Appendix A. Pandemic H1N1 vaccine sequencing by participating provinces with roll out beginning on the specified dates*

	British Columbia	Alberta	Ontario	Quebec
Week 43 Oct 25-31, 2009	October 26 • People < 65 yoa with chronic conditions • Women in the second half of pregnancy and any pregnant women with chronic conditions • Residents of remote and isolated communities	October 26 • General campaign started but halted October 31 to enable sequencing	October 26 • People < 65 yoa with chronic conditions • Pregnant women • Healthy children 6 moa to < 5 yoa • Residents of remote and isolated communities • HCW • Household contacts and care providers of persons at high risk who may not mount their own response to vaccine	October 26 • HCW
Week 44 Nov 1-7, 2009	November 2 • Children 6 moa to < 5 yoa • Household contacts (< 65 yoa) of infants < 6 moa and of immuno-compromised people • HCW delivering critical care (i.e. Emergency Room, Intensive Care, specialized units)	November 5 • Children 6 moa to < 5 yoa • HCW with direct patient contact November 6 • Pregnant women		November 2 • Children 6 moa to 4 yoa • Families of infants < 6 moa November 6 • Pregnant women
Week 45 Nov 8-14, 2009	November 9 • Women in the first half of pregnancy (unadjuvanted formulation available) • Other HCW	November 10 • Children 5 to 9 yoa with chronic conditions • Parents and caregivers of infants < 6 moa November 12 • Children 10 to 17 yoa with chronic conditions • Adults 55 to 64 yoa with chronic conditions November 13 • Adults 45 to 54 yoa with chronic conditions November 14 • Adults 18 to 44 yoa with chronic conditions • Household contacts of persons who cannot be immunized	November 9 • Adults ≥ 65 yoa in health care institutions • First responders and frontline workers in adult and youth facilities	November 10 • People with chronic conditions 6 moa to < 65 yoa November 12 • Children 5 to 19 yoa
Week 46 Nov 15-21, 2009	November 16 • People ≥ 65 yoa with chronic conditions • Healthy children 5 to 18 yoa • First responders not covered above November 19 • General population	November 16 • HCW and first responders November 17 • Adults > 75 yoa and spouses/partners November 19 • Adults ≥ 65 yoa and spouses/partners November 20 • Healthy children 5 to 18 yoa and their household contacts	November 16 • Children ≤ 13 yoa • Adults ≥ 65 yoa with chronic conditions November 19 • General population	November 20 • Adults ≥ 65 yoa
Week 47 Nov 22-28, 2009		November 23 • General population		November 25 • General population

***NOTE:** Sequencing was cumulative – those included earlier in the campaign remained eligible in subsequent weeks.
HCW = health care worker; yoa = years of age; moa = months of age.

Appendix B – Laboratory methods by province November 1 - December 31, 2009

1. BRITISH COLUMBIA

In British Columbia all respiratory specimens were first tested for all influenza A viruses by a single real-time reverse transcription-PCR (RT-PCR) targeting the matrix (M) gene[1] with the addition of a second probe specific for pH1N1 (**VIC-aaagacactttccagtctc-MGB-NFQ**). Sub-typing for seasonal human influenza A (A/H1 or A/H3) was performed by real-time RT-PCR targeting the HA gene (CDC real-time PCR protocol). Specimens in which influenza A could not be detected were then tested by the Luminex RVP Assay (Luminex, Canada) which detects common respiratory viruses including seasonal influenza A and B viruses[2,3].

2. ALBERTA:

In Alberta, all specimens were tested by an RT-PCR to the M gene of influenza A[4] followed by the Luminex RVP Assay. Although pandemic influenza A/H1N1 is detected in the RVP assay it is reported as non-typable. All influenza A positives arising from the screening singleplex RT-PCR and non-typables from the Luminex RVP were tested in the in-house subtyping assay for pandemic influenza A/H1N1 [5].

3. ONTARIO:

Testing for seasonal influenza consisted of screening using an RT-PCR assay targeting the matrix (M) gene of influenza A virus[1,2,5] and the NS1 gene of influenza B virus (CDC real-time RT-PCR protocol). Sub-typing for seasonal human influenza A (A/H1 or A/H3) was performed by real-time RT-PCR targeting the HA gene (CDC protocol). Specimens were also tested using a multiplex molecular kit (Luminex® RVP or Seeplex® RV) in addition to the CDC real-time RT-PCR assay. Specimens were prospectively tested for influenza A alone by the screening real-time RT-PCR assay and were retrospectively tested for influenza B by real-time RT-PCR or a multiplex molecular kit. Influenza A positive specimens were subtyped by realtime RT-PCR. The A/H1 and A/H3 subtypes were detected as described above. Pandemic influenza A/H1N1 was confirmed using an in-house real-time RT-PCR targeting the HA gene segment using the forward primer **cctgggaaatccagagtgtga**, reverse primer **cgttccattgtctgaactagrtgtt** and probe **tcactctccacagcaagctcatgg**.

4. QUEBEC

All respiratory samples were tested using real-time RT-PCR TaqMan based assays targeting M gene sequences conserved among influenza A virus, the NS1 gene of influenza B virus and M gene specific sequences of the pandemic influenza A/H1N1 subtype. Influenza A positive, pandemic H1N1 negative samples were further tested for seasonal human influenza A (A/H1 or A/H3) by real-time TaqMan RT-PCR targeting the HA gene. All protocols but the pandemic H1N1 assay were according to CDC methods distributed via the Association of Public Health Laboratories. The pandemic influenza A/H1N1 protocol was an in-house developed TaqMan based RT-PCR assay using primers **tagacgctttgtccaaaatg** and **tgaatagcttagtgacacctcc** and the probe **acccgaacaacatggatagagcagtta.**

References for laboratory methods:

1. Fouchier, R.A, Bestebroer, TM, Herfst S, Van Der Kemp L, Rimmelzwaan GF et al. (2000) Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. J. Clin. Microbiol. 38:4096–4101.
2. Mahony JS, Chong F, Merante S, Yaghoubian T, Sinha C, et al. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. J. Clin. Microbiol. 2007;45:2965-2970.
3. Mahony JB, Hatchette T, Ojkic D, Drews SJ, Gubbay J, et al. Multiplex PCR tests sentinel the appearance of pandemic influenza viruses including H1N1 swine influenza. J Clin Virol 2009;45:200-202.
4. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans N Engl J Med. 2009;360:2605-15
5. Pabbaraju K, Wong S, Wong AA, Appleyard GD, Chui L et al. Design and validation of real-time reverse transcription-PCR assays for detection of pandemic (H1N1) 2009 virus. J Clin Microbiol 2009;47:3454-60.

Appendix C – Characteristics of controls by pandemic H1N1 vaccine status during the primary analysis period spanning illness onset from week 45 (November 8, 2009) through week 48 (December 5, 2009)

	Vaccinated[a] against pandemic H1N1	Not vaccinated[b] against pandemic H1N1	Overall
	n/N (%)	n/N (%)	n/N (%)
Age category	58	285	343
6 months - 9 years	22 (38)	49 (17)	71 (21)
10 -19 years	3 (5)	53 (19)	56 (16)
20 - 49 years	21 (36)	127 (45)	148 (43)
50 - 64 years	11 (19)	36 (13)	47 (14)
65+ years	1 (2)	20 (7)	21 (6)
Median (Range), years	22 (<1 -88)	27 (<1-87)	27 (<1 -88)
Sex			
Female	37 (64)	163 (57)	200 (58)
Male	20 (34)	117 (41)	137 (40)
Unknown	1 (2)	5 (2)	6 (2)
Chronic Conditions			
No	37 (64)	226 (79)	263 (77)
Yes	21 (36)	59 (21)	80 (23)
Province			
Alberta	11 (19)	55 (19)	66 (19)
British Columbia	19 (33)	114 (40)	133 (39)
Ontario	7 (12)	24 (8)	31 (9)
Quebec	21 (36)	92 (32)	113 (33)
Received 2009-10 seasonal vaccine			
No	14 (52)	179 (95)	193 (90)
Yes	13 (48)	9 (5)	22 (10)
Received 2008-09 seasonal vaccine*			
No	14 (35)	199 (78)	213 (72)
Yes	26 (65)	57 (22)	83 (28)
Specimen collection interval			
≤4 days	46 (79)	251 (88)	297 (87)
>4 days	12 (21)	34 (12)	46 (13)
Median (Range), days	3 (0-7)	2 (0-7)	2 (0-7)

NOTE: Inclusion criteria as specified in Table 1 of main manuscript apply to this table. Participants who received vaccine within 2 weeks of illness onset excluded from the analysis.

[a]Received vaccine ≥2weeks prior to onset of influenza-like illness

[b]Did not receive vaccine

*Excludes children too young to have received the 2008-09 seasonal vaccine