Updates to CDC Human Influenza Diagnostic RT-PCR Panel

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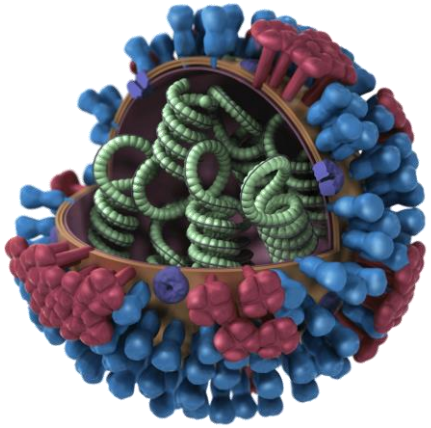

Human Influenza RT-PCR Diagnostic Panel

- DDT developed and maintains a panel of FDA-approved RT-PCR assays to detect/distinguish Influenza A and Influenza B viruses and further classify them into the following subtypes/lineages:
  - Influenza A Subtypes
    - A(H1)pdm09
    - A(H3)
    - A(H5) (Asian lineage)
  - Influenza B Subtypes
    - B/Victoria
    - B/Yamagata

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel		
Kit	**Contents**	**Applications**
A/B Typing Kit	**Primer and Probe Sets:** InfA, InfB, RP **Controls:** Seasonal Influenza Positive Control (SIPC), Human Specimen Control (HSC)	• Detects Influenza A and Influenza B viruses
A Subtyping Kit	**Primer and Probe Sets:** InfA, H3, pdminfA, pdmH1, RP **Controls:** SIPC	• Detects and differentiates seasonal influenza subtypes H3 and H1pdm09 • Detects classical swine influenza viruses and swine triple reassortant viruses (note: must be referred to CDC for further confirmation)
A/H5 Subtyping Kit	**Primer and Probe Sets:** InfA, H5a, H5b, RP **Controls:** H5VC, HSC	• Specifically detects influenza A(H5) Asian lineage viruses
B Lineage Genotyping Kit	**Primer and Probe Sets:** InfB, VIC, YAM, RP **Controls:** Influenza B Positive Control (IBPC)	• Detects and differentiates influenza B lineage genotypes; B/Victoria, B/Yamagata

Recent Increase in Fixed Mutations Within Target Regions

- Recent analysis has shown an increase in influenza viral sequences with fixed mutations within the target regions for a subset of the primers and probes within the influenza RT-PCR diagnostic panel:
  - InfA
  - pdmH1
  - pdmInfA

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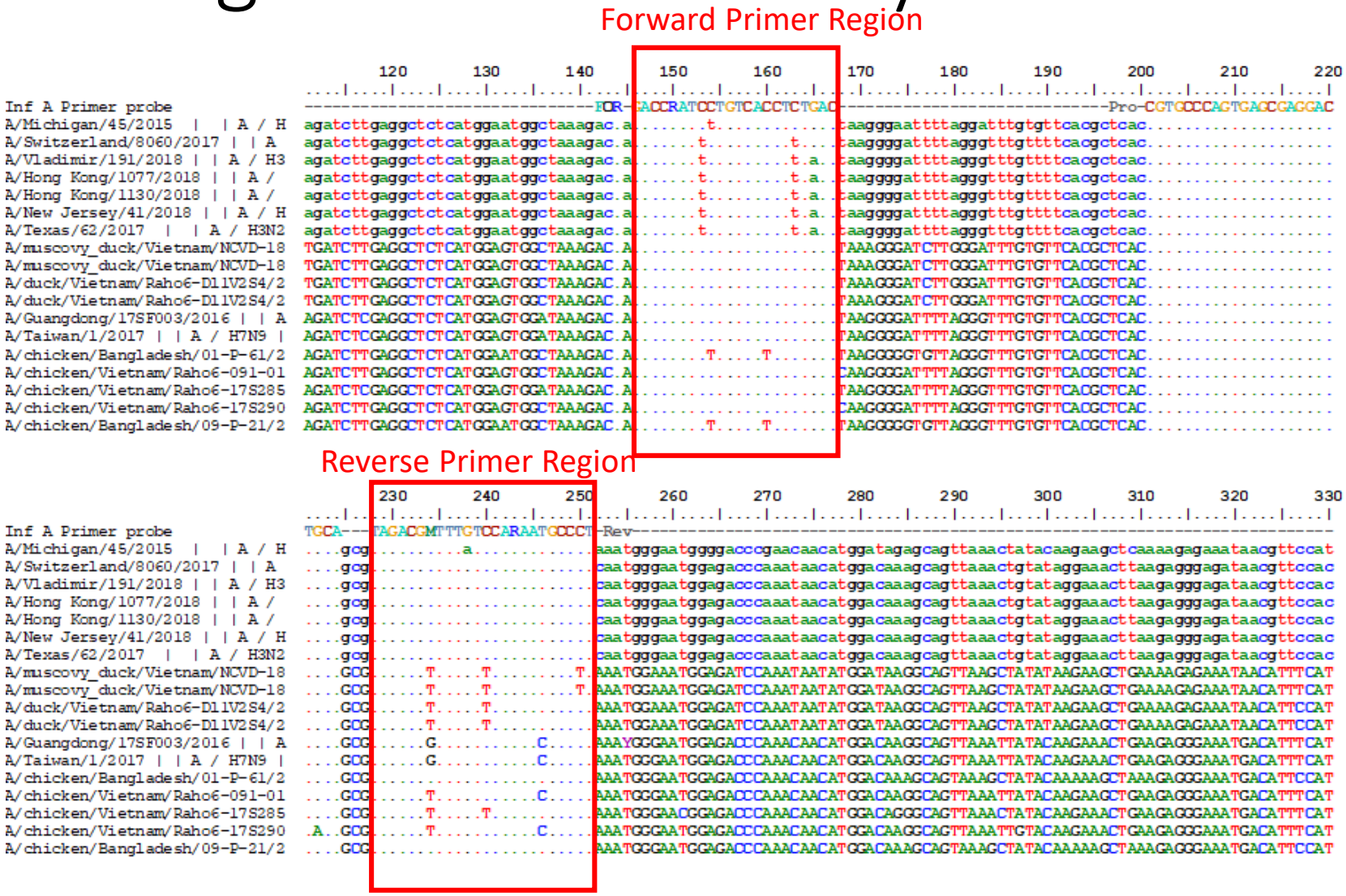
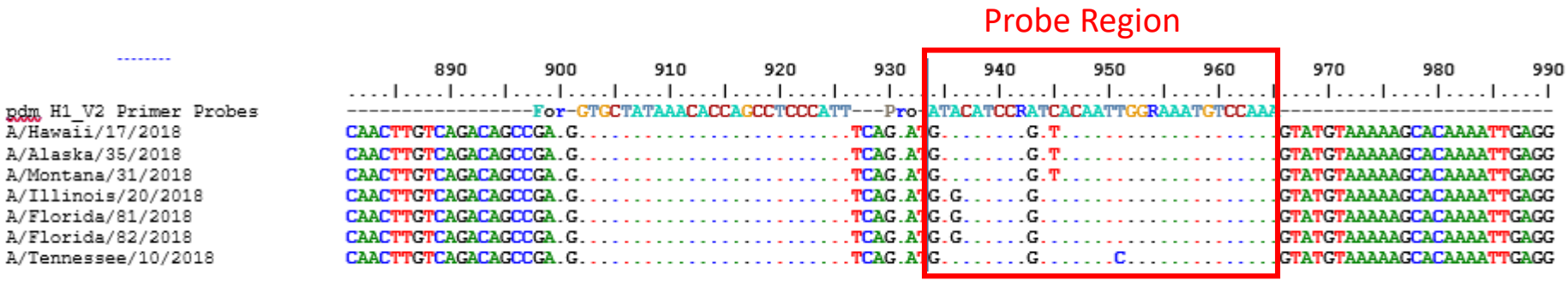
Mutations Within Forward and Reverse Primer Regions of InfA Assay



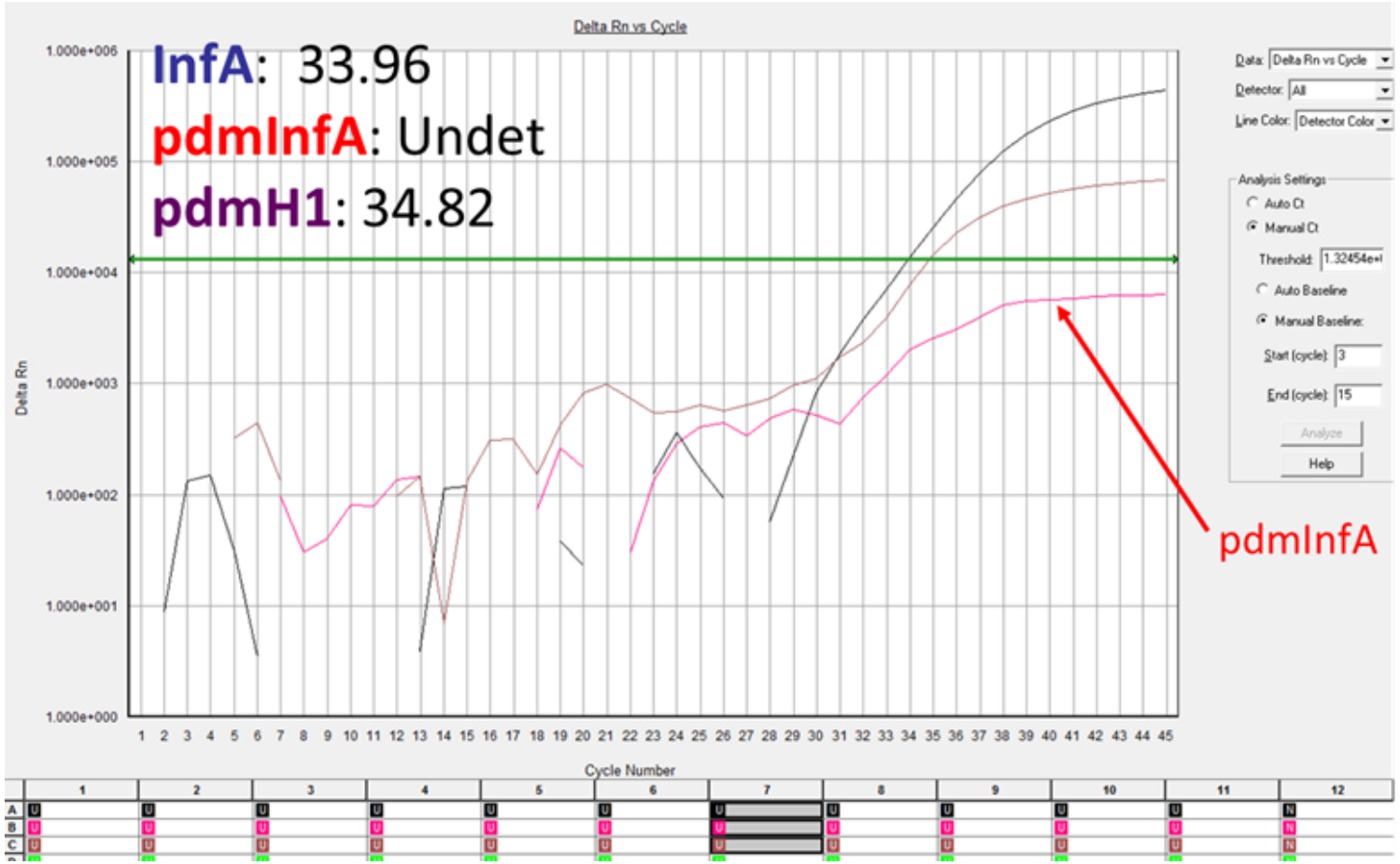
Mutations Within Probe Region of pdmH1 Assay



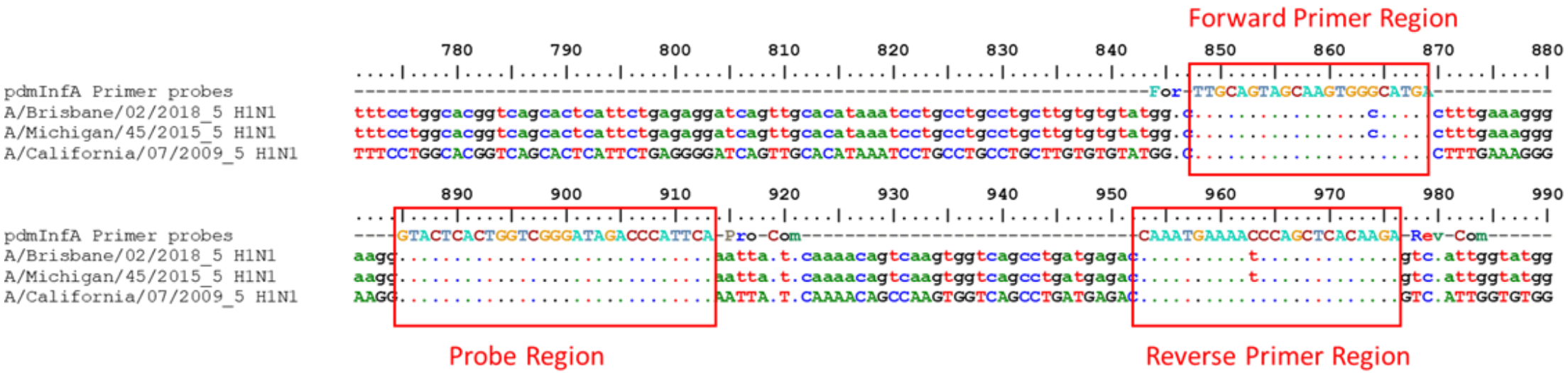
- Steadily increasing numbers of late or failed pdmH1within submissions to CDC
- All mutations seem to be in the Probe region of pdmH1

Loss of florescence in pdmInfA

- pdmInfA lost florescent signal intensity, curve shape, and was often hard to discern from background
- Hard to pinpoint mutations in the seasonal viruses that seemed to account for the loss in signal
- Added a “pad” to the primers to increase primer efficiency

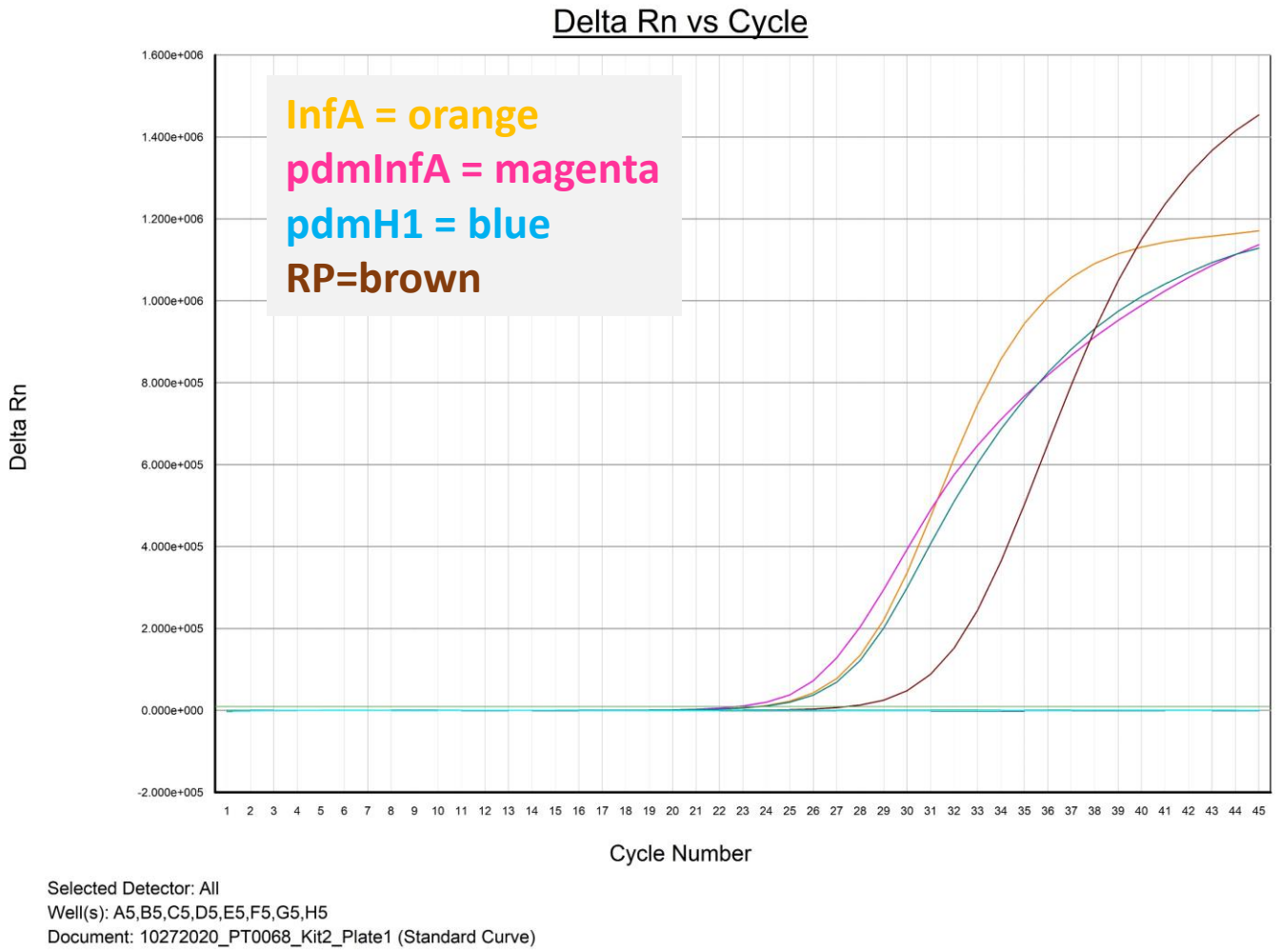


Mutations Within Primer and Probe Regions of pdmInfA Assay



Adding a pad to the pdmInfA primers returns the florescence and curve shape

- Added 12nt AT rich pad sequence to each primer restores performance
- 12 nt sequence does not match influenza and just is placed on primer to increase primer efficiency
- No loss in performance of the assay seen
- No increase in cross reactivity



Updates/Redesigns of Impacted Primers/Probes Are In Testing Phase

- **Inf A assay**: Updates have been made to both the forward and reverse primers
  - Forward primer now has two designs – one specifically targeting the H3N2 subtype and the other to capture all other InfA subtypes
  - Reverse primer also now has two designs – one design targets pdmH1 and the other design captures all other InfA subtypes
  - No updates currently needed to probe sequence
- **PdmInfA assay**: Updates made to the forward and reverse primer sequences
- **PdmH1 assay**: Update made to the probe sequence; no updates currently needed in the forward and reverse primers

Name	Version	Sequence 5' – 3'	Location[1]
InfA Forward Primer (M gene)	**Original**	**GAC CRA TCC TGT CAC CTC TGAC**	146 - 167
	Update for all but H3N2	caa GAC CAA TCY TGT CAC CTC TGAC	143 - 167
	Update for H3N2	caa GAC CAA TYC TGT CAC CTY TGAC	
InfA Reverse Primer (M gene)	**Original**	**AGG GCA TTY TGG ACA AAK CGT CTA**	251 - 228
	Update for all but pdmH1	GCA TTY TGG ACA AAV CGT CTA cg	248-226
	Update for pdmH1	GCA TTT TGG ATA AAG CGT CTA cg	
PdmInfA Forward Primer (NP gene)	**Original**	**TTG CAG TAG CAA GTG GGC ATG A**	848-869
	Update	AATAAATCATAA[2] TTG CAG TAG CAA GTG GsC ATG A	
PdmInfA Reverse Primer (NP gene)	**Original**	**TCT TGT GAG CTG GGT TTT CAT TTG**	976-953
	Update	AATAAATCATAA TCT TGT GAG CTG GrT TTT CAT TTG	
PdmH1 Probe (HA gene)	**Original**	**ATA CAT CCR ATC ACA ATT GGR AAA TGT CCA AA**	934-965
	Update	TGG CCA GYC TCA ATT TTG TGC TTT TTA CAT A	997-966

[1]Accoring to M (FJ966975), NP(FJ969536) and HA (FJ966974) of A/California/07/2009
[2]The 12-bp 5' AT-rich flap sequence to improve rRT-PCR performance

Questions

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Submit samples to:
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Atlanta, GA 30329