IPQ INTERNATIONAL PHARMACEUTICAL QUALITY
Inside the Global Regulatory Dialogue

COVID-19 Vaccine Urgency Throws Spotlight on Next-Generation Sequencing for Adventitious Virus Control

[Editor's Note: The following is IPQ's coverage of the May 2020 CMC Strategy Forum Europe. The coverage appeared as the first two parts of a five-part IPQ story on the collaborative efforts now going on globally to advance the use of in vitro testing in assessing and controlling adventitious viruses for vaccines and biotherapeutics – efforts for which the COVID-19 pandemic has provided a strong catalyst. Parts III and IV of the story focus on the adventitious virus discussions that took place at the December 2019 CASSS Japan forum. The full five-part story was included in the April/May IPQ Monthly Update. The issue has been made publicly available so that its content can be as helpful as possible in meeting the COVID-19 pandemic challenges. CLICK HERE for the full April/May issue.]

PART I

Adventitious Virus Control with NGS Draws Global Dialogue

The urgency of delivering a vaccine for COVID-19 in previously unattainable timeframes and volumes is throwing the spotlight on the expanded role next-generation sequencing (NGS) needs to play to avoid the time delays and other limitations of *in vivo*/animal testing.

The wave of COVID-19 vaccine development projects now underway is driving sponsors and regulators to further intensify their focus on how the full potential of NGS technology in assuring the safety and control of the vaccines can be realized.

Under review is how far the methodology has advanced over the past decade, how to best fill in and/or adjust to the knowledge and experience limitations that remain, and what needs to be communicated between sponsors and regulators to assure that the technology is performing its intended purpose.

> **These pivotal issues were explored at a very timely session on "the use of next generation sequencing to characterize and detect adventitious viral agents in biological products" at the mid-May 2020 CASSS CMC Strategy Forum Europe.**

The forum was originally scheduled to be held in Stockholm, Sweden, but was conducted virtually due to the pandemic. The conference agenda took shape in the latter part of 2019 before the pandemic was in view, making the choice of NGS as a topic of importance particularly prescient.

A session on "technologies related to viral safety" had been held at the December 2019 CASSS CMC strategy forum in Tokyo, Japan, in the wake of ICH's release in November of a concept paper on updating its Q5A(R2) guideline on evaluating viral safety in biotech products ***(see Part IV).***

The first session of the CASSS Europe forum, held on Monday May 11, included the traditional European Federation of Pharmaceutical Industry Associations (EFPIA) satellite session – formerly held under the "European Biopharmaceutical Enterprises" moniker. EBE has now been folded into EFPIA's Manufacturing & Quality Experts Group (MQEG). The session covered the current CMC issues for polysorbates, antibody drug conjugates (ADCs), and adeno-associated viruses (AAVs).

On Tuesday, attention at the CASSS forum was on ICH developments regarding Q12, Q13, Q14, and Q2(R), before shifting later in the afternoon onto NGS. Wednesday sessions addressed bioassays for monoclonals and patient-centric quality standards, respectively.

Academic, Industry, and Regulator Perspectives Offered

In focus at the NGS session at the CASSS Europe forum was the expanding use of the methodology in qualifying vector and virus seed sequences and the absence of adventitious viral agents that may impact the quality and safety of cell-based products and vaccines.

Explored were the significant advantages NGS offers over the conventional *in vivo* methods, as well as the technical and regulatory challenges involved and the heightened urgency of addressing them in the COVID vaccine development context. Included was a review of the ongoing regulator/industry discussions regarding the validation of NGS assays, and what data should be submitted and in what format.

> **The session included insights on the NGS progress and technical/regulatory challenges from the academic, industry, and regulatory perspectives.**

Introducing the speakers was EDQM European Pharmacopeia (Ph. Eur.) Division B Head Emmanuelle Charton, who co-chaired the session along with CBER Office of Vaccines Research and Review (OVVR) Division of Viral Products Deputy Director Robin Levis. Noting that falling under the "next generation" designation are "massive parallel," "deep" and "high throughput" sequencing (HTS), Charton stressed that "if PCR revolutionized the world of molecular biology as first-generation sequencing methods in the 20th century, NGS revolutionized this world in the 21st century."

Throwing strong light on the technology's potential, she explained, was its use in 2010 in detecting the unexpected presence of porcine circovirus (PCV) in a rotavirus vaccine. Since then, she pointed out, its development and use has expanded as a replacement for *in vivo* methods in identifying, characterizing, and controlling contaminants in biological raw materials and cell substrates.

The lead-off speaker was Ghent University researcher Sebastiaan Theuns, who explored the evolution in the sequencing technologies over the past decade and their expanded use by pharmaceutical manufacturers and veterinarians. Theuns heads a new Ghent University research spin-off company, PathoSense, that will serve industry in viral/bacterial detection with NGS.

Sanofi Pasteur Virology Analytical Expert Carine Logvinoff followed with a discussion of the evolving regulatory environment for HTS and the interactions Sanofi has had with health authorities – and particularly with FDA's Center for Biologics Evaluation and Research (CBER) – regarding substituting HTS for *in vivo* tests in detecting adventitious viruses for its new viral vaccine candidates. ***[Logvinoff's and Theuns' presentations are reviewed in Part II.]***

Italy National Center for the Control and Evaluation of Medicines Senior Researcher Domenico Genovese provided a European regulator's perspective on the current regulatory landscape for evaluating the safety of vaccines and biological products and the challenges of balancing "regulation and innovation" in applying NGS to the task ***(see Part III)***.

The Impact of the Wave of Coronavirus Vaccines Under Development

Session co-chair Levis then offered some highly valuable insights on CBER's substantial engagement with NGS and the impact of the current pandemic.

She reviewed the research her Division of Viral Products has been doing under the leadership of Supervisory Microbiologist Arifa Kahn to expand virus reference resources – stressing the challenges and importance of the bioinformatics piece in particular – and highlighted the sponsor interactions with Sanofi and others through "technical working groups" that have led to the ability to use NGS in replacing *in vivo* testing.

She then brought the audience squarely into the present with the wave of coronavirus vaccines that her office is now involved with and the important discussions that are taking place on how to deploy NGS in assuring their safety in eclipsed development timelines where full validation is not an option.

COMMENTS BY CBER'S ROBIN LEVIS AT THE CASSS EUROPE NGS SESSION

I want to just spend two or three minutes talking about FDA interactions in the world of NGS. First, I am in the Division of Viral Products. We are a research division at the FDA.

Probably most of you who are involved in the field of NGS know Arifa Khan. A lot of her laboratory efforts are related to the work of the working group and contributions. So I just want to start out first to just kind of introduce some of her contributions to the world of NGS. She is kind of our pivot point at the FDA for interactions with sponsors.

Virus Reference Resources

One of the things she has been really involved in is the development of the **reference panel** of, I think, five or six viruses that have been used for looking at standardization and validation. I will say that initially a very limited number of vials of each virus was made and characterized. But Arifa has just gotten a nice grant to renew and remake the reference panel, and she will have thousands of vials of each. So, that can be a global resource that can be made available to academics, to industry, and to anybody who wants them now that we have a good supply.

In conjunction with that, I know **NIBSC** [UK's National Institute for Biological Standards and Control] is working on making a reference panel that is much more extensive, I think up to 25 viruses. But that work is really ongoing, and that panel will not be available for some time. But that is also in the works, just so people know.

The other thing that Arifa and our division have been involved in is the development and modernization of the **virus-specific database**. And that is just one database. I know that certain sponsors have their own databases, and I think that is kind of one of the discussions for downstream. I do not know if we will have that discussion today.

I think one of the things that needs to be addressed is the **bioinformatics** part of this whole thing. It scares most of us who do not really understand how that works. I think it really is a critical piece – how we utilize the bioinformatics and how we kind of harmonize that across all of the different people who are utilizing these technologies and potentially are using common databases versus having their own moderated databases. From a regulatory point of view, I think that is a really important question about how we use that data, and I will speak to that in a minute.

Sponsor Interactions

So the second thing I wanted to mention is kind of what our sponsor interactions have been. Carine gave a really nice introduction to that. I want to thank Carine and the Sanofi team, because we really have had what we call **technical working group discussions**, which are not regulatory meetings. They are just scientists who are engaged in the problem-solving as it pertains to the CMC development of a product. We have really had some fruitful and important and successful dialogues, which has led to Sanofi really moving the field forward with respect to the use of NGS for characterizing testing samples for use in product development.

As Carine said, these kind of started out with the use of NGS to complement existing strategies. Now we have moved to where NGS can actually replace or substitute for those *in vivo* strategies and be the sole testing. Some of the PCR assays and things can be supplemented or replaced using the NGS. We have moved forward with the acceptance of that.

Applications for COVID-19

You can well imagine that in the days of coronavirus vaccine development, we have many, many vaccines coming across our doorstep to analyze and get into clinical trial. And, how do we effectively evaluate these vaccines, ensure they are safe, and get them into the clinic when our traditional pathway for entry into phase I of a trial is showing on the sponsor's behalf that it is safe to go into first-in-humans?

Part of that is a complete adventitious agent set of assays done both on all raw materials of biological origin, such as in cell substrates, such as the virus seed banks. With an *in vivo* test taking up to four to five weeks, how do we move these products into trial in a way that we still know they are safe?

So, we have been engaging with a lot of sponsors about fast-forwarding the use of NGS to test these new products and the new raw material substrates, in terms of cell substrate and virus banks using NGS. It has been a little bit of a challenge for us because, given the breadth and number of sponsors we have, they do not all have the expertise that Sanofi has in terms of how to develop these assays.

And really, nobody has time to actually validate the assay. So how well qualified does it have to be? These are some of the issues that we are kind of just working through in terms of this short notice in this really critical situation where it is important to get these vaccines into the clinic. How are we going to utilize and rely on NGS data? That is something that is really foremost for us.

Data Sets Needed for Submissions

Another thing I wanted to touch on, which is a little bit more technical – I just will say this quickly maybe for possibly this year's discussion or next year – but how do regulators interact with the actual NGS data?

In the Division of Viral Products, we have made the decision that we are not going to reanalyze the data sets and do our own bioinformatics analysis. We just don’t have the computing capacity yet. We have the intellectual capacity kind of. But we would essentially have to set up a bioinformatics lab or division, and we just do not have those resources at this time. So, we have not been asking sponsors to submit the original data sets.

What we have been asking sponsors to do is to retain the data set – and as the libraries become updated, that potentially they reanalyze data to keep the data current. Like I said, those I think are discussions for a little bit farther downstream, when we get more experience with that.

Keeping Up with the Science

The final thing I want to say before we get to our discussion and questions is that I really appreciate and wanted to thank Domenico for the comments on really the limitations on our current assays. At any one point in time, we develop assays based on the existing technology. The assays that have been in use for some decades to ensure the safety of our products with respect to bioburden and adventitious agents were all developed at a time when what we could do is look in animals, what we could do is look in tissue culture. What we could do when PCR came around was to look at specific things by PCR.

Really now, all of that can be supplanted with this new technology as it moves forward. I just wanted to end my comments on saying we really should always use the current science to move both our product development from a sponsor's point of view and product review from a regulatory point of view. Also, for the researchers who are developing all of these assays and how to use them, it is really important for us to stay current with the technologies.

Levis and Charton Stress the Harmonization Opportunity

Levis then moderated the panel discussion that followed ***(see Part II for a review of the full panel discussion)***. At its conclusion, she stressed that the introduction of NGS presents a "real opportunity" for harmonization between industry and regulators across the human and veterinary arenas and for sharing in the effort to maximize NGS's effectiveness.

> **EDQM's Charton agreed on the harmonization opportunity and benefits. Levis asked her fellow session moderator about the potential benefit of a pharmacopeial monograph for NGS.**

Charton responded that in view of how quickly the technologies evolve, she did not envision having product-specific tests, but "maybe more guidance on how to validate the techniques" and how to demonstrate equivalence. She noted that how to further the use of these technologies was under active discussion at EDQM and that "there is lots to work on."

She pointed to the appreciation for the NGS references in Ph. Eur. expressed by stakeholders at the major International Alliance for Biological Standardization (IABS) conference on "NGS for adventitious virus detection in human and veterinary biologics" held at the University of Ghent in November 2019.

"Of course, it is always a question of what to write and what not to write," she commented, "because if we write too much, then you could be seen as preventing innovation. That is surely not what we want to do, but to continue to encourage the use of these techniques for the characterization or the identification of adventitious agents in biological medicines."

[In Part II, highlights of the 2019 IABS meeting were provided by CBER's Khan as part of her presentation on NGS in viral detection at the CASSS Japan Strategy Forum in December 2019, which is reviewed in Part III.]

PART II

Sanofi Pasteur and Ghent University Experience with NGS

At the May CASSS CMC Strategy Forum Europe, Sanofi Pasteur Virology Analytical Expert Carine Logvinoff began a presentation on her company's engagement with next-generation sequencing (NGS) by highlighting the "noisy entry made by HTS [high-throughput sequencing] in the vaccine world" in 2010 with the discovery that the existing testing package could miss viral contaminants.

The impact on the regulatory environment, she explained, included vaccine manufacturers being requested to consider additional adventitious agent testing methods.

> **Sanofi Pasteur, in turn, made the decision to accelerate the exploration of new molecular technologies – finding HTS to be an "effective and valuable tool for both identification and reduction of unknown adventitious virus."**

Logvinoff outlined in detail the validation steps the company took to run the testing under GMP, with consideration of the new chapter 5.2.14 in the European Pharmacopoiea (Ph. Eur.).

The chapter recommends selection of a panel of representative, well-characterised model viruses to demonstrate that a proposed new method is equivalent in sensitivity to the *in vivo* methods. In view of the challenges in assessing the sensitivity of the broad *in vivo* methods, Logvinoff's team took advantage of an NIH study published in 2014 to assess their test against the panel of 16 model viruses used in the research paper.

> **At the same time, the international regulatory environment was evolving, with publication of a series of technical reports by WHO and revision of the Ph. Eur. chapters on cell substrates and tests for extraneous agents in viral vaccines. Logvinoff explained that these revised texts supported Sanofi in changing its approach to the testing package – enabling a justification of the new technology based on the viral risk assessment.**

She reviewed Sanofi's extensive participation in international meetings and conferences – in particular, the International Alliance for Biological Standardization (IABS) meeting on NGS for adventitious virus detection in biologics in November 2019, held at the University of Ghent. "I think it was a really important meeting" she said, "both for the technology aspect and the interface with the regulatory authorities."

Sanofi's collaborative engagement around NGS has included active participation in PDA's Advanced Virus Detection Technologies Interest Group (AVDTIG). Sanofi participants are now co-leading the IG sub-groups on bioinformatics and follow-up investigations.

[Editor's Note: See Part III for further discussion of the 2019 IABS meeting and AVDTIG by FDA's Arifa Khan at the CASSS CMC Strategy Forum Japan in December 2019.]

> **Logvinoff concluded with a review of the valuable meetings Sanofi has had with FDA and other regulatory authorities on its use of HTS.**

At the initial meeting with CBER in 2017 on Sanofi's proposal to replace *in vivo* tests for a new vaccine, the company was invited to have "any type of technical meeting as we felt the need," she said.

A pre-IND meeting was held to discuss data submission requirements in 2019, followed by another at which the testing challenges were further addressed. These "open and extremely fruitful discussions" ensured that there was "mutual confidence" in the HTS test and that "CBER expectations" were being met. ***[A link to Logvinoff's full remarks at the forum is provided below.]***

Researcher Theuns Explores Fast Moving NGS Technology

In offering his extensive research experience with the rapidly evolving NGS technologies at the CASSS Europe session, Ghent University researcher and PathoSense leader Sebastiaan Theuns explored the wide range of analytical applications now in play and discussed the innovations of major sequencing companies such as Illumina, PacBio, ThermoFisher, Oxford Nanopore Technologies, and MGI (BGI).

Theuns was a member of the scientific planning committee for the November 2019 IABS conference. This second IABS meeting expanded the scope from a 2017 meeting to consider both human and veterinary biologicals. The planning committee for the 2019 conference included representatives from industry, European regulatory agencies, FDA and WHO.

In his presentation at the CASSS forum, Theuns noted the range of genome sizes being studied by NGS for adventitious agent testing, diagnosis of pathogens, and whole human genome sequencing, including: ● viruses at 1.5-150 kilobases ● bacteria in the 1-5 megabases range and ● human genomes at 3.2 gigabases. Depending on the sample source and purpose of the testing, amplification and both short- and long-read NGS technologies may be needed to investigate genome sequences of interest.

A common challenge for everyone working in the field of NGS, he stressed – whether in fundamental or applied research in academia, industry or regulatory agencies – is the rapid evolution of sequencing technologies, with weekly improvements in accuracy, the release of new equipment models, and the strong competition among manufacturers.

> **After discussing the technologies, Theuns moved on to the 'major hurdle' of the bioinformatics expertise and hardware needed for storage and real-time analysis of the data generated.**

"In the past," he explained, "we have mainly been using central processing units (CPUs), which are the typical chips in your computer." But increasingly, graphic processing unit (GPU) hardware – the graphical cards in a computer that are used by gamers – is crucial to speed up analysis, and is "really the way forward."

As a current example of the utility of NGS in diagnosis in real-time, Theuns spoke about use of the technique to diagnose and monitor the spread of COVID-19 in people crossing the border into the Netherlands, as well as at the local level in hospital departments.

Questions Posed by Panel Moderators and Participants

During the latter part of the session, a panel discussion provided an opportunity for presenters and moderators to further explore some of the issues in deploying NGS technologies for virus control that had been raised in the presentations.

Joining Logvinoff and Theuns on the panel was Italy National Center for the Control and Evaluation of Medicines Senior Researcher Domenico Genovese, who presented on balancing regulation with innovation ***(see Part III for more on Genovese's remarks).*** Moderating the discussion were CBER Division of Viral Products Deputy Director Robin Levis and EDQM Ph. Eur. Division B Head Emmanuelle Charton.

The NGS issues drawing discussion were:

- whether system cost and capabilities related to data quality
- the level of accuracy needed for adventitious testing and how to validate it
- how to maintain continuity of data quality in a GMP environment with rapidly evolving technology
- whether NGS could be used for quantifying bioburden as well as adventitious virus testing
- how to ensure complete DNA/RNA extraction by using two kits
- ease of use of different platforms and the need for appropriate expertise to interpret the big data generated
- the importance of virus standards in the early implementation phase of NGS with the many variables involved

- what discussions there were with other regulatory bodies besides FDA – in particular, European agencies
- product-specific validation using model viruses and during method transfer
- the opportunities to speed up coronavirus vaccine development using NGS with appropriate bioinformatics
- the critical need to follow up positive results to confirm they are 'true' positives
- the use of nuclease treatments to enrich the selection of encapsidated nucleic acids, and
- the evolution of the Ph. Eur. monographs and eventual harmonization of requirements and guidance

[CLICK HERE for the panel's discussion of these issues.]

Reinforced during the panel discussion was the importance of engaging with regulators before deployment of new and complex analytical technology like NGS to align on expectations. Also critical, participants agreed, is keeping up with and engaging in the open dialogue between academia, industry and regulators in various fora and interest groups.

Several of the issues debated by the panel – including validation, standardization, bioinformatics expertise and need for follow up of positive results – are being considered by AVDTIG. These include ● sample selection, preparation, and processing ● reference materials and virus standards ● development and evaluation of a publicly available virus reference database, and ● bioinformatics and follow up strategies to confirm an NGS "hit."

[CLICK HERE for Logvinoff's presentation at the forum.]
