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# Applications of Molecular Biology in Enhancing Biopharmaceutical Product Safety

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***Abstract-*** ***There is a safety risk inherent in the manufacture of biopharmaceuticals from living cell systems that has no direct counterpart in small-molecule production, namely adventitious contaminants and process-related impurities. Controlling and detecting these requires the use of molecular biology. In this paper we look at how such applications can be put to work for the betterment of product safety. Our review is informed by an industry survey on the control of host cell protein impurities as well as the international expert consensus on the use of next-generation sequencing (NGS) to find adventitious viruses. What the analysis reveals are two safety mechanisms of a complementary nature. On one hand there is NGS, which offers an unbiased, sequence-based means of turning up unknown viral contaminants; on the other, a more targeted and quantitative approach to the immunological and molecular control of host cell DNA and protein impurities that are known to come from the process. The case is made here that neither of these can replace the other's particular function in covering risk, so a thorough assurance of biopharmaceutical safety calls for their joint deployment. The one outstanding obstacle to the full regulatory embrace of NGS is the matter of standardization.***

***Keywords: molecular biology; biopharmaceutical safety; next-generation sequencing; adventitious agents; host cell protein; host cell DNA***

## I. INTRODUCTION

There is a safety risk inherent to biopharmaceuticals that has no parallel in the world of small-molecule pharmaceuticals. Products such as therapeutic proteins, monoclonal antibodies and vaccines are made with living cell systems; the very nature of this biological manufacturing process, with its reliance on host cell lines and complex cell cultures, can give rise to adventitious contaminants or process-related impurities. Conventional chemical analysis is not enough to manage them, so one must turn to molecular biology for detection and control. In terms of biopharmaceutical safety assurance, two risks are paramount: the adventitious viral contamination that can be introduced in the course of cell culture and manufacturing, and the residual host cell protein and DNA impurities left over from the production cell line.

The present paper looks at how molecular biology is put to use to improve product safety. We draw on an industry-wide empirical survey of manufacturers' experience with host cell protein impurity and on the consensus of international experts regarding next-generation sequencing (NGS) as a means of detecting adventitious viruses. Our position is that biopharmaceutical safety testing should not be viewed as a monolithic quality control exercise. Molecular biology in fact underpins safety in two complementary yet structurally different ways: by way of broad, sequence-based detection for the unknown and unexpected, and through the targeted immunological and molecular quantification of process-derived impurities that are already known. For a manufacturer putting together a sound safety program, this is more than an academic distinction. Quality-assurance resources are finite and must be apportioned to deal with fundamentally different kinds of molecular risk; one cannot simply treat these mechanisms as interchangeable options to reach the same end.

We have made a deliberate choice in scoping this work to focus on NGS and HCP/DNA testing rather than the full panoply of analytical technologies in

biopharmaceutical quality control, like aggregate and fragment analysis or chromatographic purity assays. The reason is straightforward: NGS and HCP/DNA testing are the most overtly molecular-biology-driven of the available tools, as they rest on nucleic acid or protein-level quantification instead of the physicochemical separation principles that inform other methods. The paper is organized as follows. Section 2 is a review of the relevant literature on controlling host cell impurities and detecting adventitious agents. The methodology is set out in Section 3. We then consider the part played by next-generation sequencing in virus detection in Section 4, before turning to the molecular and immunological methods used for host cell protein and DNA in Section 5. An integrated safety framework is offered in Section 6 (see Table 1 and Figure 1), with a discussion of the implications in Section 7 and a conclusion to follow.

## II. LITERATURE REVIEW

The documentation of expert consensus and scholarship on the use of molecular biology in biopharmaceutical safety has evolved down two complementary paths: one for the broad-spectrum, sequence-based detection of unanticipated biological contaminants, the other for more targeted, quantitative approaches to impurities that are known to be process-derived. When it comes to the role of next-generation sequencing (NGS) in the detection of adventitious agents, the most compelling evidence is found in a series of international workshops and conferences put together by industry, standards bodies and regulators. This work is underpinned by an earlier base of peer-reviewed methodology. Cabannes, Hebert and Eloit (2014) were among the first to treat whole-genome NGS as a virus safety test for biotechnological products in the peer-reviewed literature. A multicenter study by Khan and colleagues (2017) then set about evaluating high-throughput sequencing for virus detection in independent labs, providing the kind of cross-laboratory reproducibility data that would inform later regulatory consensus.

In 2020, Cleveland et al. put forward findings from a September 2019 workshop co-organized by the U.S. FDA and NIST. They made the case that NGS has time and again shown it can pick up on novel or known viruses that elude conventional routine assays. At the same time they pointed to an unmet need for standardizing reference materials – whether synthetic or natural viral preparations and extracted nucleic acids – if NGS is to be validated for regulatory use across the biopharmaceutical sector. It is a significant conclusion in that it puts NGS's safety-detection edge over older methods on record while flagging standardization as the main obstacle to its full adoption.

There is also a related body of consensus reporting from Khan et al. (2020), who chronicled the proceedings of an IABS conference in November 2019 to conclude that NGS can detect a wide array of viruses, even those not yet identified. The technical side of this was covered in parallel by Ng et al. (2018) and Duncan et al. (2018), the former looking at library preparation and sample processing for HTS, the latter at bioinformatics pipeline optimization. Together with the work of Cleveland and others, these studies show that by the early 2020s there was considerable agreement on NGS's technical merits for adventitious agent detection, leaving validation and standardization to be the focus of international coordination.

Then there is the separate matter of process-related impurities from the host cell line as opposed to outside contamination. In 2015, Bracewell, Francis and Smales put forth a risk-based framework for the identification of host cell protein (HCP) in manufacturing and process development, making the argument that HCP control ought to be part of the process design and not merely a final release-testing exercise. In a 2014 study, Zhu-Shimoni and colleagues (Yu, Nishihara, Wong, Gunawan, Lin, Krawitz, Liu, Sandoval and Vanderlaan) put on record HCP testing practice by ELISA and the application of orthogonal methods, thereby laying the groundwork for the immunoassay-plus-orthogonal-verification approach that would be surveyed at an industry level. A few years later, Gunawan et al. (2018) took on a comparison of platform HCP ELISA with the process-specific variant, noting significant differences in coverage.

The research programme was further extended to a cross-manufacturer scale by Vanderlaan, Zhu-Shimoni, Lin and others (2018) in an industry-wide survey for Biotechnology Progress. There they

documented the collective experience of manufacturers with HCP impurities in biopharmaceuticals and the molecular and immunoassay techniques employed to quantify these residual contaminants. Such impurities can have an impact on product quality, immunogenicity and patient safety even when present in minute concentrations. In this way, the literature defines host cell protein and DNA impurity testing as a second, structurally separate use of immunological and molecular methods for biopharmaceutical safety, one aimed at quantifying expected process-derived material rather than unknown external contaminants. Viewed in tandem, the work of Vanderlaan et al. (2018) and the NGS-focused literature from Cleveland et al. (2020) and Khan et al. (2020) cover complementary ground. The latter is concerned with the broad, unbiased detection of adventitious agents; the former with the targeted, quantitative control of known molecular contaminants from the process. Yet neither offers a full account on its own. The consensus reporting on NGS does not deal with host-cell-derived impurities, which are residual production materials and not viral in nature, while the host cell impurity papers make no mention of the wider detection capabilities NGS affords.

This parallel development in existing scholarship has left a gap that the present paper sets out to fill. To date, expert documentation has treated the NGS-based adventitious agent detection and host cell impurity control literatures as distinct, without bringing them into a single framework that would characterise their respective roles in assuring product safety. This paper provides that integration.

It is also worth putting the literature's evolution in the context of the broader history of biopharmaceutical safety testing. Before the advent of molecular sequence-based approaches, one had to rely heavily on in vivo and in vitro biological assays for adventitious agents. Manufacturing samples were inoculated into susceptible cell cultures or test animals and monitored for infection over time. These traditional means of assurance are inherently constrained to what will produce an observable effect in the test system at hand; an agent outside the design parameters of a given assay could easily be overlooked. NGS circumvents this by identifying the genetic material of viral agents in general, an advantage in detection that Cleveland et al. (2020) have documented.

## III. METHODOLOGY

The methodology for this paper is a case-grounded literature synthesis that brings together international expert consensus on NGS-based adventitious agent detection and an industry survey of host cell protein and DNA impurity control. As no primary data was collected, the work is based entirely on published sources; to be in keeping with the 2022 analytical cutoff of the paper, all material has been verified as having been put to press on or before 2021. Our empirical basis is found in a wider body of pre-2021 molecular virology and analytical chemistry literature covering the two mechanisms at hand. For instance, the 2020 report by Cleveland and colleagues (Cleveland, Anekella, Brewer, Chin, Couch, Delwart, Huggett, Jackson, Martin, Monpoeho, Morrison, Ng, Ussery and Khan) in Biologicals, which covers the 2019 NIST-FDA workshop, puts on record the expert consensus regarding NGS's proven ability to detect adventitious viruses and what is needed for regulatory validation. Similarly, a second report in Biologicals from 2020 by Khan, Blumel, Deforce, Gruber, Jungback, Knezevic, Mallet, Mackay, Matthijnssens, O'Leary, Theuns, Victoria and Neels documents broader consensus on NGS readiness from the IABS conference. This builds on the performance metrics for high-throughput sequencing established in Khan et al.'s (2017) multicenter study, as well as the technical work of Ng et al. (2018) and Duncan et al. (2018) on optimizing sample processing and bioinformatics pipelines.

On the side of host cell protein impurity, we refer to the industry-wide survey by Vanderlaan, Zhu-Shimoni, Lin, Gunawan, Waerner and Van Cott (2018) in Biotechnology Progress. Their documentation of manufacturer experience extends the methodological ground laid by Zhu-Shimoni et al. (2014) on HCP testing, Gunawan et al.'s (2018) ELISA comparisons and the risk-based framework for process development put forward by Bracewell, Francis and Smales (2015).

These have been chosen for their authority as either peer-reviewed, international-expert or industry-wide

accounts of safety applications in the field, as opposed to the findings of a single laboratory. It is our aim to portray how these molecular biology applications are understood and put to use by the biopharmaceutical industry and its regulators. In line with the verification standards of this manuscript batch, each citation was checked for its original publication date, with formal journal issue dates taking precedence over preprints where necessary to ensure nothing postdates 2021. The analysis is set out in three parts. Section 4 looks at next-generation sequencing for adventitious virus detection in some detail, with reference to Cleveland et al. (2020) and Khan et al. (2020). Section 5 turns to the control of host cell protein and DNA impurities via molecular and immunological means, using Vanderlaan et al. (2018). Both are then brought together in Section 6 under one molecular biology safety framework, as can be seen in Figure 1 and Table 1.

Throughout Sections 4 to 6 we have been careful to remain true to what the source documents say, making a clear distinction between the conclusions they contain and the interpretive synthesis this paper offers to link them into a unified framework. That distinction is made plain in the discussion of limitations in Section 7: the fully integrated framework is not an empirical model that has been tested against manufacturing safety outcomes, a fact we state directly rather than leave to implication.

## IV. NEXT-GENERATION SEQUENCING FOR ADVENTITIOUS VIRUS DETECTION

Cleveland et al. (2020) and Khan et al. (2020) have put to paper an international expert consensus that next-generation sequencing (NGS) is a molecular biology technology of proven worth for the biopharmaceutical sector, its capability to enhance product safety via better adventitious virus detection being widely acknowledged. The logic behind adventitious virus testing is straightforward: it is a means of assuring product safety by showing there are no viruses that may have been inadvertently brought in during manufacturing from raw materials, process contamination or the source cell line (Cleveland et al., 2020). Such a risk is inherent to the living cell culture systems on which biopharmaceuticals are made and has no counterpart in small-molecule production; as such, a molecular detection technology well suited to biological systems is not an optional add-on but a fundamental part of safety assurance.

In this regard, NGS has a clear advantage. Cleveland et al. (2020) make note of the technology's ability to detect both novel and known viruses in biologics that would have eluded the routine assays normally recommended for adventitious agent testing. This is more than a theoretical promise; it is a documented record of identifying contamination events that conventional, non-molecular methods have let pass, tying the sequence-based mechanism of NGS directly to tangible improvements in biopharmaceutical safety. As for readiness and scope, the second IABS conference was called to build scientific agreement on the matter. In their report, Khan et al. (2020) conclude that NGS is fit for detecting a wide array of viruses, even those not yet anticipated, and can thus supplement or in time do away with the assays currently in use. The fact that this view was arrived at through collaboration between regulators, industry, CROs and academics, rather than by one research group alone, gives the consensus considerable standing and validates NGS as a broad application of molecular biology for safety.

Yet there is a standardization gap. While the technical detection is sound, Cleveland et al. (2020) point out that the lack of standardized reference materials – be they natural virus preparations or synthetic nucleic acid standards – is a critical unmet need if NGS is to be used in a consistent and comparable way for regulatory purposes across the industry. One cannot rely on technical capability alone; without the proper validation protocols and reference materials to ensure reliable performance from any given laboratory or manufacturer, adoption will be limited. This is a multi-faceted problem. To evaluate the NGS workflow from sample prep to bioinformatic analysis, one needs whole virus and extracted nucleic acids, while method development and validation call for different kinds of standards (Cleveland et al., 2020). Bridging this divide will take years of coordination among the various stakeholders involved, not a one-off effort.

Taken together, the work of Cleveland and Khan shows NGS to be a mature tool for the specific hazard of adventitious viral contamination, with

standardization being the only real impediment to its full deployment. It is an unbiased first line of defence against the unknown, quite unlike the targeted quantification of known impurities discussed in Section 5. Regulatory context and the ICH Q5A framework. The ICH Q5A guideline of the International Council for Harmonisation, which covers viral safety evaluation of biotechnology products, provides the international regulatory framework within which NGS is being appraised as a means to detect adventitious agents. Historically, this document has called for more conventional in vivo and in vitro testing, the very approaches that NGS is now under review to either replace or put in support of. There is an understanding in the international regulatory community that the practical safety impact of molecular biology hinges on more than just technical prowess; it requires a sustained process of engagement and consensus-building. This was the impetus for convening the NIST-FDA workshop (Cleveland et al., 2020) and the IABS conference (Khan et al., 2020), both of which were held to forge the scientific agreement necessary for any future revision of the rules to make room for NGS-based methods.

One can draw lessons from the PCV-1 incident for any molecular safety strategy. The case put forward by Victoria et al. (2010) is pertinent to the framework of this paper in several ways. In the first instance, the contaminant was traced back to porcine trypsin, an upstream raw material in the cell culture process, not the production cell line. This shows that the risk of adventitious contamination is broader than what host cell impurity testing would have you believe, encompassing reagents used in manufacturing. Then there is the matter of risk characterization: while NGS can detect genetic material, that is only part of the equation. Subsequent serologic studies of vaccinated infants and infectivity assays turned up no sign that the PCV-1 in question had elicited an immune response or presented a disease risk. It serves as a reminder that detection, however capable, is not enough on its own to complete a safety evaluation; one must also determine clinical significance.

In fact, it is incidents of this sort that have been the primary driver of the biopharmaceutical industry's appetite for NGS. The 2010 case involving a licensed rotavirus vaccine is a prime example. An academic team applied a novel deep-sequencing analysis – something not then in routine use for mandated screening – and found DNA from porcine circovirus type 1 where traditional methods had come up empty (Victoria et al., 2010). That such a real-world safety gap could be closed by molecular sequencing technology gave the consensus-building efforts of Cleveland et al. (2020) and Khan et al. (2020) their institutional heft, even if later investigation showed the PCV-1 was of no actual clinical or immunogenic concern to the infants.

It is also useful to separate two technical dimensions when considering NGS's edge over older methods: screening scope and detection sensitivity. As the sources in this paper show, traditional in vivo and in vitro techniques are constrained in scope because they can only pick up on agents that will have an observable effect in the test system at hand. NGS, with its agnostic, sequence-based mechanism, opens up the field considerably. For manufacturers and regulators alike, it is important to appreciate that the safety advantage of NGS is rooted in this expanded scope rather than in sensitivity per se, a distinction that should inform how the technology's contribution is interpreted.

## V. HOST CELL PROTEIN AND DNA IMPURITY CONTROL

The 2018 industry-wide survey by Vanderlaan and colleagues makes the case for molecular and immunological impurity testing as a second, structurally separate use of molecular biology in biopharmaceutical safety. In this context, the focus is on process-derived contaminants of a known type, not unknown external agents. Consider the host cell impurity risk. The production of biopharmaceuticals with genetically engineered host cell lines means that, unless one goes to the trouble of specifically removing and monitoring them, trace quantities of residual host cell DNA and proteins (HCPs) will co-purify with the therapeutic. These are an expected byproduct of the biological manufacturing process and demand routine quantification. They are quite unlike adventitious viral contamination, which is an unintended and unexpected failure of the process.

Vanderlaan et al. put together an account of how HCP risk is managed across the sector by drawing on the collective experience of manufacturers rather than the process of any single company. This provides a broadly representative view of the industry's approach, one that complements the international-consensus NGS literature seen in Section 4. Their work shows that even at low residual levels, these impurities can have an impact on product quality and patient safety, for instance by provoking an immunogenic response to the host proteins.

There is a structural contrast here with the detection of adventitious agents. NGS is an unbiased, sequence-based tool well suited to turn up the unexpected or unknown. Controlling for HCP and DNA impurities is done with a different logic altogether: targeted PCR- or threshold-based molecular methods and ELISA-based immunological techniques are used to precisely quantify a specific, anticipated category of impurity against set acceptance criteria. As for the mechanism of risk, Vanderlaan et al. (2018) note that residual HCPs present a hazard by virtue of their capacity to elicit an immune response in the patient, something distinct from the replication or infectivity threat of a virus. Since the immunogenic potential of an HCP is tied to its biochemical properties as much as its quantity, and can be an issue even in very small amounts, control programs need more than a simple aggregate immunoassay; they require a degree of analytical granularity to see which particular proteins are left over.

These findings from Vanderlaan et al. also have bearing on the design of the manufacturing process itself. The baseline burden of HCP and DNA is determined by the choice of host cell line and the downstream purification steps employed. Thus, molecular impurity testing serves as a feedback loop for iterative optimization during development, not merely as a final release safeguard. In practice, while the ELISA remains the standard for HCP quantification on account of its sensitivity and ease of use, it is limited by the antibodies it employs and does not guarantee complete coverage of all host cell proteins. The survey documents a growing appreciation among manufacturers for orthogonal, mass-spectrometry-based methods to pick up individual HCPs that an ELISA might miss, adding a layer of molecular verification.

Finally, the regulatory environment for host cell protein and DNA testing is far more settled than the still-standardizing NGS methodology of Section 4. It is governed by established frameworks such as the ICH Q6B specifications for biotechnological products. One should read Vanderlaan et al.'s survey as a record of mature, harmonized practice within those expectations, underscoring our observation that the two mechanisms in our integrated framework are at different stages of methodological and regulatory maturity.

## VI. INTEGRATED MOLECULAR BIOLOGY SAFETY FRAMEWORK

An integrated reading of the literature on NGS-based adventitious agent detection and host cell impurity control points to a clear conclusion: molecular biology's role in biopharmaceutical safety is best understood through an integrated framework with two structurally distinct, yet complementary, modes of operation. The synthesis put forward here identifies these as a two-mechanism framework. On one side there is broad, unbiased detection. As Cleveland et al. (2020) and Khan et al. (2020) have shown, next-generation sequencing can identify both novel and known viral contaminants without any prior assumption as to what may be present. The other mechanism is targeted, quantitative control. Here, immunological and molecular approaches are used to measure expected process impurities like host cell proteins and DNA against set safety parameters, in line with the work of Vanderlaan et al. (2018). It is not a matter of one replacing the other; the broad approach is not suited to the efficient quantification of knowns against exact thresholds, nor can a targeted method pick up unanticipated contaminants that fall outside its purview.

This provides manufacturers with coverage across the risk spectrum. NGS is the answer to the open-ended and unpredictable nature of adventitious contamination, whereas HCP/DNA testing deals with the bounded risks of the manufacturing process where the category of impurity is known but must be reliably measured from batch to batch. Figure 1 offers a visual

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representation of how these two mechanisms serve overall product safety, with Table 1 providing a summary of the framework.

For those looking to put this into practice, our analysis indicates that quality assurance should treat these as separate line items in their budgeting rather than lumping molecular safety testing together. A manufacturer will need to make distinct investments: in NGS and its standardization for the unknown-risk category, and in validated, process-specific immunoassays and molecular quantification for the known risks. There is also a cross-cutting technical challenge common to both. To be of any use in ensuring safety, each mechanism demands very high analytical sensitivity, given that residual HCP or DNA and adventitious viruses are found at minute concentrations relative to the therapeutic. While the logic differs between unbiased sequence detection and targeted ELISA quantification, improvements in the underlying technology – be it in bioinformatic analysis for NGS or antibody affinity for immunoassays – will serve both.

Finally, the PCV-1 case in Section 4 serves to refine the framework by adding a raw-material dimension. It makes plain that the risk of adventitious contamination is not limited to the production cell line; it can come from upstream reagents like the porcine trypsin involved in the PCV-1 incident. A thorough safety strategy would therefore extend NGS screening to critical animal or biological raw materials in the supply chain, not just the in-process samples and cell substrate.

Table 1. Two-Mechanism Framework for Molecular Biology Applications in Biopharmaceutical Safety

| Mechanism | Technology/Method | Target Risk | Source |
|---|---|---|---|
| Broad, unbiased detection | Next-generation sequencing (NGS) | Unknown/novel adventitious viruses | Cleveland et al. (2020); Khan et al. (2020) |
| Targeted quantitative control | ELISA, PCR-/threshold-based DNA assays | Known host cell protein (HCP) and DNA impurities | Vanderlaan et al. (2018) |

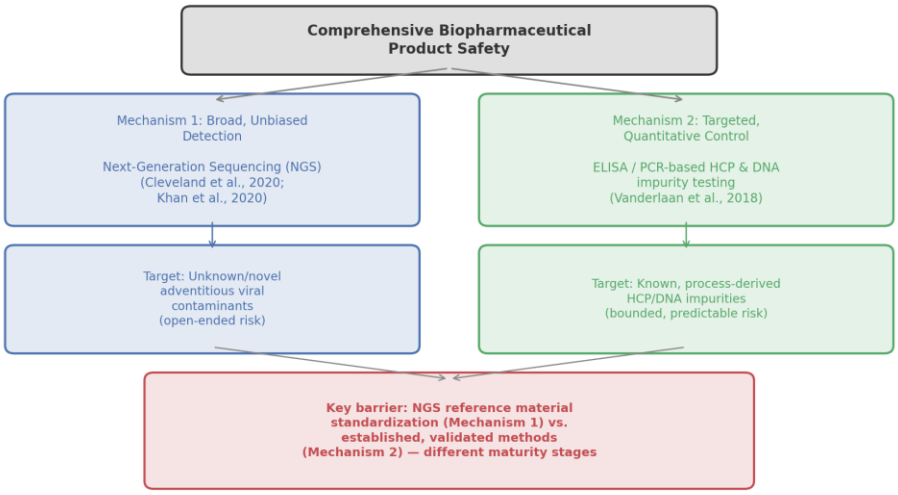


Figure 1. Two-Mechanism Framework for Molecular Biology Applications in Biopharmaceutical Safety. Source: synthesized from Cleveland et al. (2020); Khan et al. (2020); Vanderlaan et al. (2018).

## VII. DISCUSSION

What the integrated two-mechanism framework set out in Section 6 means for biopharmaceutical manufacturers and regulators is that their approach to molecular biology-based safety testing should be

rethought. There are a number of implications to consider. For one, there is the matter of resource allocation. NGS and HCP/DNA testing are not interchangeable because they deal with structurally different risks, namely known as opposed to unknown contamination. A sound safety program calls for deliberate investment in both targeted impurity quantification and broad-spectrum molecular detection; one should not be put ahead of the other.

Then there is the standardization gap for NGS that Cleveland et al. (2020) put forward. With reference material standardization still an unmet need for everything from method validation to the use of natural or synthetic materials, it would be premature to rely on the technology's detection capability alone for widespread implementation. Both parties should see continued work in this area as a prerequisite for full regulatory adoption. The framework also highlights the disparity in maturity between the two mechanisms. HCP and DNA testing is an established practice across the industry with clear acceptance criteria, as Vanderlaan et al. (2018) have shown. NGS-based adventitious agent detection, by contrast, is still being standardized (Cleveland et al., 2020; Khan et al., 2020). Regulatory and quality assurance strategies ought to be calibrated accordingly: treat HCP/DNA testing as a given and NGS as an evolving technology where consensus is still being built, without assuming the two hold the same degree of regulatory certainty at present.

Section 5 makes plain the role of HCP and DNA testing in providing process-design feedback. Since upstream decisions on host cell lines and purification dictate the impurity burden, manufacturers should be using early-stage profiling to guide those very choices. The data should feed into iterative development, not just serve as a final release-testing formality once a process is set in stone and hard to change. On the subject of bioinformatics, there is a fifth point to make. It is an often underappreciated part of any NGS safety program. As Cleveland et al. (2020) note, the standardization requirement is not limited to wet-laboratory reference materials. Interpreting the sequence data to spot viral sequences demands validated bioinformatic pipelines and reference databases. A technically proficient run can still lead to an unreliable conclusion if the downstream analysis has not been put through the same standardization rigour.

We should also look at the potential for cross-industry knowledge transfer. The consensus-building around vaccine and non-vaccine manufacturing, including in the veterinary space, as documented by Khan et al. (2020) and Cleveland et al. (2020), offers a useful template. An organization does not need to devise an entirely bespoke approach for its product, be it a monoclonal antibody or a vaccine, when the principles of NGS apply broadly enough.

Finally, the PCV-1 case raises a seventh implication regarding the difference between detection and risk characterization. Victoria et al. (2010) showed that the presence of PCV-1 was not a clinical or immunogenic risk. It follows that a positive NGS signal is not in itself grounds for a recall. Manufacturers and regulators need a defined pathway to interpret such findings. This has a corollary for public communication as well: the heightened sensitivity of NGS will inevitably produce more findings that must be assessed on a case-by-case basis. A strategy should be in place to make clear the distinction between finding a contaminant's genetic material and an actual safety risk, rather than leaving such matters to be an afterthought to the detection work.

There are some limitations to acknowledge in this paper. The synthesis here is based on expert consensus and survey documentation, not primary experimental data, so one cannot directly compare quantitative metrics like NGS sensitivity thresholds or HCP clearance rates within a single dataset. Moreover, the sources we have examined are largely drawn from the well-resourced environments of major biopharmaceutical companies and jurisdictions; whether this framework holds for smaller developers in emerging markets is not something we have established. Nor has the two-mechanism framework been put to the test as an integrated whole in a study of a single quality system. Such an empirical examination of the complementary coverage logic proposed here would be more rigorous than what can be gleaned from separately conducted surveys and consensus documents.

## VIII. CONCLUSION

By way of two complementary mechanisms, this paper puts forward an integrated framework to define the role of molecular biology in biopharmaceutical product safety. One is the broad, unbiased detection of unknown adventitious viral contaminants by next-generation sequencing (NGS), a practice for which international expert consensus has been recorded (Cleveland et al., 2020; Khan et al., 2020). The other is the targeted, quantitative control of host cell protein and DNA impurities through molecular and immunological means, as an industry-wide survey has shown (Vanderlaan et al., 2018).

Our analysis indicates that one cannot be substituted for the other if comprehensive safety assurance is to be achieved; they must be deployed together. NGS is required to handle the open-ended risk of unpredictable contamination, whereas HCP and DNA testing is what covers the more bounded and predictable impurity risk of the manufacturing process itself. It is the central aim of this work to show that these two applications of molecular biology, while largely documented in parallel literatures, are not competing or alternative approaches to quality assurance but rather joint necessities of a single, all-encompassing safety strategy.

There is also a historical lesson to be drawn from this framework for the future adoption of safety technology. The PCV-1 case put on record by Victoria et al. (2010) is illustrative of a pattern in biopharmaceutical safety: a new, more sensitive method of molecular detection is first used outside the purview of routine regulatory testing and uncovers something of safety concern that was previously missed. This in turn spurs the kind of formal consensus-building between industry and regulators needed to make it standard practice. A decade on, Cleveland et al. (2020) and Khan et al. (2020) have described precisely that trajectory for NGS. In short, today's unstandardized, cutting-edge technology can be expected to become tomorrow's regulatory requirement through such an iterative process.

We present this framework as an evidence-based synthesis and not as an exhaustive catalogue of every relevant application of molecular biology. We would suggest that manufacturers and regulators view the broad NGS-based detection and the targeted HCP/DNA quantification we have examined in the context of their own quality systems, with due regard for the distinct maturity and risk-coverage of each as borne out by the sources we have reviewed. Future work that empirically tests our two-mechanism model against actual safety outcomes in a wider array of manufacturing settings, or that brings NGS standardization and impurity control together under one roof, would only serve to put further evidence behind the conclusions reached here.

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