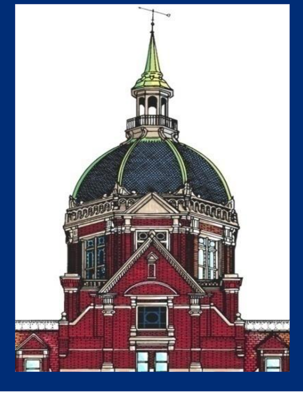
# Use of RT-PCR/Electrospray Ionization Mass Spectrometry (RT-PCR/ESI-MS) to Monitor Evolution of Genotypes, and Surveillance of Pandemic H1N1 Influenza in Realtime



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## Introduction

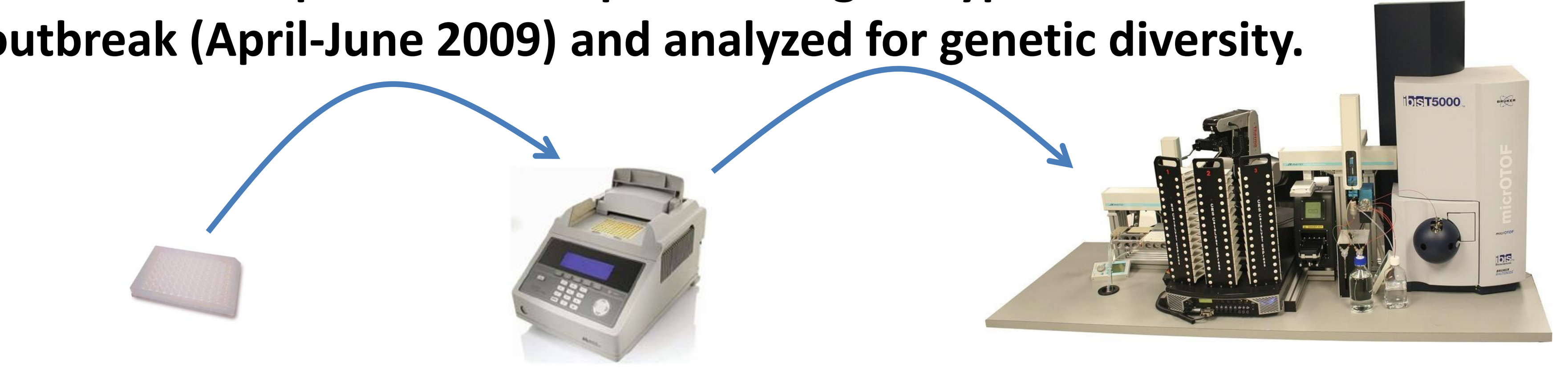
The novel H1N1 influenza pandemic (pH1N1) demonstrated the requirement for accurate, rapid tests to differentiate influenza-like illnesses (ILI), as well as a need for molecular surveillance for monitoring influenza A evolution. RT-PCR with electrospray ionization mass spectrometry (RT-PCR/ESI-MS) allows identification, strain-typing, and genetic analysis of influenza by using base composition characterization of amplified fragments from six target genes (PB1, NP, M1, PA, NS1, and NS2). We used this assay to identify genetic changes of pandemic H1N1 isolates over the later phases of the outbreak compared to earlier genotypes.

## Objective

In our laboratory, we studied an RT-PCR coupled to electrospray ionization mass spectrometry (ESI-MS) assay for identification, strain-typing and genetic surveillance of influenza from different phases of the 2009 outbreak.

## Methods

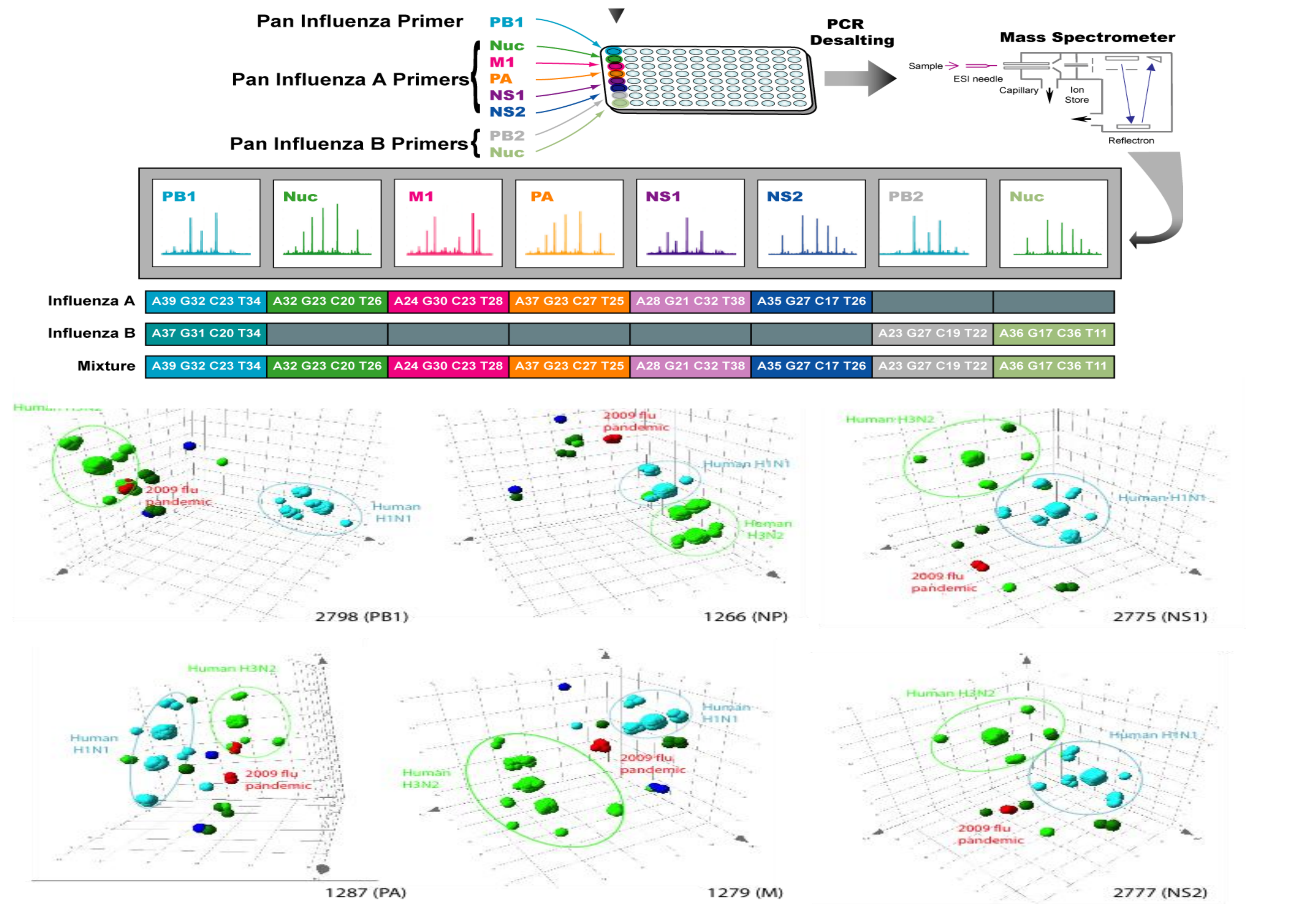
Nasopharyngeal aspirates (N =70) from patients at the Johns Hopkins Hospital that previously tested positive for influenza A pH1N1 between October - December, 2009 were tested using influenza-specific primers followed by base composition analysis of the amplicons by ESI-MS for pathogen identification. Positive controls (pH1N1, seasonal H1N1, H3N2) from Zeptometrix, Inc. were used as positive controls for assay validation. Results were compared to original CDC-approved RT-PCR. The genotyping data was compared to the published genotypes from earlier in the H1N1 outbreak (April-June 2009) and analyzed for genetic diversity.




1. Broad-Range Primer Selection
2. RT-PCR
3. Electrospray Ionization-Mass Spectrometry (ESI-MS)

## Methods

Laboratory Methods: Primers targeting eight genes (1 pan influenza target, 5 Influenza A primers, and 2 Influenza B primers) were designed to identify all known H and N subtypes. Base composition characterization by electrospray ionization mass spectrometry (ESI-MS) (Abbott Molecular) of amplified nucleic acids allowed for H and N identification.




## Results

Compared to CDC RT-PCR results for typing, 69/70 (98.6%) samples were identified as pH1N1 (1 sample was insufficient quantity for analysis), and RT-PCR/ESI-MS correctly identified the pH1N1 A/California/04/2009 strain in all 69 samples. The total time to identification and genotype was 8 hours. Late year samples exhibited greater genetic diversity (11 genotypes vs. 7 genotypes). The proportion of pandemic H1N1 samples with genotypes divergent from the reference strain increased from the uniform genotype of early samples to 61.4% in our later samples.

## Results

Representative comparison of early and late influenza genotyping data. (Yellow indicates an identical matches to the original reference strain. All other colors indicate at least one base pair change from the reference strain.)

| CDC ID | T5000 Inferred Subtype | PB1 | NP | M1 | PA | NS1 | NS2 |
|---|---|---|---|---|---|---|---|
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | - |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | - | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | - |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
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| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | - |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
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| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |

Representative Influenza Samples (April – June 2009)

from Zembower, T., Deyde, V., Gubareva, L., Klimov, A., Penugonda, S., Kunstman, K., Bolon, M., Dittmann, D., Blyn, L. B., Ecker, D. J., Wolinsky, S., Sampath, R. 2010. PCR/ESI-MS Approach for High-Throughput Identification of 2009 Pandemic Influenza A H1N1 Viruses. Presented at IDSA 2010.

| CDC ID | T5000 Inferred Subtype | PB1 | NP | M1 | PA | NS1 | NS2 |
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| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 36 25 27 24 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 34 22 21 25 | 24 28 24 29 | 36 25 27 24 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 37 34 25 32 | 35 21 20 25 | 24 28 24 29 | 39 24 28 21 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 37 34 25 32 | 35 21 20 25 | 24 28 24 29 | 39 24 28 21 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 37 34 25 32 | 35 21 20 25 | 24 28 24 29 | 39 24 28 21 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 37 34 25 32 | 35 21 20 25 | 24 28 24 29 | 39 24 28 21 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | - | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 38 25 29 20 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 33 24 32 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 37 34 25 32 | 35 21 20 25 | 24 28 24 29 | 39 24 28 21 | 36 35 20 28 | 34 29 16 26 |
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| Swine H1N1 | Swine H1N1 | - | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 38 33 25 32 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |
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| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 22 30 25 28 | 35 26 28 23 | 36 35 20 28 | 34 29 16 26 |
| Swine H1N1 | Swine H1N1 | 39 32 24 33 | 35 21 20 25 | 24 28 24 29 | 35 26 28 23 | 35 35 21 28 | 34 29 16 26 |

Representative Influenza Samples (October – December 2009)

## Discussion

RT-PCR/ESI-MS accurately detected and typed pH1N1 influenza samples, and provided evidence that genetic mutations were occurring in the gene segments. Spectrometry data revealed an increase in genetic diversity of samples collected over time, demonstrating its potential use for molecular epidemiology of emerging influenza viruses.

## Acknowledgements

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