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Report of the international conference on next generation sequencing for adventitious virus detection in biologicals☆

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ABSTRACT

A fundamental aspect of biological product safety is to assure absence of adventitious agents in the final product. Next-generation or high-throughput sequencing (NGS/HTS) has recently demonstrated detection of viruses that were previously missed using the recommended routine assays for adventitious agent testing of biological products. This meeting was co-organized by the International Alliance for Biological Standardization (IABS) and the U.S. Food and Drug Administration (FDA) to assess the current status and discuss the readiness of NGS for adventitious virus detection in biologics. The presentations included efforts for standardization, case studies on applications in biologics, comparison with routine virus detection assays, and current regulatory thinking. Participants identified the need for standard reference reagents, well-annotated databases, large data storage and transfer capacity, personnel with relevant expertise, particularly in bioinformatics; and harmonization of international regulations for testing biologic products and reagents used for their manufacturing. We hope this meeting summary will be of value to regulators and industry for considerations of NGS applications for adventitious virus detection in biologics.

1. Introduction

Dr. Pieter Neels (IABS) opened the meeting by acknowledging the Organizing Committee for developing the scientific program for the conference and the funding support of the sponsors for the meeting.

Dr. Arifa Khan (FDA) provided the background and goals of the meeting and presented an introduction on next-generation sequencing (NGS), also referred to as high-throughput sequencing (HTS), massively parallel sequencing (MPS) and deep-sequencing (DS). Her talk indicated the important role of PCR and microarrays for detection of known viruses, and how the recently developed NGS technologies can generate large volumes of data for detection of known and novel viruses, without prior sequence knowledge [1]. However, NGS technology is complex and sequencing platforms are rapidly evolving and new ones emerging: the early instruments were large bench-top models, whereas the 4th generation devices are hand-held; the chemistry has also progressed from generating short fragments/reads that have a higher sequence accuracy but rely on accurate assembly to produce a longer fragment (e.g. viral genome), to single-molecule sequencing for directly generating a long fragment of a viral genome, the latter at this time, has a higher error rate and therefore needs alignment for correction with a reference genome or short reads. Approaches to address the technical and bioinformatics challenges for NGS applications for virus detection in biologics will be discussed during the meeting.

Dr. Khan also presented the history leading to the organization of the current conference. She outlined various public discussions since 2009 related to the need for using advanced nucleic acid based-technologies for broad virus detection and their challenges for evaluating safety of biomedical products. These included: (1) 2009 Parenteral Drug Association (PDA)-sponsored Cell Substrate Workshop, which introduced some novel cell substrates used for manufacturing biological products [2,3]; (2) 2011 IABS-sponsored meeting on Adventitious Agents, New Technologies and Risk Assessment [4]; (3) 2011 PDA/FDA-sponsored meeting on Adventitious Agents and Novel Cell Substrates: Emerging Technologies and New Challenges, which discussed applications and identified knowledge and technical gaps that needed to be addressed for further development and applications [5]; (4) 2012 FDA VRBPAC discussions on the use of human tumor cell lines and potential use of advanced virus detection technologies [6]; and (5) the 2013 PDA/FDA-sponsored meeting on Advanced Technologies for Virus Detection in the Evaluation of Biologicals: Applications and Challenges, which identified priority areas to be addressed for applications to biological products [7]. The latter led to the formation of the current Advanced Virus Detection Technologies Interest Group, which provides an international scientific forum for collaborations and discussions to address the challenges of NGS applications in biologics [8].

The conference objectives were to review the current progress on NGS for virus detection and discuss the readiness and challenges of

Abbreviations: NGS, next-generation sequencing; HTS, high-throughput sequencing; FDA, U.S. Food and Drug Administration; CBER, Center for Biologics Evaluation and Research (FDA); WHO, World Health Organization; IABS, International Alliance for Biological Standardization; NIBSC, National Institute for Biological Standards and Control (U.K.); HC, Health Canada; PEI, Paul Ehrlich Institute; GSK, GlaxoSmithKline; APHA, Animal and Plant Health Agency (U.S); USDA, United States Department of Agriculture; APHIS, Animal and Plant Health Inspection Service (U.S.)

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applying NGS to supplement or replace the current testing assays for adventitious virus evaluation in biologics. These were the goals of the talks and discussions in the plenary sessions on: (1) Current Perspectives on NGS, (2) Efforts on NGS Standardization for Virus Detection, (3) NGS Applications for Biologics, and (4) Current Virus Detection Assays and NGS. The final session in the conference was a Panel Discussion to assess the current developments in standardization and validation of NGS, discuss the readiness of the technologies to supplement or replace current adventitious virus detection assays, and identify what else would be needed for future applications in biologics.

This meeting was co-organized by IABS and the FDA in Rockville, MD on October 26–27, 2017. One hundred twenty-eight scientists from 16 different countries attended the meeting, including representatives from regulatory agencies and health authorities, industry (including vaccines, biotherapeutics, and gene therapy), service providers, and academia. This report summarizes the presentations and discussions and thus may be helpful for considering regulatory applications of NGS for virus detection in biologics.

2. Current perspectives on NGS

This session was co-chaired by Drs. Phil Minor (NIBSC) and Ivana Knezevic (WHO). Dr. Knezevic provided an introduction on the evolution of nucleic acid technologies and the associated instrumentation. She noted that NGS technologies have the promise of facilitating the detection and identification of adventitious agents in biological products, by methods that are potentially more sensitive, more rapid, and less costly. However, in order to deploy these new methods to their fullest potential in the production and safety testing of biologics, particularly vaccines, the relevant international and national regulatory agencies and bodies must be convinced that they are suitable for and robust enough for the tasks proposed for them.

Dr. Knezevic further indicated that the pathway for NGS to move forward to become a key element in biologics production involved meeting the criteria put forth by international and national bodies and regulators, including the World Health Organization (WHO), the European Pharmacopoeia Regulations (Ph.Eur.), the European Medicines Agency (EMA), the U.S. Food and Drug Administration (FDA), and the Animal and Plant Health Agency (APHA), United Kingdom. The focus of Session 1 was to present perspectives from these different agencies as well as from industry for using NGS for virus detection in biological materials.

Dr. Arifa Khan (CBER, U.S Food and Drug Administration) presented a regulatory perspective on high-throughput sequencing (HTS) for virus detection. Dr. Khan enumerated the general strategies to mitigate risk of adventitious viruses as follows: (1) by conducting risk assessment through identifying potential sources of virus introduction for developing a comprehensive risk mitigation strategy and testing plan; (2) by prevention through the use of well-characterized cell banks and certified/tested animal-derived biological materials; (3) by undertaking process validation through incorporation of robust viral clearance steps during manufacture and by reduction of residual cellular materials (DNA, RNA, proteins); and (4) by conducting extensive testing using various sensitive and broad assays for detection of known and unknown agents in the starting materials as well as at different stages of the manufacturing process. Currently, there are a range of tests, both *in vivo* and *in vitro* for infectious adventitious viruses, which are detailed in the FDA "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)" [9]. The currently recommended assays have been generally effective for demonstrating the absence of adventitious viruses in biological products. However, there have been some cases in the past where adventitious viruses have been detected in biological raw materials used for product manufacturing, for example: bovine viruses in serum, porcine parvovirus in trypsin, murine parvovirus in media, and species-specific viruses in some cell substrates.

Recommended testing for adventitious viruses has been updated with the development of more sensitive assays, discovery of new viruses, and the use of novel cell substrates that have potential concerns for unknown viruses. Examples include: the PERT assay for retroviruses; virus-specific PCR assays, such as one for minute virus of mice (MVM); and *in vitro* virus-specific cell culture assays, such as the 324K assay for MVM. Additionally, chemical induction assays may activate latent, unknown viruses, thereby facilitating their detection. In general, it is recommended that validated assays be used, however, there are situations in which validated assays are not available, such as chemical induction assays and oncogenicity assays for *in vivo* detection of oncogenic viruses; these are evaluated by the FDA Office of Vaccine Research and Review (OVRR) on a case-by-case basis for complementing other assays so as to provide additional assurance for product safety.

It was noted, however, that the routine assays for adventitious agents have several limitations, including that the cell-culture assays are based upon detection of a visible effect by virus replication; and the molecular assays such as PCR are based upon designing primers using available virus sequences, can use only a small test volume, and requires a cell culture assay to follow up a positive result in order to determine infectivity for evaluating potential risk. These limitations have been highlighted with the discovery of unexpected viruses using new advanced technologies such as microarrays and HTS for detection of porcine circovirus type 1 in a licensed rotavirus vaccines [10] and degenerate PCR and HTS for detection of a novel rhabdovirus in the Sf9 insect cell line [11], discussed later in this talk.

The FDA has made several efforts related to HTS including: establishment of the FDA Genomics Working Groups in 2013, for strengthening a research and regulatory infrastructure to support policy development and decision-making related to applications of HTS; and the formation of the Advanced Virus Detection Technologies Interest Group (AVDTIG), an FDA/industry group sponsored by the PDA. AVDTIG's mission is to advance the tools for the next generation of viral risk evaluation by providing an informal, scientific forum for discussions and scientific collaborations. It has 5 subgroups and more than 100 members [8]. The subgroups include: Subgroup A: Sample selection/preparation/processing; Subgroup B: Virus standards and reference materials; Subgroup C: Database evaluations and development of a complete and correctly annotated, publicly available virus reference database; Subgroup D: Bioinformatics pipelines analysis; and Subgroup E: Follow-up strategies to confirm the identity of a "hit."

Dr. Khan highlighted some of the challenges of HTS application for virus detection in biologics, which include: (1) assay standardization and validation, as the methods are complex and evolving; (2) bioinformatics, in terms of data analysis and data submission, and storage and transfer. Furthermore, in terms of data analysis, there is a need for pipeline optimization, guidelines for acceptable quality of reads, approaches to identifying a novel virus that has minimal nucleic acid sequence homology to known viruses, and development of a complete and correctly annotated, publicly available, reference virus database; and (3) follow-up strategies for determining the biological relevance and significance of a positive signal, which are key aspects in the use of HTS in the biologics context.

Dr. Khan reported on 3 efforts that her laboratory has made towards HTS standardization. (1) A collaborative spiking study with GlaxoSmithKline (GSK), and Sanofi Pasteur using 4–5 viruses to evaluate breadth and sensitivity of virus detection by different NGS platforms. This study was recently published and the datasets are available in NCBI [12]. The details are provided in the presentation by Dr. Jean-Pol Cassart (Session 2). (2) Development of five large-scale, well-characterized, reference virus stocks, which have been prepared at ATCC and are available for distribution to evaluate the performance of HTS platforms. (3) Generation of a complete Reference Virus Database