IPQ INTERNATIONAL PHARMACEUTICAL QUALITY
Inside the Global Regulatory Dialogue

FDA's ARIFA KHAN ON USING NGS FOR ADVENTITIOUS VIRUS DETECTION IN BIOLOGICS

At the CASSS Japan CMC strategy forum session on technologies related to viral safety, CBER Office of Vaccines Research and Review [OVRR] Senior Investigator Arifa Khan offered "a regulatory perspective on using NGS for adventitious virus detection in biologics." She discussed: ● the background for NGS in the biologics space ● FDA's collaborative efforts ● themes of the International Alliance for Biological Standardization (IABS) November 2019 meeting ● current CBER thinking around NGS for adventitious virus detection, and ● NGS considerations for updating ICH Q5A(R2). Khan presented remotely and referred to her slides as she progressed through the presentation. The number of the slide to which she is referring is indicated in brackets. CLICK HERE for the full slide deck. Formatting changes and other minor edits have been made by IPQ for clarity and readability. The normal disclaimer that the presentation represents the views of the speaker and not necessarily that of his/her organization is not included.

I would like to thank the session chairs for inviting me to give my perspective on using next generation sequencing for adventitious virus detection in biologics. The perspective that I will be giving today is from the Office of Vaccines Research and Review in the Center for Biologics Evaluation and Research in the US FDA. I have to apologize that I was not able to give this presentation in Japanese, but I will be speaking in English.

● I will first present the background for NGS moving into the biologics space ● and then go into some efforts for NGS and progress made towards application in biologics. ● I will also present highlights from the recent meeting in Ghent, Belgium, held from November 13-14 and sponsored by the IABS [International Alliance for Biological Standardization] – the conference on NGS for adventitious virus detection in biologics. ● Then I will present some of the current thinking in our office related to the use of NGS for adventitious virus detection. ● I will end with a brief description including NGS for updating the ICH Q5A (R2) document that you just heard from Dr. Sakurai ***[see Part V].***

Background for NGS in the Biologics Space

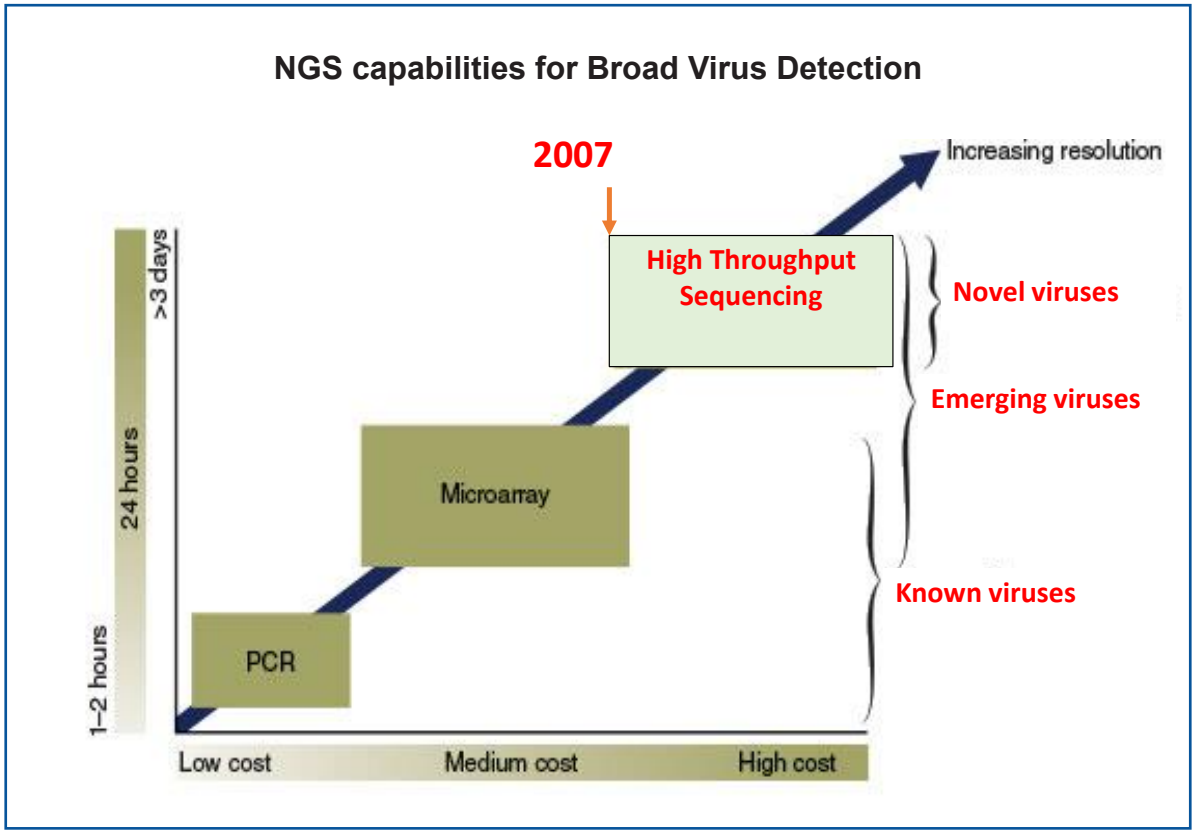
The next slide ***[#3]*** presents some background for the need to consider adventitious virus detection technologies. This started in our office mainly due to the introduction of many novel cell substrates that were needed for development of new vaccines – almost 20 years ago now – that were against emerging and re-emerging diseases such as influenza and AIDS.

These included several mammalian cell lines that were tumorigenic, tumor-derived, and also cells such as CHO that were producing retroviral particles. This was new for vaccines, although they have long been used in therapeutics. Additionally, new species such as avian, insect, plant, and bacteria were introduced that were not previously used in viral vaccines.

This raised safety concerns for known and unknown viruses that were particularly of concern, because virus vaccines cannot undergo the rigors of inactivation and removal of clearance steps as for therapeutics.

The discussions related to transformed tumorigenic cells were initiated early in 2001. And we also had discussions on tumor-derived cells in 2012. These discussions were in the public format in our Vaccines and Related Biological Products Advisory Committee or the VRBPAC.

The next slide highlights the emergence of NGS, which is also called high throughput sequencing, gene sequencing, or rapidly parallel sequencing. This new technology emerged in 2007, and it demonstrated capabilities for broad virus detection, including known viruses, novel viruses, and also for detection of emerging viruses. This was just in time to address our concerns related to adventitious virus detection in new cell substrates.



Challenges Include Standardization, Validation and Bioinformatics

The next slide ***[#5]*** shows some of the public discussions related to the use of new technologies for the use of adventitious detection in biologics in which the FDA has been involved. There have been several meetings starting in 2009 and through 2013.

All of these meetings focused on the challenges of using new technologies for virus detection. The discussions and the presentations in these meetings resulted in identifying the challenges which are shown in the next slide ***[#6]:***

- The main challenge for using NGS was related to the method standardization and validation. Basically, there was a need for appropriate model viruses and other relevant reference materials and standards to evaluate the efficiency of the different steps involved in the methodology and to determine the sensitivity and specificity of the technology.

- Additionally, there were challenges related to the bioinformatics, which is a large part of using NGS. This includes data analysis, pipeline optimization, and using a complete and correctly annotated reference virus database. Additionally, the data submission, storage, and transfer of a large amount of data from NGS also needs to be considered with regards to the format and the security.

- Also, when you are using NGS, it is expected that you will get a hit. However, it is important to develop a follow-up strategy so you can confirm the nature of a 'true' hit versus a false hit or a background signal – also then to follow up to determine the biological relevance and significance of the positive signal.

NGS Potential for Broad Adventitious Virus Detection

The next slide ***[#7]*** highlights the application of NGS in biologics. The discovery of the porcine circovirus [PCV] in a licensed rotavirus vaccine by an academic group at the University of California, San Francisco was an important discovery, because the vaccine had undergone all of the rigorous testing that was required using the conventional assays for licensure of a vaccine – which was very extensive testing.

However, the testing missed the presence of this porcine circovirus. It is not a new virus. It is a known virus, but the tests were not able to detect it because there was a gap in the current testing, which NGS was able to fill for the discovery of the virus.

Additionally, more recently, my lab has discovered a novel rhabdovirus in the Sf9 insect cell line that is used for baculovirus-expressed products. So these findings in biological materials have demonstrated the power of NGS for virus discovery, and this is recognized by both industry and regulatory authorities.

FDA's Collaborative Efforts

The next slide ***[#8]*** shows some of the FDA efforts on NGS for applications in biologics. The FDA has established both agency-level as well as CBER-level genomic working groups to support our research and regulatory infrastructure to support policy development and decision-making related to NGS applications.

There is also a strengthening of our in-house laboratory and bioinformatics expertise for NGS analysis, as well as being involved in co-organizing scientific public meetings that involve data presentations and discussions on the readiness of NGS for adventitious virus detection in biologics.

FDA/Industry Interest Group Sponsored by PDA

One of these efforts is highlighted in the next slide ***[#9].*** This describes the FDA and industry coordinated efforts in establishing an Advanced Virus Detection Technology Interest Group [AVDTIG], which is sponsored by the Parenteral Drug Association, the PDA. It was initiated as a 'user's group' in 2012 and then changed to an 'interest group' in 2014.

The mission of the group is to advance next generation tools for virus risk evaluation by providing an informal scientific forum for open discussions and scientific collaborations.

The current co-chairs include myself, as well as Dominick Vacante from the US, Jean-Pol Cassart from Europe, and Keisuke Yusa from Japan. We have open public participation with more than 150 international scientists from industry, regulatory and other government agencies, as well as national authorities, academia, CROs, and others. We have meetings and discussion by teleconference every month. We have subgroups that focus on additional points targeted to address the challenges. I am mentioning this because anyone is welcome to join who is interested in being involved with efforts towards NGS standardization and other efforts to facilitate its application in biologics for virus detection.

IABS/FDA 2017 Meeting

The next slide ***[#10]*** highlights the meeting that was focused on NGS in 2017. This was cosponsored by the IABS and the FDA in Rockville Maryland, in the USA. The meeting focused on data presentations and discussions for developing a scientific consensus for using NGS for virus detection in selected applications of biologics.

The next slide ***[#11]*** shows the publication of the summary of the meeting. ***[A link to the summary is provided below.]*** It details the information that was presented in the talks themselves so please, look into the paper in Biologicals from 2018 for further details about this meeting.

The next slide ***[#12]*** highlights some of the outcomes of this meeting. The participants identified the need for: ● standard reference reagents ● well-annotated databases ● large data storage and transfer capacity ● a follow-up strategy for NGS hits ● the need for personnel with relevant expertise in bioinformatics and virology, and ● a need to have early harmonization of international regulations for testing biologics and the availability of reagents for standardization.

It was noted that continued collaborative efforts and scientific exchange was needed to move the field forward with the goal of assuring the safety of biologics.

AVDTIG Subgroups in 2019

The current organizations in the Advanced Virus Detection Interest Groups is shown on the next slide. Currently there are three subgroups, and each of these are focused on addressing the need to facilitate NGS standardization.

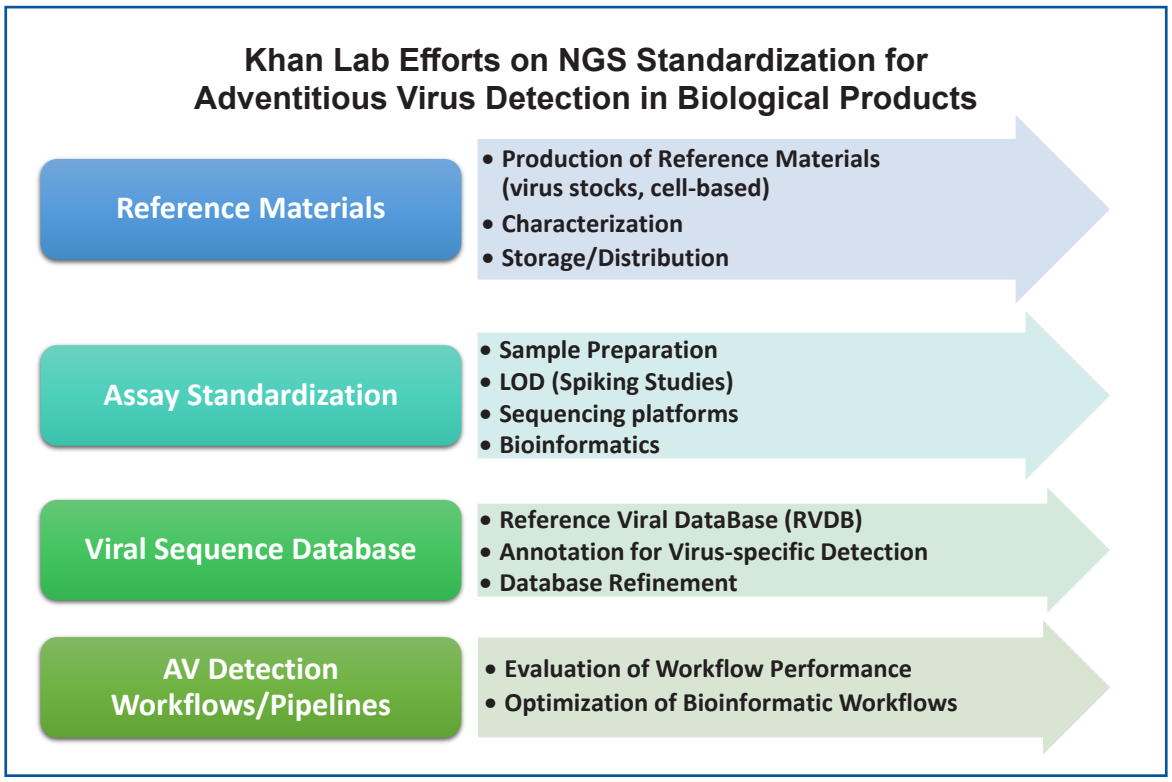
The co-leaders of the groups are indicated here [see slide #13 for names and email addresses]. The subgroup A/B focuses on: ● sample selection, preparation, and processing, and ● reference materials, and development of standards. Subgroup C is focused on: ● database evaluations and ● continuing to develop and refine a publicly available virus reference database. Subgroup D/E focuses on: ● bioinformatic pipeline analysis and ● follow-up strategies to confirm an NGS 'hit.'

Again, anyone who is interested in these efforts is welcome to join these subgroups and please email me or any of the subgroup leaders.

The next slide ***[#14]*** shows papers published by some of the IG members. These are considerations and current perspectives related to using NGS for: ● upstream sample processing and library preparation – the workflow – as well as the ● downstream with regards to the bioinformatics and optimization of the pipeline for virus detection.

Standardization for NGS Detection of Viral Adventitious Agents

The current efforts in my lab are focused on NGS standardization for adventitious virus detection in biologics. The topics that I will present briefly are related to: ● reference materials ● assay standardization, and ● the viral sequence database.



This slide ***[#16]*** shows a publication from a collaborative, multicenter study that involved my lab, GSK, and Sanofi. This was a small-scale study in which we used four selected viruses that were publicly available. The viruses were distinct based on the physiochemical properties. They represented the potential viruses present in biologics.

These virus stocks were used to evaluate the performance of HTS for virus detection. The experiments were done independently by the three labs. Interestingly, we found that regardless of the complex matrices that were used, the sample processing, the library preparation, the different sequencing platforms, and the different bioinformatic pipelines, we got similar results in terms of virus detection of the four model viruses that were spiked in the matrices. Please look into this paper for further details about the results.

Virus Stocks for NGS Evaluation and Standardization

However, more importantly, this study demonstrated that well-characterized model viruses could be used as reference stock for evaluating NGS analysis in different laboratories. So this was the basis for my laboratory, in consultation with the interest group, to develop five large virus stocks that are shown in the next slide ***[#17]*** – the porcine circovirus, the reovirus, feline leukemia virus, human respiratory syncytial virus, and Epstein-Barr virus.

This represents the number of vials that were prepared, and each were about .5ml. We prepared them through a contract at the American Type Culture Collection, ATCC.

These have been distributed to the members of the interest group for their ongoing spiking studies – for larger studies with more participants. And they are also now available to others who are interested in using standardized NGS for viral detection. You can request them by emailing me, and they are available through an MTA, material transfer agreement.

The next slide ***[#18]*** shows the potential use of these virus stocks. They can be useful to **perform spiking studies** to evaluate total NGS workflow for virus detection in different matrices relevant for biological materials in production of biologics.

They may be also used to **compare NGS** with current nucleic acid-based assays or even infectivity assays for virus detection, because these stocks have been characterized in terms of their genome copy numbers, as well as their infectivity titer, and particle counts. Also the stocks have been characterized by NGS in terms of the background host-cell sequences. So they are well-characterized stocks.

More recently, stability studies have been performed – a twenty-four month stability study on the virus stock as well. The results of these spiking studies using the virus stock can also generate well-characterized data sets for **evaluating bioinformatic pipelines**.

New Reference Virus Database (RVDB)

The next slide ***[#19]*** describes my lab's efforts on creating a new reference virus database. There was a need recognized in doing NGS analysis with the current databases that there were gaps…with regards to sequences representing all viruses.

So, we developed a new reference virus database. This database has been found to be more sensitive and specific for virus detection. It contains viral sequences that have been obtained from GenBank [including] viruses from all different species that are being used as cell substrates in biologics.

This database has reduced nonspecific cellular hits, which results in less data volume for bioinformatic analysis and less computational time. We have both the unclustered version and a clustered version that has been clustered at 98% identity. They were available at the George Washington University HIVE since 2017.

Proteic versions of the same databases have been kindly provided by Marc Eloit and Thomas Bigot at the Institut Pasteur and more recently – in October 2019 – we have moved the reference virus database to the University of Delaware, where it can be presented in a more user-friendly and blast-searchable format.

The next slide shows the current status of this database. It is the website for the University of Delaware. My lab continues to update the database quarterly coincident with the GenBank update We now have annotation efforts initiated to characterize some of the viral entries that may also contain non-viral sequences because of how they were deposited in GenBank.

RVDB Current Status

- **Reference Viral DataBase: https://rvdb.dbi.udel.edu/**
- **Continues to be updated quarterly and coincidently with a GenBank update**
- **Annotation efforts initiated to characterize some viral entries that also contain non-viral sequences**

RVDBv17.0 and 1st Annotation File (v.16.0)

- **Available since Oct. 29, 2019**
- **Based on GenBank Oct. 2019 release version 234 and RefSeq Oct. 2019 release version 96**
- **Number of sequences (U-RVDB/C-RVDB): 2,820,860 / 717,145.**
- **Proteic versions are available at RVDB and https://rvdb-prot.pasteur.fr/**

Currently, version 17.0 is available, and we also put in the first annotation file of the website…. The similar version for the proteic arc is available at the RVDB as well as the Pasteur Institute.

The applications of RVDB are shown in the next slide ***[#21]***. This is aimed to have complete representation of diverse virus families specifically from host species used in cell substrates for production of biologics and accurately annotated entries indicating non-viral/viral regions. This is what we are working on now. So you can get rapid and accurate results for adventitious virus detection using the database.

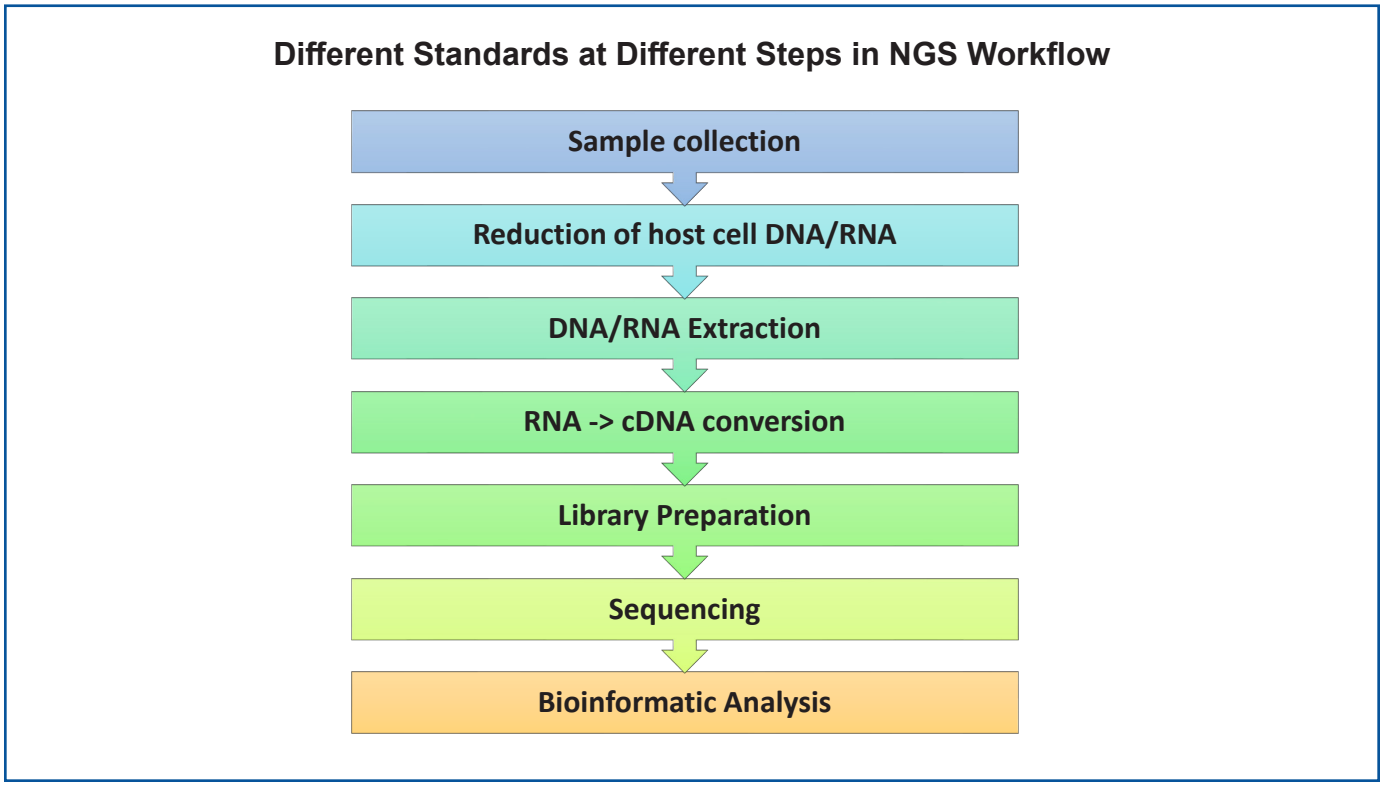
The availability of both the unclustered and the clustered nucleotidic database as well as the corresponding proteic versions can facilitate detection of known, and distantly-related as well as novel viruses. This can also allow development of strategies for NGS large data analysis for adventitious virus detection by facilitating the general use of NGS for broad virus detection. Our regular updating with new entries in GenBank will aid in the detection of the emerging viruses.

NIST/FDA 2019 Meeting on Standards for NGS Detection

The next slide ***[#22]*** indicates a recent meeting with NIST [National Institute of Standards and Technology] on NGS standards. This was held September 18-19 of this year. The main topics that we discussed here were related to the currently available reference materials or standards for NGS virus detection and what else may be needed to fill the gap for standardizing NGS.

The next slide ***[#23]*** shows the different types of standards that were discussed for NGS virus detection. They may be **natural** standards that are extracted from whole, intact viral particles or could be extracted viral nucleic acid. Or they could even be **synthetically made** standards of the virus-like particles or they could be naked nucleic acids like plasmid.

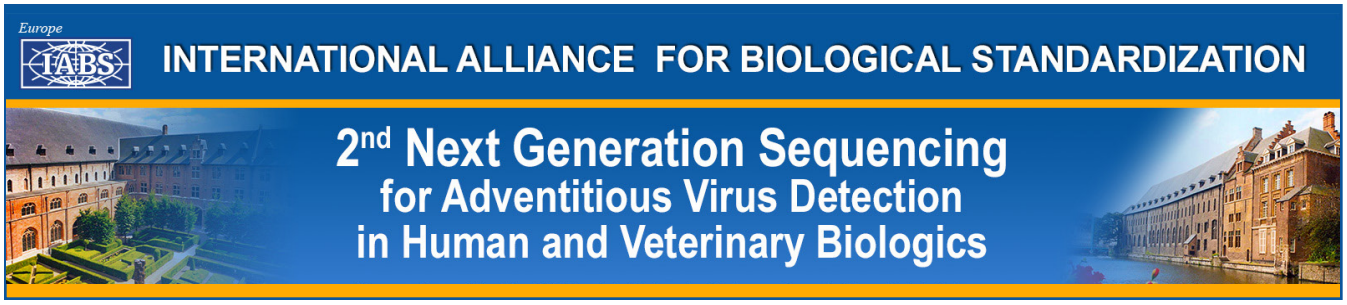
The next slide just shows that there may be different standards that may be used at different steps in the NGS workflow, and we are currently preparing a summary of the meeting. We will publish that soon. ***[A link to the summary, published in Biologicals in March 2020, is provided below.]***



Themes of the IABS Meeting in November 2019

The next slide shows a recent meeting on NGS with the IABS. This was a meeting in Ghent, Belgium in November of this year [2019].

There we heard ongoing efforts on standardization and validation of both the technical and bioinformatic steps of NGS for its application in characterizing and safety evaluation of biologics. This included both human and animal vaccines. The goal of this was to develop a consensus, a scientific consensus, regarding the readiness of NGS for detection of adventitious virus in biologics.



Scientific Committee

Dieter Deforce, Ghent University/Federal Agency for Medicines and Health Products of Belgium (FAMHP)

Sebastiaan Theuns, Ghent University/PROVAXS

Arifa Khan, US Food and Drug Administration (FDA)

Pieter Neels, International Alliance for Biological Standardization (IABS)

Sven Arnouts, Ghent University/PROVAXS

Johannes Blümel, Paul-Ehrlich Institut (PEI)

William Egan, GlaxoSmithKline Vaccines

Carmen Jungbäck, International Alliance for Biological Standardization (IABS)

Ivana Knezevic, World Heath Organization (WHO)

Laurent Mallet, Sanofi Pasteur

Gerald Schumann, Paul-Ehrlich Institut (PEI)

David Mackay, Advisor Veterinary Vaccinology

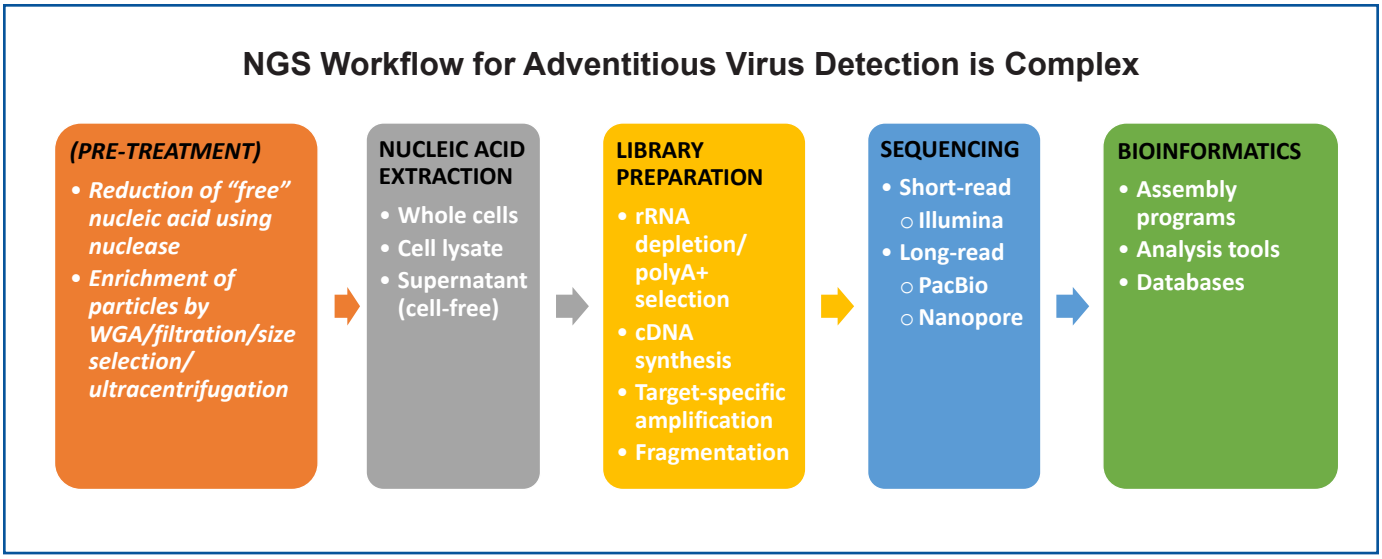
Joseph Victoria, Boehringer-Ingelheim

- **Bring together industry, academia, technology providers, and international regulatory bodies to discuss current status of NGS for adventitious virus detection in biologics**
- **Present ongoing efforts on standardization and validation of the technical and bioinformatics steps in NGS for its applications in characterization and safety evaluation of biologics, including human and animal vaccines.**
- **Develop a scientific consensus regarding readiness of NGS for detection of adventitious viruses in biologics.**

The next slide ***[#26]*** highlights some of the key issues discussed at the meeting. It was recognized that NGS is complex. The questions that were discussed are indicated here. They are:

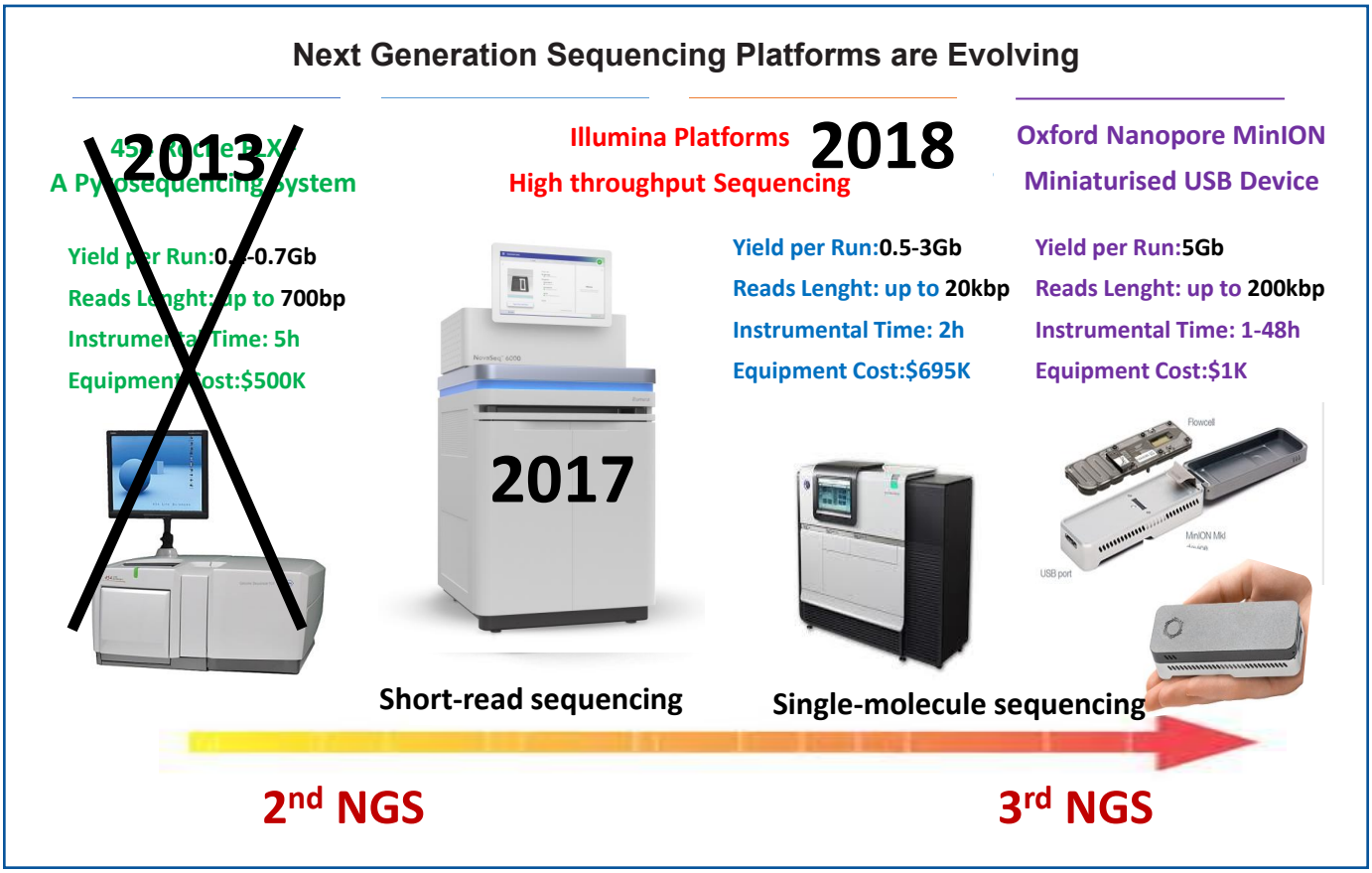
- What is the readiness of NGS to currently supplement and/or replace assays?
- In which situation can NGS be used to supplement or replace current assays? Which assays?
- Is there a difference in the expectations for NGS data for supplementing or replacing assays?
- What is needed for broader (routine) applications of NGS?
- And can the different regulatory authorities develop the same expectations?

This is really important since we are still in the early phases of NGS applications in the regulatory arena. So, as I mentioned, it was recognized that NGS is complex. This is shown in the next slide. There are multiple steps to the general workflow of NGS, and each of these may need to be standardized or the performance evaluated for the overall standardization of the workflow.



Evolution of NGS Platforms

An additional challenge with NGS is shown in the next slide. The technology is still evolving. As you can see, the first-generation platform which was the Roche 4S4 platform became obsolete in 2013. And even now, one of the Illumina short-read platforms has been changed. Now they have higher proof of platforms and new technology has emerged in terms of the long-read platform for single molecules, such as the PacBio as well as the MinION.



So the technology is moving. However, the challenges are being met, because the power of NGS is recognized, and its benefits for potential applications in biologics is recognized. Therefore, regardless of these challenges, there has been much progress made towards the standardization and applications of NGS.

Some of the potential applications are shown in the next slide ***[#29]***. It may be used as a strategy **to mitigate the risk** of adventitious virus introduction, for example in terms of the: ● raw material testing ● cell banks ● virus seeds and it can be used to **monitor the absence** of adventitious viruses during production, for example, in testing the:● bulk harvest or even ● characterizing the final product.

Currently Recommended Conventional Assays

The next slide ***[#30]*** shows the currently recommended conventional assays used for adventitious virus detection. These are detailed in the 2010 FDA guidance document for using cell substrates for preventative vaccines.

- This includes **general detection assays** including *in vivo* assays, *in vitro* assays of general actives like TEM [transmission electron microscopy] as well as retrovirus specific assays like the PERT [product-enhanced reverse transcriptase] assay.

- It includes **species-specific assays** based upon if your manufacturing process has included any animal-derived material.

- In the case of novel cell substrates, recommended additionally in our Office of Vaccines on a case-by-case basis are extended PCR assays, oncogenicity assays, as well as chemical induction assays with endogenous retroviruses and latent DNA virus detection.

Potential Applications of NGS

This next slide ***[#31]*** shows the potential of NGS as a replacement alternate assay or even a supplementary assay. It may be used for an **alternate assa**y. These are under discussion and consideration. For example, for the *in vivo* assays, the use of NGS can provide defined sensitivity and retrovirus detection and also meet the '3R' objectives to reduce the use of animals. NGS can also have similar or greater sensitivity than PCR assays.

And it can be a single assay compared to the long list of PCR assays that may need to be used for specific viruses. It can be used as a **supplementary assay** possibly for *in vitro* assays for characterizing cell substrates.

Also, in terms of the *in vitro* conventional adventitious virus detection assay where there may be interference due of lack of effective neutralization of vaccine virus. It could even potentially supplement *in vitro* adventitious assay as a read-out assay to broaden virus detection. However, the use of NGS needs to have a **follow-up strategy** established in terms of how to determine the positive signal is a true hit and to determine the biological relevance and significance as with any nucleic-based assay.

Current CBER Thinking Around NGS

The next slide ***[#32]*** shows the current status of using NGS in our Office of Vaccines. NGS data is currently under review in CBER. The use of NGS for product characterization and testing has been presented: ● for adventitious virus testing for master and working seeds and viral harvests ● for the genetic stability of vaccine virus, and ● for cell substrate characterization.

We are considering NGS on a case-by-case basis currently – often complementary to traditional testing both to characterize products and in regard to adventitious agents – and efforts are still underway to standardize and validate NGS.

Within our office, we highly recommend that sponsors request early technical working group discussions related to the use of NGS and the product characterization – also to discuss the plans for using NGS in a non-regulatory meeting and to reach a consensus prior to initiating lengthy, expensive studies.

Assay Standardization and/or Validation

The next slide ***[#33]*** shows what is needed for assay standardization and validation. This is for any assay including NGS. You need: ● relevant controls ● to determine sensitivity and specificity ● to demonstrate precision ● to valuate assay robustness, and ● to demonstrate the reliability of the assay. More details on the validation of analytical assay and statistical analysis are described in the ICH Q2(R1) document.

This slide emphasizes that testing is one component of a strategy to mitigate the risk of adventitious viruses. This is one prong of a multi-prong scheme. You also need to do **risk assessment** with **prevention** of introduction of agents as well as **process validation,** if your process can incorporate all those viral clearance steps during the manufacturing. So, the focus has been on **testing**, but of course there is a need to also include other strategies to mitigate the risk of adventitious viruses.

Testing is One Component of the Strategies to Mitigate Risk of Adventitious Viruses

- **Risk assessment- Identity potential sources of virus introduction to develop a comprehensive risk mitigation strategy and testing plan**
  - **Know the spectrum of infectious viruses that could potentially be in the host species of source materials (naturally-occurring, animal vaccines)**
  - **Gain cell culture passage history and characterization**
  - **Examine potential for virus exposure in the supplier's facilities (including chemically-derived materials)**
- **Prevention**
  - **Use well-characterized cell banks**
  - **Use certified/tested animal-derived biological materials (e.g. serum, trypsin, antibodies)**
- **Process validation**
  - **Incorporation robust viral clearance steps steps during manufacture for viral clearance and purity (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 improved sensitive and broad detection assays**

For the testing, it is extensive – and redundant in some cases – to assure safety at different stages, and we recommend using **various improved sensitive assays** for broad virus detection.

The next slide ***[#35]*** shows the path to introduce improved assays. This can increase the efficiency in terms of **time** for testing. It can also address **ethical issues** with regards to reducing animal use. And in some cases it can also be a **superior assay** in terms of limit of detection, specificity, repeatability, and accuracy.

I should also mention that the current guidelines and regulatory documents already provide flexibility for using alternate approaches to broad virus detection capabilities and fit for purpose. This was in our US FDA cell substrate guidance of 2012. It is in the WHO cell substrate guidance of 2012. And it is also in various European Pharmacopoeia documents of 2017.

Updating ICH Q5A(R2)

The next slide ***[#36]*** now goes into the ICH Q5A that you already heard details about. I am not going to go over this because Dr. Sakurai already very nicely went through the details of the current update with regards the ICH Q5A(R2) related to viral safety evaluation. I just want to highlight with regards to the new virus assay, an alternative analytical method for virus testing ***[slide #37]***.

Nucleic acid-based assays are being considered, such as PCR and also next generation sequencing, since they can provide rapid and sensitive detection of adventitious endogenous viruses in the starting and harvest materials. And the general principle for the inclusion of new assays and potential replacement supplement of existing assays should be presented in order to continue to support future development of new technology.

And with that, I thank you for your patience on this teleconference. I know it is difficult over the phone. And then the next slide ***[#38]*** just shows my appreciation. And thank you for listening.

LINKS:

NIST/FDA September 2019 NGS Workshop

- Agenda and slides
- Report