTACTCCCT-BHQ-3'	
COV NL63		Gunson, R N et al,
8A forward	5'-ACGTACTTCTATTATGAAGCATGATATTAA-3'	2005. SA
8B reverse	5'-AGCAGATCTAATGTTATACTTAAAACTACG-3'	
8P1 probe	5'-FAM-ATTGCCAAGGCTCCTAAACGTACAGGTGTT-BHQ-3'	
COV HKU1		Raymond, F et al,
9A forward	5'-TTGAAGGCTCAGGAAGGTCTGCT-3'	2009. LT
9B reverse	5'-TGCTTAGTKACTTGCTGAGGTTTAG -3'	
9P probe	5'-6FAM-TAAAACAAGATTAGCGATCTC-MGBNFQ-3'	
ADV		Heim, A et al, 2003.
3A forward	5'-GCCACGGTGGGGTTTCTAAACTT-3'	SA
3B reverse	5'-GCCCCAGTGGTCTTACATGCACATC-3'	
3P probe	5'-FAM-TGCACCAGMCCSGGGCTCAGGTACTCCGA-BHQ-3'	
HRV 1		In-house development.
7A forward	5'-AGCCTGCGTGGCTGCCTG-3'	Regional Virus
7A2 forward	5'-CCTGCGTGGCGGCCARC-3'	Laboratory, Belfast.
7B reverse	5'-CCCAAAGTAGTYGGTCCCRTCC-3'	LT
7P probe	5'-FAM-TCCTCCGGCYCCTGAATG-3'-MGBNFQ	
HRV 2		Scheltinga, S A et al,
8A forward	5'-GACARGGTGTGAAGAGCC-3'	2005. SA
8B forward	5'-GACATGGTGTGAAGACYC-3'	
8C reverse	5'-CAAAGTAGTYGGTCCCATCC'-3	
8P probe	5'-FAM-TCCTCCGGCCCCTGAATGYGGCTAA-3'-BHQ1	
BOV		Allander, T et al, 2007.
2A forward	5'-GGAAGAGACACTGGCAGACAA-3'	SA
2B reverse	5'-GGGTGTTCCTGATGATATGAGC-3'	
2P probe	5'-FAM-CTGCGGCTCCTGCTCCTGTGAT-BHQ-3'	
RNP		World Health
1A forward	5'-AGATTTGGACCTGCGAGCG-3'	Organisation, 2009.
1B reverse	5'-GAGCGGCTGTCTCCACAAGT-3'	SA
1P probe	5'-6FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ-3'	

For each target in the test panel, the forward and reverse primers, probe sequences, reporters and quenchers are presented, along with their initial source references and supplier details.

Abbreviations: “ADV: adenovirus; BHQ: Blackhole Quencher;BOV: bocavirus; FAM: 6-carboxyfluorescein (reporter molecule); COV: coronavirus; FLU A: influenza A; FLU B: influenza B; **LT**: Life Technologies (Paisley, UK); MGBNFQ: minor groove binding nonfluorescent quencher; MPV: metapneumovirus; PF:

parainfluenza; RHV: rhinovirus; RNP: Ribonuclease P; RSV: Respiratory Syncytial Virus;SA: Sigma-Aldrich Company Ltd(Dorset, UK); TAMRA: Carboxytetramethylrhodamine".

Table 1. List of reagents used in the preparation of respiratory viral-related amplification mixes.

Each provides instruction for the preparation of 1000 tests when reaction volume used is 8µl. All volumes shown are µl.

All RNA target TaqMan master mixes were prepared using SuperScript® III Platinum® One-Step Quantitative RT-PCR System (Life Technologies, Paisley, UK).

Influenza A Matrix

Nuclease free water	**1580**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
FLA 11A @ 200µM (final 0.4µM)	**20**
FLA 11B @ 200µM (final 0.4µM)	**20**
FLA 11P @ 100µM (final 0.2µM) FAM-BHQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

Influenza A H1(seasonal)

NFW	**1580**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
FLA 16A @ 200µM (final 0.4µM)	**20**
FLA 16B @ 200µM (final 0.4µM)	**20**
FLA 16P @ 100µM (final 0.2µM) FAM-TAMRA	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

Influenza A H3

NFW	**1580**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
FLA 17A @ 200µM (final 0.4µM)	**20**
FLA 17B @ 200µM (final 0.4µM)	**20**
FLA 17P @ 100µM (final 0.2µM) FAM-TAMRA	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

Influenza A H5

NFW	**1580**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
FLA 9A @ 200µM (final 0.4µM)	**20**
FLA 9B @ 200µM (final 0.4µM)	**20**
FLA 9P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Influenza B

NFW	**1580**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**

FLB 4A @ 200µM (final 0.4µM)	**20**
FLB 4B @ 200µM (final 0.4µM)	**20**
FLB 4P @ 100µM (final 0.2µM) FAM-TAMRA	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: RSV Types A&B

NFW	**1680**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
RSV 4A @ 200µM (final 0.4µM)	**20**
RSV 4B @ 200µM (final 0.4µM)	**20**
RSV 4P @ 100µM (final 0.2µM) FAM-BHQ	**20**
RSV 5A @ 200µM (final 0.4µM)	**20**
RSV 5B @ 200µM (final 0.4µM)	**20**
RSV 5P1 @ 100µM (final 0.2µM) FAM-BHQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Metapneumovirus

NFW	**1520**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
MPV 3A @ 200µM (final 0.4µM)	**20**
MPV 3B @ 200µM (final 0.4µM)	**20**
MPV 3P @ 100µM (final 0.2µM) FAM-MGB	**20**
MPV 3A @ 200µM (final 0.4µM)	**20**
MPV 4B @ 200µM (final 0.4µM)	**20**
MPV 4P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Rhinovirus1

NFW	**1560**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
HRV 7A @ 200µM (final 0.4µM)	**20**
HRV 7A2 @ 200µM (final 0.4µM)	**20**
HRV 7B @ 200µM (final 0.4µM)	**20**
HRV 7P @ 100µM (final 0.2µM) FAM-MGB	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Rhinovirus2

NFW	**1660**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 3.5mM)	**80**
HRV 8A @ 200µM (final 0.2µM)	**10**
HRV 8B @ 200µM (final 0.2µM)	**10**
HRV 8C @ 200µM (final 0.4µM)	**20**
HRV 8P @ 100µM (final 0.2µM) FAM-BHQ	**20**

Superscript III RT/Platinum® *Taq* Mix	**200**

RNP

NFW	**1700**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
RNP 1A @ 200µM (final 0.8µM)	**40**
RNP 1B @ 200µM (final 0.8µM)	**40**
RNP 1P @ 100µM (0.2µM) FAM-BHQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Parainfluenza 1

NFW	**1580**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
PF1 2A @ 200µM (final 0.4µM)	**20**
PF1 2B @ 200µM (final 0.4µM)	**20**
PF1 2P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Parainfluenza 2

NFW	**1580**
Reaction mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
PF2 2A @ 200µM (final 0.4µM)	**20**
PF2 2B @ 200µM (final 0.4µM)	**20**
PF2 2P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Parainfluenza 3

NFW	**1580**
Reaction Mix x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50mM (final 4mM)	**160**
PF3 3A @ 200µM (final 0.4µM)	**20**
PF3 3B @ 200µM (final 0.4µM)	**20**
PF3 3P @ 100µM (final 0.2µM) FAM-BHQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Parainfluenza MULTIPLX

NFW	**1460**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
PF1 2A @ 200µM (final 0.4µM)	**20**
PF1 2B @ 200µM (final 0.4µM)	**20**
PF1 2P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
PF2 2A @ 200µM (final 0.4µM)	**20**

PF2 2B @ 200µM (final 0.4µM)	**20**
PF2 2P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
PF3 3A @ 200µM (final 0.4µM)	**20**
PF3 3B @ 200µM (final 0.4µM)	**20**
PF3 3P @ 100µM (final 0.2µM) FAM-BHQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Coronavirus 229E, OC43, NL63and HKU1 MULTIPLEX

NFW	**1400**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
COV 6A @ 200µM (final 0.4µM)	**20**
COV 6B @ 200µM (final 0.4µM)	**20**
COV 6P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
COV 7A @ 200µM (final 0.4µM)	**20**
COV 7B @ 200µM (final 0.4µM)	**20**
COV 7P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
COV 8A @ 200µM (final 0.4µM)	**20**
COV 8B @ 200µM (final 0.4µM)	**20**
COV 8P1@ 100µM (final 0.2µM) FAM-BHQ	**20**
COV 9A @ 200µM (final 0.4µM)	**20**
COV 9B @ 200µM (final 0.4µM)	**20**
COV 9P@ 100µM (final 0.2µM) FAM-MGB	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Coronavirus 229E

NFW	**1580**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
COV 6A @ 200µM (final 0.4µM)	**20**
COV 6B @ 200µM (final 0.4µM)	**20**
COV 6P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Coronavirus OC43

NFW	**1580**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
COV 7A @ 200µM (final 0.4µM)	**20**
COV 7B @ 200µM (final 0.4µM)	**20**
COV 7P @ 100µM (final 0.2µM) FAM-MGBNFQ	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

TaqMan PCR: Single Mix: Coronavirus NL63

NFW	**1580**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
COV 8A @ 200µM (final 0.4µM)	**20**
COV 8B @ 200µM (final 0.4µM)	**20**

COV 8P1 @ 100µM (final 0.2µM) FAM-BHQ1	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

Taqman PCR: Single Mix: Coronavirus HKU1

NFW	**1580**
Buffer @ x2	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgSO_4$ @ 50m (final 4mM)	**160**
COV 9A @ 200µM (final 0.4µM)	**20**
COV 9B @ 200µM (final 0.4µM)	**20**
COV 9P @ 100µM (final 0.2µM) FAM-BHQ1	**20**
Superscript III RT/Platinum® *Taq* Mix	**200**

All DNA target TaqMan amplification mixes were prepared with Platinum® Quantitative PCR SuperMix-UDG (Life Technologies, Paisley, UK).

TaqMan PCR: Bocavirus DNA

NFW	**1740**
Platinum® qPCR Supermix-UDG	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgCl_2$ 50mM (final 4mM)	**200**
BOV 2A @ 200µM (final 0.4µM)	**20**
BOV 2B @ 200µM (final 0.4µM)	**20**
BOV 2P @ 100µM (final 0.2µM) FAM-TAMRA	**20**

TaqMan PCR: Adenovirus DNA

NFW	**1740**
Platinum® qPCR Supermix-UDG	**5000**
BSA @ 2µg/µl (final 0.2µg/µl)	**1000**
$MgCl_2$ @ 50mM (final 4mM)	**200**
ADV 3A @ 200µM (final 0.4µM)	**20**
ADV 3B @ 200µM (final 0.4µM)	**20**
ADV 3P @ 100µM (final 0.2µM) FAM-BHQ	**20**

Abbreviations: “ADV: adenovirus; BHQ: Blackhole Quencher; BOV: bocavirus; BSA: Bovine serum albumin; COV: coronavirus; FAM: 6-carboxyfluorescein; FLU A: influenza A; FLU B: influenza B; MGBNFQ: minor groove binding nonfluorescent quencher; $MgCl_2$: Magnesium chloride; $MgSO_4$: Magnesium sulphate; MPV: metapneumovirus; NFW: nuclease free water; PF: parainfluenza; RHV: rhinovirus; RSV: Respiratory Syncytial Virus;TAMRA: Carboxytetramethylrhodamine.

Table 3. Primers used in reverse-tagging step (PCR 2).

Name	Sequence	Length
806R_ f1	GTGAaGGAGHcAGAcGTGTGCTCCGATCTN NNNNACGGACTACHV GGG TW TCTAAT	61
806R_ f2	GTGACTGGAGUCAGACGTGTGCTCUCCGATCTNNTNNNACGGACTACHV GGGTWTCTAAT	62
806R_ f3	GTGAaGGAGUcAGACGTGTGamCCGATCT NNCTNN NAC GGACTACHV GCGTWTCTAAT	63
806R_f4	GTGACTGGAGUCAGACGTGTGCTmCCGATCTNNACTNNNACGGACTACHV GGGTWTCTAA	64
806R_f5	GTGACTGGAGTrCAGACGTGTGCTCTrCCGATCTNNGAaNNNACGGAaACHW GGGTWTCTAAT	65
806R_ f6	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNGACTNNNACGGACTACHV GGGTWTCTAAT	66

Table 4. Primers for forward-tagging step (PCR 3).

Name	Sequence	Length
515F_f1	GCCTCCCTCGCGCCATCAGAGATGTG TATAAGAGACAG NNNN NNNNGA GTGCCA GCMGCCGCGGTAA	67
515F_f2	GCCTCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG NNNNNNNN GAGTGCCA GCM GCCGCGGTAA	68
515F_f3	GCCTCCCTCGCGCCATCAGAGATGTG TATAAGAGACAG NNNN T NNNN GA GTGCCAGCMGCCGCGGTAA GCCTCCCTCGCGCCATCAGAGATGTG	69
515F_f4	TATAAGAGACAG NNNN CT NNNN GAGTGCCAGCMGCCGC GGTAAGCCTCCCTC GCGC C ATCAGAGATGTG TATAAGAGACAG NNNN ACT NNNN GA	70
515F_f5	GTGCCAGCMGCCGCGGTAA GCCTCCCTCGCGCCATC AGAGATGTG TATAAGA GACAG NNNN GACT NNNN GA	71
515F_f6	GTGCCAGCMGCCGCGGTAAGCCTCCCTCGCGCCATCAGAGATGTG TATAAGAG ACAG NNNNTGACT NNNN GA GTGCCAGCMGCCGCGGTAA	72