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Challenges and Ongoing Efforts for Using NGS for Adventitious Agent Detection

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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

Outline of Talk

- Need for considering new virus detection methods
- Ongoing efforts for advancing new technologies for evaluation of biologicals
- Current status and challenges for regulatory applications of NGS

Strategies to Mitigate Risk of Adventitious Agents

- **Identification of 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**

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)

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)*

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

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**

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** (*pub. 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** (*pub. 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) (**joint statements by NIAID and EuroVacc Foundation *http://vaccinenewsdaily.com/stories/510544948-nyvac-hiv-vaccine-applied-in-hvtn-092-and-hvtn-096-clinical-trials***)**

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*

Discussions for Using New Virus Detection Methods in 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 https://www.google.com/search?hl=en&source=hp&q=sept+19+2012+vrbpac&gbv=2&oq=sept+19+2012+vrbpac&gs_l=heirloom-hp.12...6584.17316.0.19173.37.19.4.14.0.0.202.2277.2j16j1.19.0....0...1ac.1.34.heirloom-hp..17.20.2136.FUzWJdSNfDA
- **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

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

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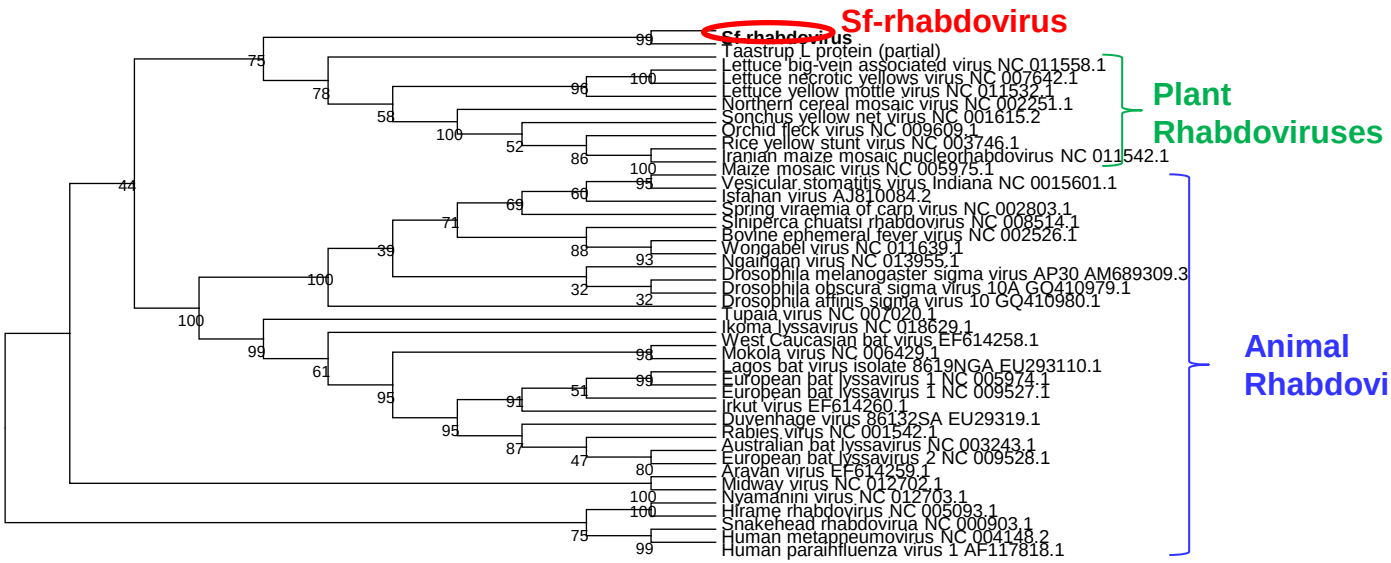
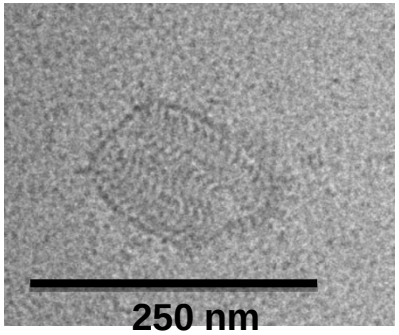
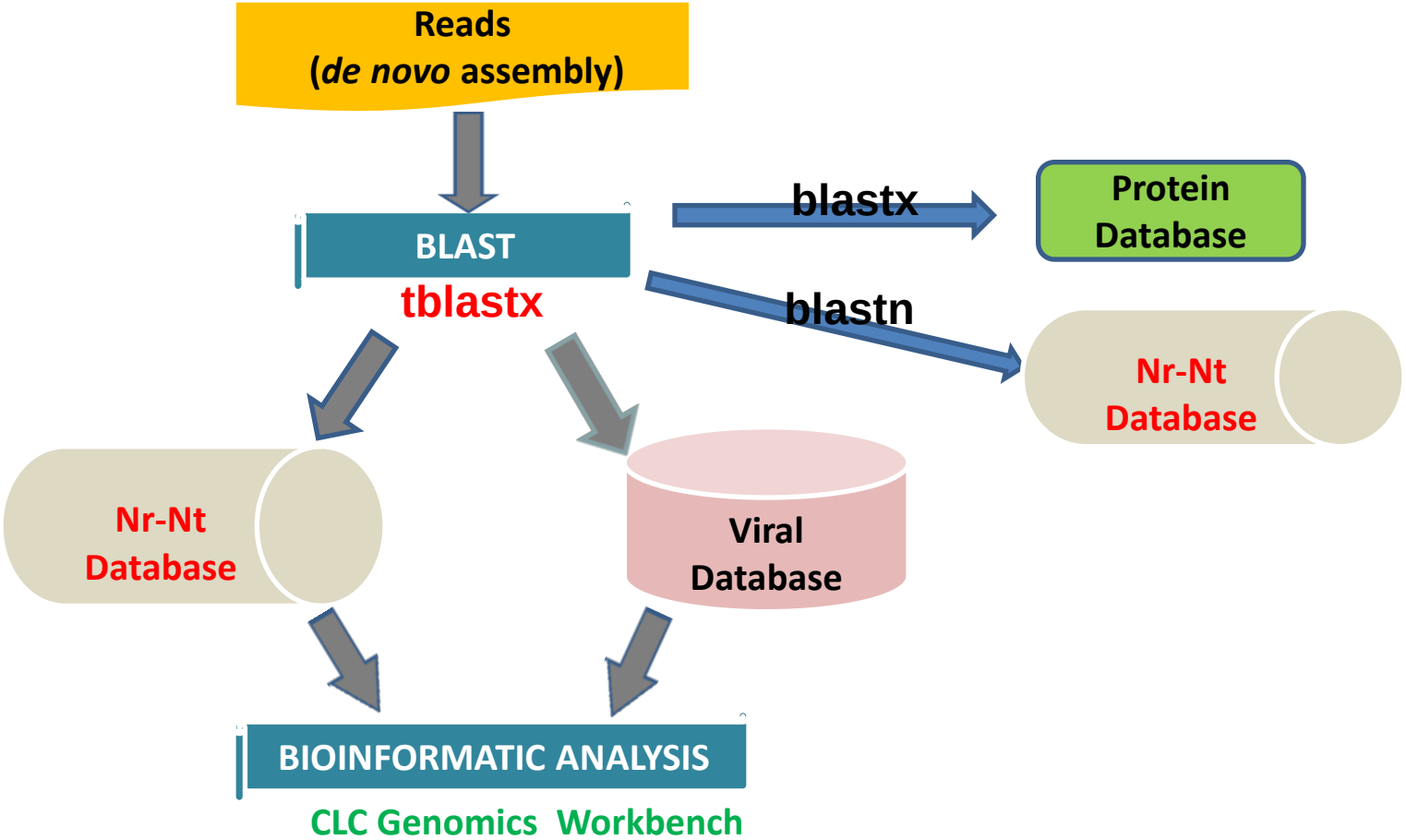




No infection in mammalian cell lines; Limited infection in insect cell lines

Bioinformatic Analysis of MPS Data



Mapping reference sequences to reads / contigs to confirm the specificity of the viral hits
Sequence alignment for phylogenetic relationship to determine diversity of reads

NOVEL VIRUS DETECTION

Some General Considerations for MPS Applications

- **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
- **Library preparation**
  - Various library preparation methods
- **Bioinformatics analysis**
  - Analysis pipelines
  - Reference sequence or de novo assembly
  - Databases
  - Data submission and transfer
- **Follow-up strategy** for a positive signal

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, new technologies for broad virus detection are more challenging due to the high complexity of the methodology**

Some Challenges for Regulatory Applications of MPS

- **Assay standardization and validation: methods are complex and evolving**
  - **Sensitivity and specificity**
  - **Appropriate model viruses (*virus spiking studies*)**
  - **Sample type: processing and virus recovery**

- **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**

Ongoing Effort for Advanced Technologies for Virus Detection in the Evaluation of Biologicals-I

- **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*)**
  - Current issues for cell substrates
  - DNA based detection of adventitious agents

Ongoing Efforts for Advanced Technologies for Virus Detection in the Evaluation of Biologicals-II

- **PDA Advanced Virus Detection Technologies Interest Group** (established in October, 2012 as a Users group)
  - Co-chairs: Arifa Khan, CBER/FDA and Dominick Vacante, Janssen R&D

    Coordinator: Josh Eaton, PDA
  - Comprises of scientists from industry, regulatory and other government agencies, academia, and technology service developers and providers
  - "Mission" – to advance the tools for the next generation of viral risk evaluation by providing an informal, scientific forum for open discussions and scientific collaborations
    - Exchanging technical experiences & best practices Identifying common issues regarding use of the technologies and develop approaches to address them
    - Leverage resources, where necessary

Advanced Virus Detection Technologies Interest Group: *Current Activities and Future Goals*

AVDTIG: Principles of Participation

- Facilitate open, scientific discussions for advancing the tools for next generation of viral risk evaluation and testing
- Information gathering by knowledge exchange and experimentation
- Data sharing (non-proprietary)
- Maintain confidentiality of information presented or discussed
- No archiving or distribution of presentations (unless approved by presenter)

AVDTIG Subgroups

- **Subgroup A:** Sample selection (type)/preparation/processing (e.g. w/wo nuclease treatment, concentration, cDNA lib prep): and nucleic acid recovery

  Siemon.Ng@sanofipasteur.com

- **Subgroup B:** Virus standards and reference materials (and their characterization)

  Jean-Pol.Cassart@gsk.com

- **Subgroup C:** Databases evaluations and development of a complete and correctly annotated , publically available reference database (primarily focused on Viruses)

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- **Subgroup D:** Bioinformatics pipelines analysis (primarily focused on NGS; e.g. minimum number of reads that represent a positive hit; distinguish +/-)

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- **Multi-Center Virus Spiking Study – I (FDA/GSK/SANOFI)**
- **Multi-virus spiking study (NIBSC)**

2014-2015: Overall AVDTIG Goals

- Each subgroup is working toward developing a "best practices" paper for publication toward optimization and standardization of NGS technologies
- Additional subgroups may be formed for discussions on applications of NGS
- Need discussions on regulatory expectations

Meetings

WHOLE GROUP MEETING:

1st Wed. of the month,

2015: Every other month, 9 am – 2 pm EST

SUBGROUP MEETINGS:

Generally every two weeks; 1 hour

Currently >80 members from industry (vaccine and therapeutics), regulatory and other government agencies (FDA, NIH, NIBSC), academia, CROs, and others (mostly in U.S. and Europe)

Meetings include updates from subgroups, presentations from participants, and invited others

Teleconferences facilitated through PDA

Contact: Josh Eaton, eaton@pda.org

Summary of Talk

- Currently, the new technologies may 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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Subgroup Leaders

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