De novo Profiling Strategies for Differential Mutation of Influenza Vaccines
Ryan D. Leib1, Louis Jacob2, Allis S. Chien1, Emmanuel Mignot1, & Christopher M. Adams1
Stanford University1, Université Paris Descartes2

Overview
During the 2009 H1N1 influenza pandemic, vaccines were prepared at various sites to combat infection. One of these vaccines, Pandemrix, is linked to increased onset of narcolepsy, even though the formulation is nominally identical with other contemporary vaccines. Here, we use a parallel proteomic profiling strategy to discover novel mutations, contaminants, and modifications differentially observed in 2009 H1N1 vaccines. This empirical approach leverages the strengths of database, wild card, and de novo methods to identify potential narcolepsy-triggering antigens that were not known at time of formulation.

Methods
A total of nine monovalent H1N1 influenza vaccine lots across three different tradenames were analyzed using a DDA approach on an Orbitrap Fusion mass spectrometer. The amounts and types of proteins observed in H1N1 vaccines are consistent with their formulation and dose. Interestingly, all vaccines sampled contain significant contamination by protein derived from the hen egg host. Such exogenous proteins should also be evaluated as potential sources of antigen response.

HA varies from formulation in a vaccine-specific manner

Iterative Profiling Strategy
Initial Byonic 1% FDR Database Search
- Expected H1N1 Strains (X-179A and X-181)
- Common Contaminants
- Common PTMs

Expanded Influenza Database Search
- Seven Additional Influenza Strains
- Exogenous Proteins (g. gallus, b. taurus)
- Expanded PTMs and Glycosylations

Identify Differential Expression
- Pandemrix vs. Other Vaccines
- Peptide-level Expression
- PTM/Mutant Specificity

Parallel Novel Identification
- Each possible mutation vetted by three methods

Identify PTMs/Mutations
MS/MS Validation

Select for Outliers
Peptide-specific fold changes for known and novel variant residues

Discussion
Using this iterative profiling strategy, we identified over 200 possible novel sequence variants in at least two of the parallel methods, with 44 likely variants observed by all three methods. Of these, 26 are observed to have significantly different concentrations between vaccines.

Conclusions
- Iterative profiling strategy can validate mutations
- Parallel de novo methods reduce false positives
- H1N1 vaccine composition is far more complex than formulation would indicate
- 26 targets identified for in vitro assays against narcolepsy antigen DQ0602
- Expand analysis to newly available lots

Acknowledgments
This work is supported by the Stanford Dean of Research and the Vincent and Stella Coates Foundation.

ASMS 2016 San Antonio, #272 ThP