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Challenges and Regulatory Expectations for Using Advanced Virus Detection Technologies

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Disclaimer

My comments are an informal communication and represent my own best judgement.

These comments do no bind or obligate FDA

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Outline of Talk

- Need for considering new methods for adventitious virus detection
- Challenges of method implementation
- Ongoing efforts in advanced technologies for applications to biologicals
- General considerations for regulatory applications

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Introduction of Novel Cell Substrates

- **Replace primary cell cultures with continuous cell lines**
  - meet production demands when egg supply is limited *(influenza virus vaccines)*
  - establish a well-characterized cell bank thereby reducing high risk of contamination associated with primary cultures
  - use of new manufacturing platforms
- **Provide broader susceptibility to viruses with tumorigenic cell lines** *(influenza virus vaccines and HIV-1 vaccines)*
  - for novel vaccine development
  - for higher virus yield by using tumorigenic cell lines
- **Provide complementation for some vectored virus vaccines with genetically-engineered cells** (defective adenovirus-vectored vaccines)
- **Develop well-characterized cell lines (stem cell lines) and designer cell lines (packaging cell lines, stable transfectants) for new generation products** *(vaccines, recombinant proteins, and gene therapy products)*

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Some Novel Cell Substrates and Expression Systems for Vaccines

- **Mammalian**
  - **MDCK (canine; *non-tumorigenic and tumorigenic*)**
  - **293; PER.C6; HER96 (human; *transformed, tumorigenic*)**
  - **A549; HeLa; CEM (human; *tumor-derived*)**
  - **CHO (rodent; *tumorigenic, retroviral particles*)**
- **Avian**
  - **EB66 (duck; *embryonic stem cell*)**
- **Insect cell lines**
  - **Hi-5 (*Trichoplusia ni)***
  - **Sf9 *(Spodoptera frugiperda)***
- **Plants**
  - **Tobacco**
- **Bacteria**
  - ***E. coli***

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Adventitious Virus Detection is a Major Safety Concern for Using Novel Cell Substrates

- **Known, unknown, and unexpected viruses**
  - **Tumor-inducing viruses (*in case of tumor cell lines*)**
  - **Latent viruses**
    - **DNA and RNA viruses**
    - **Endogenous retroviruses**
  - **Occult viruses**
  - **Novel viruses**

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Adventitious Virus Concerns in Cell Substrates

- **Exogenously-acquired** replicating viruses and latent viruses
- **Genetically-inherited** endogenous retroviruses
- ***de novo*-generated** novel recombinant viruses
- **VLPs containing co-packaged "unwanted" RNA or DNA**
- **Reverse transcriptase (RT) activity produced from avian and insect cells** (retroviral particles/retrotransposons?)

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Endogenous Retroviruses

- **Multiple copies present in all genomes of all species and may be constitutively expressed as particles in some**
  - Avian and rodent cell lines
- **May pose a safety concern, if biologically active**
- **Therefore, the detection of reverse transcriptase (RT) activity produced from a cell substrate needs rigorous follow-up strategy to evaluate its potential risk**
- **This may include (*in some cases*): development of reagents and assays as well as filling the knowledge gap regarding retroelements in some species, such as insects and plants**

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Strategies to Mitigate Risk of Adventitious Viruses

- **Identify potential safety concerns to enable development of a comprehensive testing plan and risk mitigation strategy**
  - **Characterization and history of the cell substrate**
- **Cell banking and use of qualified raw materials**
  - Development of well-characterized cell banks
  - Use of certified/tested animal-derived biological materials (e.g. serum, trypsin, antibodies)
- **Incorporation of steps during manufacture for viral clearance and purity**
  - **Process validation for clearance (inactivation and removal) of potential viral contaminants in final product**
  - **Reduction of residual cellular materials (DNA, RNA, proteins)**
- **Testing**
  - **Extensive testing for known and unknown agents in the starting materials (cell substrate, virus seeds, vector virus preparation)**
  - **Adventitious agent testing at different stages in manufacturing process and at steps with the greatest potential for contamination**
  - **Using various sensitive and broad detection assays**

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Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (Feb. 2010)

- **Testing of viral seeds, cell banks, unprocessed bulk, control cells and end-of-production cells**
- **http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm202439.pdf**

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Routine Adventitious Agent Tests

- **Non-viral agents**
  - **Bacteria (aerobic and anaerobic) and Fungi-**
    - **21CFR 610.12 (or an equivalent method that fulfills the requirements of 21 CFR 610.9)**
  - **Mycoplasma (cultivatable and non-cultivatable) /Spiroplasma**
    - **agar and broth media culture method (21 CFR 610.30)**
    - **indicator cell culture procedure**
    - **PCR**
  - **Mycobacteria**
    - **culture method (21 CFR 630)**
    - **guinea pig test**
    - **PCR**
- **Viruses**
  - **General Detection Assays**
    - ***In vitro* cell culture tests in cell lines of 3 species (same as cell substrate, monkey, human)**
    - ***In vivo* assays (adult mice, suckling mice, embryonated hens' eggs)**
    - **Transmission electron microscopy (TEM)**
    - **Reverse transcriptase assay for retroviruses (PERT)**
  - **Species-specific Assays**
    - **Tests for animal viruses e.g. bovine, porcine (9CFR 113.47 and 113.53)**
    - **Antibody-production assays for rodent viruses (MAP *including LCMV challenge*, HAP, RAP)**
    - **Assays for known viruses (PCR, DNA hybridization, Infectivity, antibody detection)**

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Detection by Current AA Tests

- **Currently recommended assays have been generally effective in demonstrating the absence of adventitious viruses in traditional vaccine cell substrates for vaccine production**
  - Cell-culture assays are based upon detection of a visible effect due to virus replication, such as cytopathic effect (CPE) or hemadsportion / hemagglutination
  - Molecular assays are based upon availability of virus sequences

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Recent, Unexpected Cases of AA Detection

- **June 17, 2009: Vesivirus 2117 detected CHO cell bioreactor production runs of Cerezyme and Fabrazyme by Genzyme: LC/MS detection of peptides, RT-PCR confirmation (3 events, e diferent processes at 2 different plants** (*2009 Cell Substrate Workshop, Genzyme*)
- **April 7, 2010** (*online*): **Porcine circovirus Type 1 (PCV1) sequences detected by UCSF lab and LLNL in Rotarix using virus microarrays and NGS analysis 2014:** (*Victoria et al., J. Virol. pub. June 2010, vol. 84,. 6033-6040*)
- **March 26, 2014** (*online*): **Discovery of novel rhabdovirus in Sf9 cell line using degenerated PCR and NGS** (*Ma et al., J. Virol. Pub. June 2014, vol. 88, 6576-6585*)
- **May 13, 2015: Porcine mycoplasma in clinical material of a candidate NYVAC-HIV vaccine (NYVAC-HIV-PT-1) by PCR discovered by EuroVacc and IDT (orign seed lot) using a recently developed and validated highly sensitive PCR-based assay (passed mycoplasma serility testing)** (*http://vaccinenewsdaily.com/stories/510544948-nyvac-hiv-vaccine-applied-in-hvtn-092-and-hvtn-096-clinical-trials*)

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Need for New AA Detection Methods

- **Currently recommended assays may not be sufficiently broad to address safety concerns associated with novel cell substrates**
  - **latent viruses (DNA, RNA, or endogenous retroviruses;** *uninduced and induced***)**
  - **novel (unknown) viruses**
  - **unexpected (known) viruses**

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Introduction of New Assays for Adventitious Virus Testing

- **Recommended testing has been updated over time**
  - **Availability of new methods for more sensitive virus detection**
    - **PERT assay** for retrovirus particles *(validated assays available; recommended by OVRR to replace traditional RT assay)*
  - **Novel virus discoveries**
    - **PCR assays** for virus-specific detection *(validated assays available or developed in-house; recommended or sponsor-initiated)*
  - **Use of novel cell substrates** *(validated assays not available – recommended on a case-by-case basis by OVRR)*
    - **Oncogenicity assays** for detection of oncogenic viruses *(positive controls not available)*
    - **Chemical induction assays** for latent or endogenous virus detection *(positive controls available but not species-specific)*

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New Technologies for Broad/Novel Virus Detection

- **Massively parallel sequencing (MPS)**
  - **Sequencing without prior knowledge of sequences for known and novel viruses**
  - **Several high-throughput sequencing platforms are currently available and some are emerging**
  - ***Discoveries: PCV-1 in a rotavirus vaccine; virus discovery in raw materials, the environment, and various clinical samples***
- **Microarrays**
  - **Array consists of virus-specific oligos based upon known and related virus sequences: use of long primers allows for some mismatch**
  - ***Discoveries: SARV-CoV; metapneumovirus; XMRV, PCV in a rotavirus vaccine***
- **Broad-range PCR with mass spectrometry (PCR/ESI-MS)**
  - **Long PCR primers that are specific for virus families**
  - ***Discoveries: strain-specific identification of bacteria and viruses; 2009 H1N1 pandemic; novel variant of Bluetongue virus***

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Potential Applications for Biologicals

- **Cell substrate investigations and characterization**
  - Whole genome
  - Transcriptome
  - Supernatant (Virome)
- **Evaluation of animal-derived raw materials**
- **Product: stability, purity**

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Some Discussions on New Technologies for Biologicals

- **2010: WHO** "It is probable that application of methods of this type (*nucleic acid based methods with broad detection capabilities*) will be expected or required by regulatory agencies in future. At present the methods have not been evaluated for sensitivity and specificity and should be thought of as powerful investigational tools that can reveal issues that can be explored by more established methods" TRS 978
- **2011: IABS: Adventitious Agents, *New Technologies and Risk Assessment.*** Conference Summary in Biologicals, 2011
- **Nov. 2011: PDA/FDA Adventitious Agents and Novel Cell Substrates: *Emerging Technologies and New challenges.*** Discussed applications and identified knowledge and technical gaps that needed to be address for further development and applications. Conference Report in PDA Journal, 2012
- **Sept. 19, 2012: FDA VRBPAC** discussions on use of human tumor cell lines supported use of advanced virus detection technologies to supplement currently recommended assays for novel cell substrate evaluation. Transcript pdf available: www.fda.gov
- **Nov. 2013: PDA/FDA Advanced Technologies for Virus Detection in the Evaluation of Biologicals: *Applications and Challenges.*** Identified priority areas to be addressed for regulatory applications. Conference Report in PDA Journal, 2014

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Current Efforts for Advanced Technologies for Virus Detection in the Evaluation of Biologicals

- **FDA efforts toward strengthening a research and regulatory infrastructure to support policy development and decision-making related to applications of new technologies**
  - **FDA Genomics Working Group (2013)**
    - "Mission": To prepare the FDA to address IT and scientific challenges to facilitate FDA readiness for HTS data review by establishing a robust infrastructure and through collaborations
- **WHO Cell Substrate Study Group (Beijing, *May 2013*):** Issues for cell substrates and DNA based detection of adventitious agents
- **PDA AVDTIG (2014)** *(FDA/Industry "Users Group"; Oct. 2012)*
  - "Mission" – To advance the tools for the next generation of viral risk evaluation by providing an informal, scientific forum for discussions and scientific collaborations
  - Industry, regulatory and other government agencies, academia, and technology service developers and providers

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Some Challenges for Regulatory Applications of New Technologies

- **Assay standardization and validation**
  - **Methods are complex and evolving**
    - **Sensitivity and specificity**
    - **Appropriate model viruses (*virus spiking studies*)**
    - **Sample preparation and processing**
- **Bioinformatics**
  - **Data analysis**
    - **Pipeline optimization**
      - Guidelines for acceptable quality of reads, parameters for short read assembly
      - Approaches to identify a novel virus that has minimal nucleic acid sequence homology to known viruses
    - **Development of a complete and correctly annotated, publically available, Reference Virus Database**
  - **Data submission, storage, and transfer**
    - **Format**
    - **Security**
- **Follow-up strategy**
    - **Biological relevance and significance of a positive signal**

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Some Specific Considerations for MPS Applications

- **Sample Preparation and Processing**
- **Sample type**
  - Cells: DNA genome, transcriptome
  - Supernatant (seed, harvest): virions
- **Sample processing**
  - DNase-treatment, ultracentrifugation, filtration, etc.
  - DNA/RNA extraction methods
  - Poly A+ selection, ribosomal depletion
  - PCR-amplification step
- **Selection of model viruses**
- **Library preparation**
  - Various library preparation methods
- **Bioinformatics analysis**
  - Reference sequence or de novo assembly
  - Databases (public, private)

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Introducing New Assays for Regulatory Applications

- **Assay standardization and/or validation**
  - **Development of relevant controls (*positive and negative*)**
  - **Determination of sensitivity and specificity**
  - **Demonstration of precision (*reproducibility and repeatability*)**
  - **Evaluate assay robustness (*change of assay conditions and reagents*)**
  - **Demonstrate the reliability of the assays (e.g. interference of sample matrix to the assay's intended use)**
  - **More details on the validation of analytical assays and statistical analyses are described in ICH Q2(R1)**
- **Availability of assay (establishment of method in-house or through CROs)**

- ***In general, molecular assays like virus-specific PCR assays are easier to validate compared to biological assays (less variables)***
- ***However, some new PCR-based technologies for broad virus detection are more challenging due to the high complexity of the methodology***

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AVDTIG Subgroups

- **Subgroup A: Evaluating the different approaches to sample preparation for detecting viral adventitious agents by high throughput sequencing. This includes pre-treatment of samples, different extraction methods, sequencing library generation, quality control steps and other topics of interest.** Siemon.Ng@sanofipasteur.com
- **Subgroup B: To identify virus reference standards for SPIKING studies. To define and organize the characterization of virus reference standards to be used in SPIKING studies** Jean-Pol.Cassart@gsk.com
- **Subgroup C: Development of a complete and accurately annotated, publically-available reference database (current focus Viruses)** Kavitha_bekkari@merck.com ***(mid-2015)***
- **Subgroup D: Bioinformatics pipelines analysis (reads to hits; e.g. minimum number of reads that represent a positive hit; distinguish +/-); compare piplelines output using common datasets.** Adam.Palermo@genzyme.com

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Evaluation of Sf9 Cells Using New Technologies

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Laboratory Investigations of Sf9 Cells

- **Physical Analysis**
  - **Ultracentrifugation**
  - **Size fractionation**
  - **Density gradients**
  - **TEM**
- **Infectivity analysis**
  - **Mammalian cell lines**
- **Molecular Analysis**
  - **Degenerate PCR**
  - **MPS (BioReliance; CD Genomics)**
    - Transcriptome (total RNAs from cells): Roche-454
    - Nucleic acids from filtered supernatant): Roche-454
    - Total nucleic acids from concentrated, filtered sup: Illumina-MiSeq
    - Total nucleic acids from size fractionated, filtered sup: Illumina-MiSeq
    - *Whole genome: Illumina-HiSeq*
  - **PLEX-ID (Ibis, Athogen/Abbott; CBER)**
  - **Virus arrays (LLNL)**

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Identification of a Novel Rhabdovirus in *Spodoptera frugiperda* Cell Lines

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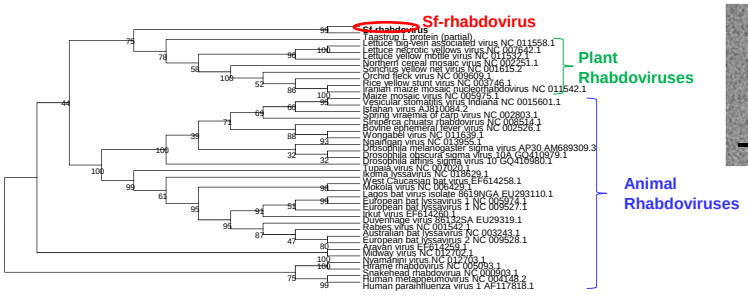

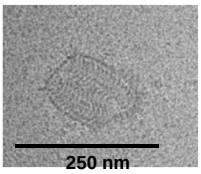

No infection in mammalian cell lines; Limited infection in insect cell lines

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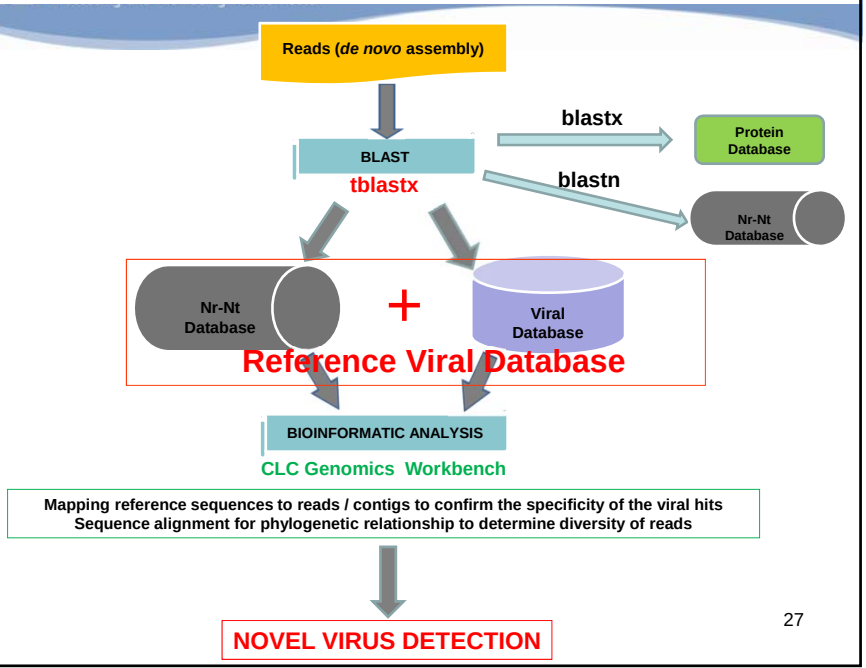
Bioinformatics Strategy for Novel Virus Detection



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Path for follow-up of a positive signal to determine its biological relevance

- **Can the results be confirmed by PCR or another assay?**
- **Is a complete viral genome present?**
- **Are particles present?**
- **Are the particles infectious?**
- **Is there a replication-competent virus?**
- **Can the nucleic acid/particles be quantified?**

❑ **WHO Guidance on scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine *(Expert Committee on Biological Standardization Geneva, October 13-17, 2014)***

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Lessons Learnt

- Need to use both conventional and new technologies for novel virus detection
  - TEM analysis of both sup and cells
  - distantly related viruses can be identified at the amino acid level
  - Reducing the cellular background in the sample may increase the efficiency of MPS analysis for virus detection using the currently available databases
  - Development of a complete virus database is necessary for increasing likelihood of novel virus detection

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Ongoing Collaborative Lab Efforts on New Virus Detection Technologies

- Determination of sensitivity of virus detection of MPS (virus spiking study with model viruses)
- Development of virus reference stocks
- Generation of a complete Reference Virus Database
- Evaluation and optimization of strategies for known and novel virus detection

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Conclusions

- Currently, the new technologies can be useful for supplementing the conventional assays for novel virus detection
- Results from ongoing studies can provide scientific direction for additional work that may be needed for advancing new technologies for regulatory applications, particularly development of virus reference materials.
- Due to the complex nature of the new methods and associated bioinformatics, collaborative efforts among industry and regulators should continue to identify and address the technical challenges and efforts towards harmonization should be initiated among Regulatory Health Agencies

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