TIME TO APPLY 3 R'S TO ADVENTITIOUS VIRUS TESTING?

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For decades, the biologicals industry has relied on mid-century clinical diagnostics methods to screen for adventitious viruses. The standard tissue culture tests, using cytopathic effects and hemadsorption and/or hemagglutination as read-outs, and the *in vivo* tests, using death/survival, "evidence" of viral infection, and hemagglutination as read-outs, have been used without validation according to the ICH guidance [Q2(R1)]. These compendial methods need only be verified, but they were never really optimized, standardized (e.g., methods differ between US and EU), and validated, including inter-laboratory reproducibility, as would be expected today. Data from a research study to evaluate the performance of the U.S. methods for the tissue culture and *in vivo* tests will be presented; however, only breadth and sensitivity were assessed. These data suggest that the *in vivo* methods are neither as broad nor as sensitive as imagined. New genomic methods have been developed, are being introduced, and eventually will become validated for the purpose of testing for adventitious viruses. But, how can one demonstrate that the new methods are comparable or better, when the old methods have unknown performance parameters? The presenter suggests a pathway forward and that it is time to apply the 3 R's to adventitious virus testing.

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INNOVATIVE INFLUENZA VACCINES, A CHALLENGING REGULATORY PATH IN PANDEMIC SITUATIONS

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Over the last decade, public health authorities invested massively to support the development of rapid responses to influenza pandemic threats. As an alternative to egg-based manufactured influenza vaccines, two cell culture-based novel influenza vaccines (FluBlok, Protein Science Corp. and Flucelvax from Novartis) were approved. Other seasonal and pandemic influenza candidate vaccines are in late phases clinical evaluations. The most advanced approaches rely on Viral-like particle (VLP) strategies as proposed with VLPs produced in insect cell cultures (Novavax) or plant cells (Medicago). Whereas the quest for a universal influenza vaccine is still a dominant line of research in many laboratories.

In spite of these remarkable progresses that can deliver a pandemic vaccine within weeks, important limitations might negatively impact the timelines for pandemic vaccine availability. For example, for cell culture produced inactivated viral vaccines, viral seed stocks are still generated using approved traditional egg based methods. Consequently, additional delays are imposed for the re-adaptation of the selected virus to cell culture. More critically, the single radial immuno-diffusion (SRID) assay remains the only method formally approved by the WHO and national regulatory bodies for the quantification of influenza and lot release of recombinant or non-recombinant influenza vaccines. SRID assay requires strain-specific antibodies that are produced within two to three months. In the case of pandemics, these delays might become very problematic.

Sustained efforts are required to accelerate the availability of viral seed stocks using recombinant technologies and to implement more rapid potency assays as an alternative to SRID.

VACCINE DESIGN AND EVALUATION WORKSHOP – THE IVAX TOOLKIT

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Key Words: Vaccine Design, Immunoinformatics, Antigen Selection, T cell epitopes

Applying Immunoinformatics to Vaccine Design and Evaluation. Emerging infectious diseases are an ongoing challenge for vaccine design, development, manufacture, clinical testing and licensure processes. Key issues facing vaccinologists today include prediction of immune response to emerging infectious diseases and cancers and design of novel and next generation vaccines. To address these challenges, we have leveraged computing power, the availability of terabytes of genomic data, and advanced immunoinformatics tools to harness T cell immunity for production of safe and effective vaccines. Highly immunogenic peptides conserved across multiple strains of input pathogen sequences are identified using the Conservatrix, EpiMatrix and EpiAssembler algorithms. Potential vaccine candidate epitopes can be aggregated into a string-of-beads design with the VaccineCAD algorithm, simultaneously minimizing deleterious junctional epitopes that may be created in the linking process. JanusMatrix, an enhanced homology analysis tool examining pathogen/host sequence similarity at the TCR interface of any given peptide, predicts potentially cross-reactive epitopes, allowing candidate sequences with potential host cross-reactivity to be preferentially excluded from vaccine constructs. Most recently, low immunogenicity H7N9 influenza antigens with high human cross-conservation1 were engineered to include epitopes more highly cross-conserved with circulating influenza strains, resulting in a 5-fold increase in post-vaccination antibody titers compared to wild type protein. The JanusMatrix tool also successfully identified the cross-reactive epitope between the MAGE A3 immunotherapeutic and human titin implicated in two fatalities among melanoma and myeloma clinical trial participants. Recent emergence of H7N9 influenza illustrates the difficulties associated with 'standard' approaches to vaccine development, while modern cancer vaccine research has underscored the danger of auto-reactive vaccines and immunotherapeutics. These studies provide an opportunity to apply immunoinformatics tools to develop safe and effective responses to these challenges. The iVAX toolkit is poised to accelerate the development of targeted, safe and efficacious vaccines, which will address important global health and biodefense challenges. 2,3

Teaching the Technology. In a unique training opportunity, members of the vaccine development community are invited to participate in a hands-on demonstration of the iVAX Toolkit. Topics covered will include Data Management, Conservation Analysis, Protein Analysis, T cell Epitope Mapping and T cell Epitope Cluster Identification, Homology Analysis, Cross-Reactivity Prediction and String of Beads Vaccine Design. iVAX comprises a suite of in silico tools for the design of genome-derived, epitope-driven vaccines generated from protein sequences. Triage, immunoinformatic analysis and manipulation of these sequences can produce high quality peptide candidates for use as components of epitope driven vaccines. The *Conservatrix* algorithm parses input sequences into 9mer frames and identifies those conserved amongst multiple whole sequences for all potential vaccine targets. *EpiMatrix* scores 9mers for potential binding affinity against a panel of Class I or II HLA alleles. *ClustiMer* identifies clusters of 9mers with a high density of putative T cell epitopes. *JanusMatrix*, an enhanced homology analysis tool examining pathogen/host sequence similarity at the TCR interface of any given epitope, predicts potentially cross-reactive epitopes. *EpiAssembler* knits together the conserved sequences to form highly immunogenic consensus sequences. *VaccineCAD* aggregates potential vaccine candidate epitopes into a string-of-beads design while minimizing deleterious junctional epitopes that may be created in the linking process.

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