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SUPPLEMENTAL INFORMATION

2 Table S1. Oligonucleotides used for the in-house RT-PCR assays targeting the M, H7 or N9

3 genes.

Assay (s)	Target	Name	Sequence (5' → 3')	Comments
CDC M (NS), CDC M (PE), and CDC M (SK)	M (FluA)	InfA Forward	GAC CRA TCC TGT CAC CTC TGA C	CDC M gene assay (ref. 1)
		InfA Reverse	AGG GCA TTY TGG ACA AAK CGT CTA	
		InfA Probe	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1	
CDC*M (AB)	M (FluA)	CDC-M-F	GAC CRA TCC TGT CAC CTC TGA C	TAMRA quencher instead of BHQ1 for the FluA probe.
		CDC-M-R	AGG GCA TTY TGG ACA AAK CGT CTA	
		CDC-M-Probe	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-TAMRA	
CDC	M (FluA)	CDCfluAf	CAT GGA RTG GCT AAA	2005 CDC FluA

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M*(QC)			GAC AAG ACC	protocol. While the forward and reverse primers differ from the current assay, the probe sequence is identical.
		CDCfluAr	AGG GCA TTT TGG ACA AAK CGT CTA	
		CDC FluA probe	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1	
CDC M*(QC2)	M (FluA)	CDCfluAH7N9f	CAT GGA GTG GMT AAA GAC AAG ACC	Modified forward and reverse sequences derived from the 2005 CDC FluA protocol.
		CDCfluAH7N9r	AGG GCR TTT TGG ACA AAB CGT CTA	
		CDC FluA probe	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1	
CDC*M (MB)	M (FluA)	FluA Forward	GAC CRA TCC TGT CAC CTC TGA C	No sequence modifications for FluA primers or probe; however, the assay is a duplex RT-PCR with FluB.
		FluA Reverse	AGG GCA TTY TGG ACA AAK CGT CTA	
		FluA Probe	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1	
	NEP/NS1	FluB Forward	TCC TCA ACT CAC TCT	

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	(FluB)		TCG AGC G	
		FluB Reverse	CGG TGC TCT TGA CCA AAT TGG	
		FluB Probe	HEX-CCA ATT CGA GCA GCT GAA ACT GCG GTG- BHQ_1	
CDC M*(NS) and CDC M*(BC)	M (FluA)	FluA F	GAC CRA TCC TGT CAC CTC TGA C	Quadruplex for FluA, FluB, RSV, and RNaseP. For FluA, the M gene forward primer is unchanged, whereas the reverse primer was shortened by 1 nucleotide at the 5' end and extended by 2 nucleotides at the 3' end. The FluA probe contains the same FAM
		FluA R	GGG CAT TYT GGA CAA AKC GTC TAC G	
		FluA pr	FAM/ZEN- TGC AGT CCT CGC TCA CTG GGC ACG- IaBkFQ	
	NP (FluB)	FluB F	TCC TCA ACT CAC TCT TCG AGC G	
		FluB R	CGG TGC TCT TGA CCA AAT TGG	
		FluB pr	MAX/ZEN-CCA ATT CGA GCA GCT GAA ACT GCG	

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			GTG-IaBkFQ	fluorophore, but the quencher(s) differ (internally quenched by ZEN and the 3' end contains IaBkFQ).
	L (RSV)	RSV F	GGA YTG TTT ATG AAT GCC TAT GGT	
		RSV R	GGR TTR TTC AAT ATA TGG TAG AAT CC	
		RSV pr	Cy5-TCC ACA ACT TGY TCC ATT TCT GC-IaBkRQ	
	RNaseP	RNaseP F	AGA TTT GGA CCT GCG AGC G	
		RNaseP R	GAG CGG CTG TCT CAA CAA GT	
		RNaseP pr	NED-TCT GAC CTG AAG GCT C-MGBNFQ	
WHO H7 (SK)	H7 (FluA)	CNIC-H7F	AGA AAT GAA ATG GCT CCT GTC AA	WHO H7 protocol (collaboration with CNIC, ref. 2)
		CNIC-H7R	GGT TTT TTC TTG TAT TTT TAT ATG ACT TAG	
		CNIC-H7P	FAM-AGA TAA TGC TGC ATT CCC GCA GAT G-BHQ1	

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CDC*H7 (MB), and CDC*H7 (SK)	H7 (FluA)	H7N9 Forward	GAA TGC ACA GGG AGA GGG AAC TG	Based on H7N9 sequences available from GISAID, the CDC H7 primers and probe were modified. The probe is labeled at the 5'-end with FAM, is quenched internally at a modified "T" residue with BHQ1, and has a 3' C3 Spacer
		H7N9 Reverse	CAT TGC TAC CAA GAG TTC AGC AT	
		H7N9 Probe	FAM-AAA GCA CTC AA"T"(BHQ1) CGG CAA TTG ATC AAA TAA CAG GA-Spacer C3	
WHO N9 (SK)		CNIC-N9F	TAG CAA TGA CAC ACA CTA GTC AAT	WHO N9 protocol (collaboration with CNIC, ref. 2)
		CNIC-N9R	ATT ACC TGG ATA AGG GTC ATT ACA CT	
		CNIC-N9P	FAM-AGA CAA TCC CCG ACC GAA TGA CCC-BHQ1	

1 Abbreviations: 6-carboxyfluorescein (FAM); Alberta (AB); Blackhole Quencher 1 (BHQ1);
2 British Columbia (BC); Centers for Disease Control and Prevention (CDC); Chinese National
3 Influenza Center (CNIC); Global Initiative on Sharing All Influenza Data (GISAID);
4 hemagglutinin 7 (H7); influenza A (FluA); influenza B (FluB); Iowa Black FQ quencher
5 (IaBkFQ); Manitoba (MB); matrix (M); minor groove binder non-fluorescent quencher
6 (MGBNFQ); neuraminidase 9 (N9); Nova Scotia (NS); nuclear export protein (NEP); non-
7 structural protein 1 (NS1); not applicable (N/A); Ontario (ON); Prince Edward Island (PE);
8 Québec (QC); respiratory syncytial virus (RSV); ribonuclease P (RNaseP); Saskatchewan (SK);
9 World Health Organization (WHO).

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11 **Supplemental References**

- 12 1. **WHO:** CDC protocol for real-time RT-PCR for swine influenza A H1N1.
13 [http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20](http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf)
14 [090428.pdf](http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf). (last accessed July 25, 2013).
15 2. [http://www.who.int/influenza/gisrs_laboratory/cnic_realtime_rt_pcr_protocol_a_h7n9.pd](http://www.who.int/influenza/gisrs_laboratory/cnic_realtime_rt_pcr_protocol_a_h7n9.pdf)
16 [f](http://www.who.int/influenza/gisrs_laboratory/cnic_realtime_rt_pcr_protocol_a_h7n9.pdf). (last accessed July 25, 2013).

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