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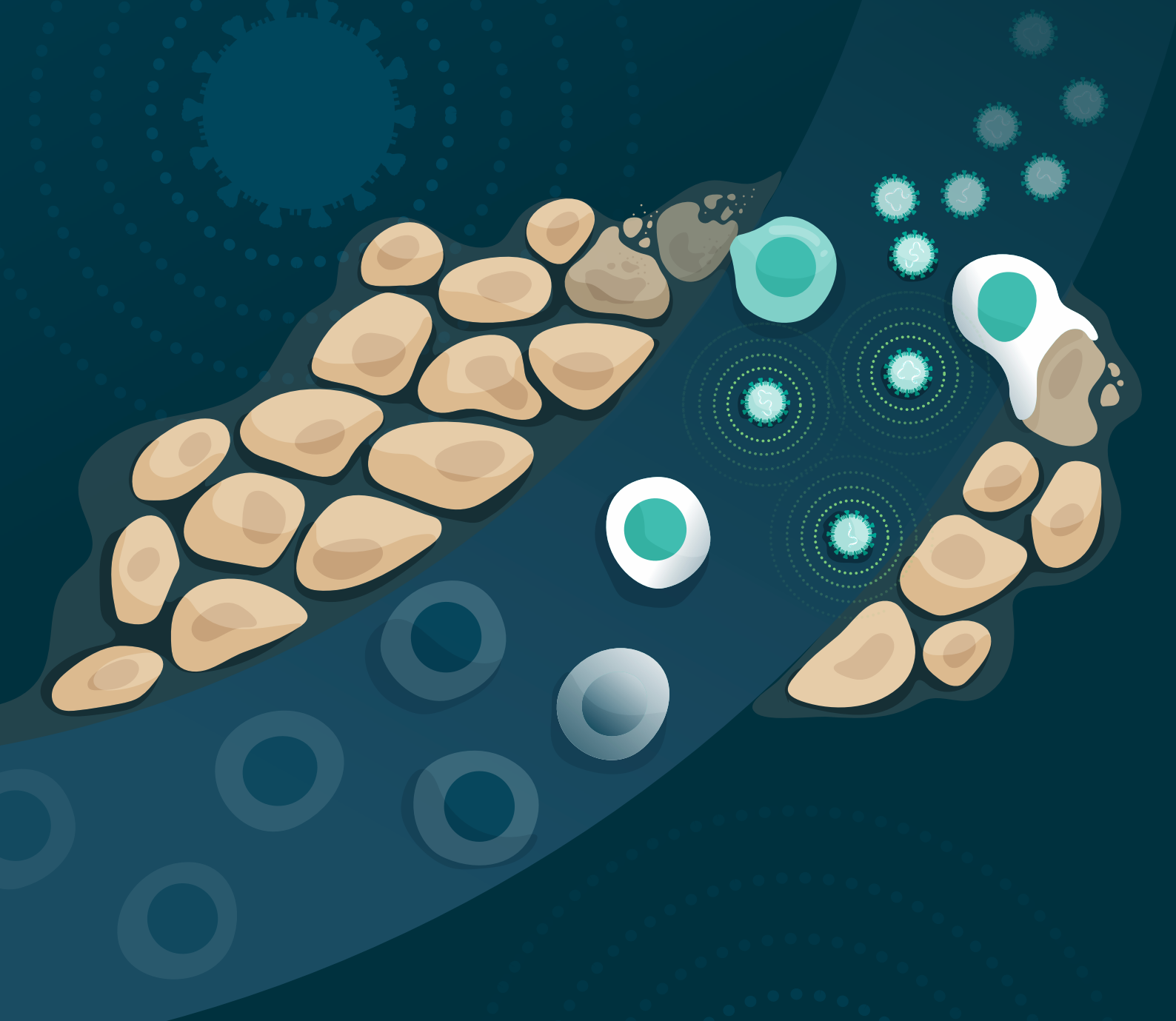

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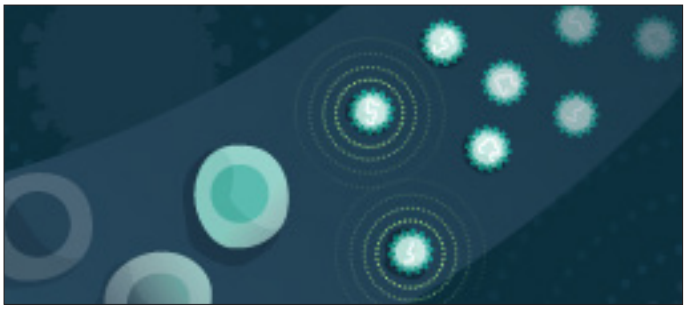

VACCINE INSIGHTS

REVIEW

TOPIC FOCUS • QC, QA & CMC
Manufacturing Regulatory

Host cell protein risks, detection, and control in vaccine manufacturing

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Host cell proteins (HCPs) are critical process-related impurities in vaccine manufacturing that can affect product safety, efficacy, and regulatory compliance. Challenges persist in detecting and controlling HCPs due to their diverse properties and interactions with vaccine components across viral vector, recombinant protein, and nucleotide vaccine platforms. This review provides a comprehensive analysis of HCP risks, detection technologies, including ELISA and advanced mass spectrometry, and control strategies encompassing upstream cell line engineering and downstream purification processes. Regulatory expectations from global authorities are discussed, emphasizing risk-based, case-by-case specification setting rather than universal numeric limits. The review further explores the operational and analytical challenges faced by manufacturers, particularly in low- and middle-income countries, and advocates for harmonized guidelines and decentralized analytical capacity to support rapid, equitable vaccine deployment. By integrating risk-informed control strategies, advanced analytics, and lifecycle monitoring, the field can ensure robust vaccine production and global access, supporting both pandemic preparedness and long-term public health.

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INTRODUCTION TO HOST CELL PROTEINS

Ensuring consistent control of process-related impurities is a foundational expectation in the development and manufacture of biological vaccines. Across all modalities, regulators require manufacturers to demonstrate that their processes remain under control, that critical quality attributes are maintained, and that batch-to-batch variability is minimized through well-designed control strategies. Impurity monitoring is central to this assurance: it provides

evidence of process robustness, supports comparability during scale-up and technology transfer, and underpins the safety and quality review of every lot released to the market. Within this broader framework, host cell proteins (HCPs) represent a key class of process-related impurities whose identity, abundance, and persistence can vary widely by modality, platform, manufacturing design, and biological context.

Against this backdrop, HCPs deserve focused consideration because of their potential, but highly product-specific, impact on vaccine quality, biological activity, and patient safety. During upstream expression, host cells generate thousands of endogenous proteins alongside the intended product. While downstream purification removes most of these proteins, a small subset may remain, interact with the product, or exhibit biological activity. The consequences of these vaccine impurities differ substantially from those relevant to therapeutic biologics, due to the typically low dose, limited dosing frequency, and inherently immunogenic nature of vaccines. Nevertheless, residual HCPs are an important marker of process control, and advancements in analytical technologies are enhancing our understanding of impurity identity and behavior. As a result, regulatory expectations regarding the characterization of these impurities are projected to become more stringent.

The objective of this review is therefore to synthesize the scientific, technological, and regulatory considerations associated with HCP risks, analytics, and control across vaccine modalities, without advocating for universal numeric limits that lack scientific or regulatory basis. This review was conducted using a narrative synthesis of peer-reviewed literature, regulatory guidelines, and publicly available vaccine and biopharmaceutical data, drawing on sources identified through PubMed, EMBASE, Google Scholar, and regulatory agency websites, with additional relevant material identified through reference screening. No confidential or proprietary information was used. Instead, the analysis focuses on how risk-based, product-specific strategies aligned with ICH principles can support consistent decision-making and global convergence, while recognizing that acceptance criteria for HCPs must be justified using process capability, clinical context, and orthogonal analytical evidence rather than prescriptive thresholds.

Understanding the types of HCPs most likely to persist or pose product quality risk provides a rationale for engineering more efficient vaccine manufacturing platforms. Identifying high-risk HCPs enables the strategic use of genome editing (e.g., CRISPR/Cas9) to selectively knock out problematic genes, thereby reducing the burden on downstream purification. This is particularly relevant for next-generation vaccine platforms that rely on complex biological constructs, such as enveloped viral vectors and glycosylated protein antigens, where product-interacting HCPs can be difficult to remove. Engineering host cells to modulate secretion pathways, lower stress responses, or reduce cell lysis can also shift the HCP profile toward lower-risk species. Moreover, improved knowledge of HCP categories informs platform-specific purification development, such as deploying affinity capture steps that avoid co-elution with chaperones, optimizing nuclease digestion for RNA platforms, or using membrane chromatography to remove persistent membrane-associated proteins from bacterial systems. By aligning cell-line engineering and purification strategies with an understanding of HCP types, vaccine manufacturers may be able to improve product quality, reduce variability, support regulatory review, and improve the sustainability of vaccine manufacturing platforms. In this context, 'sustainability' refers to the robustness, efficiency, economic viability, and resilience of vaccine manufacturing platforms across products, production sites, and scales. Through

increased knowledge, manufacturers can establish processes and control strategies that are easier to operate, more resilient to drift, and suitable for global deployment. Table 1 shows how key HCP types in vaccine production can be categorized by function [1,2,3,4], cellular localization [5,6,7], purification behavior [8,9,10], or risk level [11,12,13], and the challenges they pose to manufacturing and/or vaccine product. The risk level categories in this table are intended as conceptual groupings based on scientific literature and do not represent regulatory or pharmacopeial classifications. The actual risk associated with any HCP is highly product-specific and influenced by factors such as residual concentration, dose and dosing frequency, product modality, and supporting stability, efficacy, and safety data.

Despite advancements in purification processes, complete removal of HCPs remains a challenge due to their diverse physicochemical properties and their tendency to co-purify with the drug substance [14,15]. Interestingly, in the context of vaccines, certain residual HCPs may even provide benefits by enhancing the immune response and acting as adjuvants, potentially reducing the need for additional adjuvant supplementation [12,16]. To monitor and control HCPs, traditional enzyme-linked immunosorbent assays (ELISA) are widely used for quantification, but mass spectrometry (LC-MS/MS) and other orthogonal analytical methods are increasingly being adopted, as they provide a more comprehensive profile of HCPs [17]. As the biopharmaceutical industry evolves, companies should adopt advanced analytical tools and robust process development strategies to meet regulatory expectations and ensure product safety.

Regulatory agencies such as the US FDA, EMA, and ICH mandate stringent requirements for detecting, quantifying, and reducing HCPs to ensure biopharmaceutical quality and patient safety. However, there are currently no specific guidelines for acceptable residual HCP levels in vaccines [18]. Consequently, accurate HCP characterization is essential for risk assessment, process validation, and ensuring batch-to-batch consistency in vaccine manufacturing. This regulatory ambiguity often leaves vaccine developers uncertain about which HCPs and limits to target during downstream process development and in product specifications. Clearer regulatory guidance would help address this gap, supporting more consistent and safer vaccine production. The greatest impact of this ambiguity occurs during development, when manufacturers design purification processes and establish HCP control strategies without explicit regulatory target values. This complicates risk assessments, assay selection, and specification setting. Furthermore, global uncertainty around acceptable HCP levels can slow technology transfer, cause regulatory delays and limit regional manufacturers who may lack access to complex analytics – barriers that undermine CEPI's mission to deliver fast, equitable, 100-days ready vaccine manufacturing worldwide [19].

REGULATORY EXPECTATIONS & GUIDELINES

Regulatory authorities worldwide (e.g. US FDA, EMA, WHO) recognize the critical need to control host cell protein impurities in vaccines and other biologics.

In line with the ICH Q6B guideline [20] it is crucial to assess the purity of both the drug substance and drug product. Additionally, manufacturers must evaluate any impurities present and characterize them to the extent possible. ICH Q6B guideline stipulates that manufacturers use sensitive assays (typically immunoassays) and, when appropriate, leverage new analytical technologies capable of detecting a wide range of protein impurities to monitor residual HCPs. It further indicates that process-specific clearance studies, e.g.

TABLE 1

Host cell protein categories relevant to vaccine manufacturing.

Category	Sub-type	Description	Relevance/risk in vaccine manufacturing
By biological function	Proteases	Enzymes that degrade proteins	Can cleave vaccine antigens, viral capsid proteins, or critical excipients; risk of potency loss
	Glycosidases and hydrolases	Enzymes that modify or degrade glycans and biomolecules	May alter glycosylated antigens or impact viral vector glycan structures
	Nucleases (DNase, RNase)	Enzymes that degrade nucleic acids	Can destabilize DNA and RNA vaccine products; may interfere with plasmid DNA purity
	Chaperones/heat shock proteins	Protein folding assistants; often abundant	'Sticky' proteins that co-purify with viral vectors or antigens; commonly persistent HCPs
	Metabolic enzymes	Glycolytic, TCA cycle, and other essential enzymes	Highly abundant and frequently detected; generally low risk unless enriched in the final product
	Innate immune modulators	Cytokine-like or immunostimulatory proteins	Could induce unintended immune responses; high risk if present in final product
By cellular localization	Secreted HCPs	Naturally released to medium	Major impurity pool for mammalian cell-produced viral vectors and subunit antigens
	Cytosolic HCPs	Released upon lysis or shear	Common in processes requiring cell disruption
	Membrane/periplasmic proteins	Outer membrane components, periplasmic enzymes	Relevant for bacterial antigen expression; can be immunogenic
	Nuclear proteins	Histones, transcription factors, etc.	Tend to bind nucleic acids or viral vectors; difficult to clear in viral vaccines
By behavior in purification	Easily removed HCPs	Cleared in standard chromatography	Low concern; represents predictable background impurities
	Persistent ('problematic') HCPs	Co-elute due to charge, hydrophobicity, or folding	Often enriched in the final product; requires targeted mitigation
	Product-interacting HCPs	Physically bind antigen, capsids, or lipid nanoparticles	High-risk category; difficult to remove and requires targeted mitigation
By risk level	High risk	Proteases, immunogenic proteins, viral mimicry proteins	Potential safety and potency impact; must be monitored closely
	Medium risk	Proteins that affect analytics or stability	May interfere with potency or identity assays
	Low risk	Inert or low-abundance proteins	Minimal biological impact; controlled mainly to meet regulatory expectations

HCP: Host cell protein.

spiking experiments at lab scale, can be used to demonstrate removal of host cell proteins, potentially averting the need for strict acceptance criteria in certain cases.

According to US CFR 610.13 Purity, "Products shall be free of extraneous material except that which is unavoidable in the manufacturing process" [21]. In practice, this requires vaccine developers to aim for HCP impurity levels that are as low as reasonably achievable, alongside controlling other impurities. While the FDA does not prescribe a numeric HCP limit applicable to all products, regulators will scrutinize the

justification of any residual HCP levels in the product and the sensitivity of the assay used. During product review, manufacturers must demonstrate that HCP levels are consistently low and pose no safety risk and effect on product quality, supported by process validation data and batch analysis.

The new US Pharmacopeia chapter USP <1132.1> (2025) [22] formalizes the expectation that manufacturers use mass spectrometry not just for total HCP quantification, but also for identifying and monitoring specific HCPs that may impact product quality or safety. This reflects a regulatory transition from solely measuring total HCPs to comprehensive impurity profiling.

The European Medicines Agency (EMA) explicitly requires manufacturers to show, through routine testing and process validation, that HCPs are consistently reduced to levels justified by process capability and risk assessment [23]. In key EMA guidance [24,25], EMA states that for any recombinant product "residual HCP have to be tested for on a routine basis ... (HCP) should be routinely monitored at the purified bulk level, using suitable analytical assays" with results meeting preset specifications. Hence, batch-to-batch consistency is essential to demonstrate process control and to ensure HCP levels remain within acceptable specifications. The guidance also emphasizes that no single uniform HCP limit can be applied across all products, as the composition and quantity of HCPs vary by expression system, process design, and product type. Analytical methods are likewise not easily standardized, since the performance of HCP assays depends heavily on process-specific reagents and antibodies. It is therefore expected that acceptable HCP levels must be determined on a case-by-case basis, considering factors like the product type, dosage, route of administration, and the capability of the purification process.

The World Health Organization's guidance on recombinant biological products [26] similarly emphasizes the use of sensitive detection methods and removal of host cell-derived impurities. Following ICH Q6B, WHO recommends using broad-spectrum immunoassays for HCP detection and performing clearance validation studies during process development, generating data on the identity and risk of individual HCPs, especially those with known or suspected biological activity or immunogenicity. WHO guidance does not mandate a fixed numerical limit for HCPs in vaccines. Instead, it calls for HCP levels to be "as low as possible" in the final product, consistent with the principle of reducing impurities to ensure safety. In practice, many national regulatory authorities adopting WHO guidelines will require that manufacturers characterize residual HCP content and demonstrate that it has been reduced to a level unlikely to cause harm. This often involves setting an internal specification (acceptance criterion) for HCP content per dose of vaccine, justified by process capability and toxicological risk assessment.

Taken together, these guidelines indicate that global harmonization should focus on shared, risk-based expectations rather than a single numeric HCP limit. In practice, regulators expect manufacturers to:

- Justify acceptance criteria case-by-case using product/process understanding, dose and clinical context, and demonstrate process capability;
- Disclose method capability and, where appropriate, use orthogonal approaches to support the breadth and relevance of routine immunoassays; and
- Integrate lifecycle trending to maintain control.

This approach reflects the explicit absence of universal HCP limits in current frameworks and places emphasis on science and risk-based justification that scales across platforms and geographies [27,22].

In the absence of a single global limit, industry agreement has been to target very low HCP concentrations. A commonly cited empirical threshold is ≤100 ng HCP per mg of product (i.e. ≤100 ppm) as an acceptable upper limit for many biologics, based on the WHO guideline for monoclonal antibodies (mAbs) [28]. Eventually, regulatory authorities expect manufacturers to use the lowest level of HCP that is achievable by a well-optimized, validated purification process, and to provide justification (including any relevant toxicological data) that the residual HCPs present negligible safety and quality risks.

Assessing HCP levels across the three main modalities described here offers insights on how regulatory understanding may evolve in future. For viral vector vaccines, licensed products have been permitted to contain HCP limits in the low hundreds of nanograms per dose. As an example, the chimpanzee adenovirus-vectored COVID-19 vaccine ChAdOx1 nCoV-19 had a regulatory specification of ≤400 ng HCP per dose (5×10^{10} viral particles), providing a benchmark for this type of vaccine [29]. As manufacturing processes mature and process understanding increases, stricter limits are expected.

In contrast, recombinant protein and subunit vaccines typically require more stringent HCP control due to direct antigen exposure and frequent use of immune-potentiating adjuvants. Industry practice and published assay capabilities indicate that HCP levels are often controlled to tens of nanograms per dose or lower, with sensitive, process-specific immunoassays enabling detection at low-ng levels [30]. Until 2005, most FDA-reviewed biologics had HCP levels of 1–100 ppm measured by ELISA [31]. However, a suitable level for vaccines remains open for discussion due to differences in dosage, frequency, and administration compared to mAb treatments.

For RNA vaccines, HCPs are not considered a dominant impurity class. Residual proteins are primarily limited to trace amounts of *in vitro* transcription enzymes, and regulators expect these to be negligible or below the limit of quantification in the final formulated product, with justification based on process clearance and analytical sensitivity rather than fixed numeric limits. The EMA draft guideline on mRNA vaccines states that process-related impurities should be characterized with sufficiently sensitive methods and risk assessed [32]. For instance, the EMA assessment report on Kosative accepts a risk assessment of residual impurities in linearized pDNA, regarded as starting material, and does not require residual HCP as a release criterion for the vaccine [33].

Overall, while benchmarks exist, regulatory emphasis across vaccine modalities remains on demonstrated clearance, batch-to-batch consistency, and the absence of clinically meaningful safety or immunogenicity risk, rather than adherence to a single prescriptive HCP threshold.

IMPLICATIONS FOR SAFETY

The issues of safety for 'high-risk' HCPs arise from their ability to:

- Possess biological activity capable of altering the product or interacting with host tissues;
- Exhibit high intrinsic immunogenicity or allergenic potential;
- Stimulate innate immune pathways, or
- Form aggregates that enhance immunogenicity.

The clinical implication of these capabilities is the potential to trigger unintended and harmful immune responses [34].

Possible immune reactions to HCPs typically fall within the spectrum of immediate or delayed hypersensitivity

responses. Immediate hypersensitivity reactions (Type I), which are mediated by antigen-specific IgE bound to mast cells and basophils, typically manifest within minutes to hours after vaccination as urticaria, angioedema, bronchospasm, or anaphylaxis. These events generally require pre-existing sensitization, which is rare given the extremely low quantities of HCPs present in licensed vaccines. Delayed hypersensitivity reactions (Type IV), which are driven by antigen-specific T cells, typically present days after exposure as maculopapular rash, injection-site inflammation, or, less commonly, systemic immune-mediated symptoms. In addition to classical hypersensitivity, other immune-mediated mechanisms may be theoretically relevant, including immune complex-mediated reactions (Type III), non-IgE-mediated mast cell activation, or innate immune activation leading to exaggerated local or systemic inflammatory responses. Finally, residual host proteins might act as triggers or modulators of immune dysregulation and could contribute to aberrant immune responses in genetically susceptible individuals. While direct clinical evidence linking such mechanisms to HCPs in licensed vaccines is limited, their consideration supports a comprehensive framework for safety monitoring and signal interpretation [35].

Robust HCP monitoring may support post-marketing safety surveillance of certain types of adverse events following immunization. The heterogeneity of HCP mixtures can be challenging for safety assessment, as even at very low concentrations of these residual proteins can complicate the interpretation of adverse events, particularly when batch-to-batch variability is present. When rare immune-mediated or autoimmune-type events are reported, manufacturers and regulators can use detailed analytical data, including HCP profiles, to investigate potential quality-related causes. Even if no link is found, the availability and review of these data help strengthen public confidence by demonstrating that manufacturing-related variables have been thoroughly evaluated.

An example of a comprehensive review is the investigation by the Australian Therapeutic Goods Administration (TGA) of an increased incidence of fever and febrile convulsions in young children following seasonal trivalent influenza vaccination associated with CSL's FLUVAX products. After clinical review of post-marketing individual case reports, performance of epidemiological studies and additional reviews of clinical trial data, inspectors from the TGA audited CSL's manufacturing facility, with particular attention to three process changes introduced to increase viral yield during pandemic vaccine production, assessing change control, quality systems, and batch consistency. Extensive laboratory testing of retained and distributed vaccine batches found no evidence of contamination or manufacturing defects, nor could any single process failure be causally linked to the observed reactions. Although the precise biological mechanism remained unresolved, the findings led to regulatory action, strengthened post-market surveillance expectations, and reinforced the importance of rigorous oversight of manufacturing changes for seasonal influenza vaccines [36].

Although most HCPs are present at very low concentrations and are unlikely to be clinically relevant, rigorous characterization and control remain important to minimize aberrant immune responses and ensure consistent vaccine safety. Implementing a "platform approach", where standardized manufacturing processes are used across multiple vaccine candidates, as envisioned in CEPI's 100 Days Mission will require investment in manufacturing infrastructures that can deliver consistent product quality and, consequently, vaccines with predictable and reliable safety profiles.

HCP DETECTION & QUANTIFICATION METHODS

Stringent monitoring of HCPs is essential throughout the production process. Consequently, scientific documentation detailing methodologies and advancements in HCP analysis has become increasingly important. The field is continuously evolving, with ongoing development of novel and enhanced analytical methods [37]. Regulatory agencies are placing greater emphasis on the use of orthogonal and sensitive methods to support a broader understanding of HCP impurities, and in some cases have encouraged more extensive characterization during review. While such expectations are not formalized as universal requirements, they reflect an evolving regulatory interest in ensuring that manufacturers can justify HCP control strategies with appropriate analytical approach [38].

The following section provides a comprehensive review of analytical methods for HCPs, including a detailed analysis of the skill level and cost associated with each method, with particular attention to decentralized product release at manufacturing sites in low- and middle-income countries (LMICs), as summarized in Table 2. Regional testing capacity is essential for vaccine health sovereignty. Many LMIC manufacturers lack highcomplexity analytical infrastructure and rely on external laboratories, which prolongs timelines and reinforces dependency, limiting their ability to scale and respond quickly during emergencies [39]. In outbreak situations, centralized testing models may create bottlenecks as shipping samples across borders introduces logistical delays and exposes countries to customs or biosafety restrictions, thereby slowing batch release when speed is most critical.

The Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used immunoassay for detecting and quantifying proteins, including HCPs, based on specific antigen-antibody interactions. The assay typically involves immobilizing capture antibodies on a solid surface, followed by sample addition. If HCPs are present, they bind to these antibodies. A detection antibody, often conjugated to an enzyme, then binds to the captured HCPs. The addition of an enzyme substrate produces a measurable signal, such as a color change, proportional to the HCP amount. Variations such as sandwich, competitive, and indirect ELISAs cater to different analytical needs.

ELISA is a workhorse in HCP analysis across biopharmaceutical and vaccine development, from process monitoring to quality control and release testing. Its high sensitivity and throughput make it ideal for routine analysis. However, its effectiveness hinges on the quality and coverage of the anti-HCP antibodies used. Generic ELISAs might miss process-specific HCPs, and the method primarily provides a total HCP measurement without identifying individual proteins [37]. Antibody coverage analysis is therefore critical. Commercial ELISA kits are readily available for various expression systems, with costs per kit ranging from a few hundred to over a thousand US dollars. Custom assay development can be more costly and time-consuming. ELISA is generally easy to perform, requiring technician-level skills and standard laboratory equipment like a plate reader and curve-fitting software. However, due to its use in the diagnostic sector, there are also lab-on-a-chip options available that may be repurposed for HCP monitoring, automating the sample-to-result process for non-skilled personnel [38].

Western blotting detects specific proteins within a mixture separated by gel electrophoresis. Separated proteins are transferred to a membrane and probed with specific antibodies, followed by a secondary antibody linked to a reporter enzyme for visualization. 2D Western blot offers higher resolution by separating proteins

based on isoelectric point and molecular weight. Capillary western blot provides an automated, quantitative approach.

Western blot is applied in HCP analysis for antibody coverage assessment and detection of specific HCPs. However, it has lower sensitivity and throughput than ELISA and LC-MS/MS, and can be time-consuming, limiting the number of samples analyzed [37]. 2D Western blot improves resolution but may still lack sensitivity for low-abundance HCPs in purified samples.

Western blot is generally a low-cost technique, especially used in academia. Commercial kits are available, and services for 2D Western blot analysis are offered. It requires more technical expertise than ELISA, particularly 2D Western blot, which may necessitate experienced analysts.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combines liquid chromatography separation with tandem mass spectrometry detection for detailed HCP analysis. Samples are digested into peptides, separated by LC, and then analyzed by MS/MS to identify and quantify individual HCPs. It serves as a powerful orthogonal method to ELISA.

LC-MS/MS enables comprehensive HCP profiling and the identification of low-abundance proteins. A challenge is the high dynamic range between the drug substance and HCPs, which can be mitigated by techniques like antibody affinity extraction (AAE) and molecular weight cutoff enrichment. Data-independent acquisition (DIA) enhances HCP detection [38].

Implementing LC-MS/MS involves high costs for instrumentation, specialized consumables, and expert personnel. Analytical costs are often per injection, and sample preparation adds to the expense. Services are available for HCP analysis by LC-MS/MS. This technique requires highly skilled analysts for method development, operation, and complex data interpretation, with expertise in proteomics, chromatography, and mass spectrometry [37].

AAE is an immunoaffinity chromatography method used to assess antibody coverage and enrich HCPs. Anti-HCP antibodies are immobilized on a column, and HCP samples are passed over it to bind. Bound HCPs are then eluted and analyzed.

AAE enhances the detection of low-abundance HCPs for MS analysis and provides a more representative assessment of ELISA antibody coverage compared to Western blot [38]. The cost of AAE services is not explicitly stated but is likely higher than standard methods due to their specialized nature. Implementing and interpreting AAE data requires expertise in immunoaffinity chromatography and downstream analysis techniques like MS or 2D-PAGE.

Capillary electrophoresis (CE) separates molecules based on their charge and size in a capillary under an electric field. Techniques include capillary zone electrophoresis, capillary gel electrophoresis, and capillary isoelectric focusing. CE can analyze product concentration, quality attributes, and impurities in biopharmaceutical intermediates. CE-MS/MS has been used for trace amount HCP detection.

CE offers rapid analysis, low sample volume requirements, and high resolution [38]. It is relatively fast and has low carry-over compared to LC. Instrumentation is less expensive than LC-MS/MS. However, skilled personnel are needed for method development and operation, especially with CE-MS/MS.

The regulations emphasize the need for sensitive assays that can detect a wide range of protein impurities. Employing sensitive and validated analytical methods is crucial for meeting these regulatory requirements and ensuring vaccine safety [37]. Comprehensive HCP analytics are essential for supporting the efficacy and safety of vaccines. Regulatory agencies often request HCP antibody coverage data to support the use of HCP ELISAs. Integrating data from orthogonal methods with ELISA results provides a more complete analysis of HCP

TABLE 2

Comparison of host cell protein analysis methods.

Method	Principle of operation	Key applications in HCP analysis	Sensitivity (relative)	Specificity (relative)	Cost (relative)	Skill level required	Main advantages	Main limitations
ELISA	Antigen-antibody interaction with enzymatic signal amplification	Routine screening, process monitoring, quality control, release testing, total HCP measurement	High	Broad	Low	Technician	High throughput, relatively low cost, easy to perform	Measures total HCP, antibody dependent, may miss some HCPs
Western blot	Protein separation by size, antibody-based detection	Antibody coverage assessment, detection of specific HCPs	Low	Target-specific	Low	Experienced analyst	Visualizes specific proteins, useful for antibody validation	Lower sensitivity and throughput than ELISA and LC-MS/MS
LC-MS/MS	Peptide separation and mass spectrometric identification	Comprehensive HCP profiling, identification and quantification of individual HCPs, orthogonal method	High	High	High	Expert	High sensitivity and specificity, identifies individual HCPs	High cost, requires specialized expertise
AAE	Immunoaffinity chromatography enrichment	Enhances detection of low-abundance HCPs for MS, assesses antibody coverage	High	High	Medium-high	Expert	Enriches low-abundance HCPs, superior antibody coverage assessment to Western blot	Specialized service, likely higher cost
CE	Separation based on charge and size in an electric field	Analysis of product concentration and purity, potential for HCP detection (especially with MS coupling), charge-based separation	Moderate	Moderate	Low-medium	Experienced analyst	High separation efficiency, low sample volume, rapid analysis	May require optimization for specific HCPs, sensitivity may not be as high as ELISA or LC-MS/MS for all HCPs

HCP: Host cell protein.

content, which is generally expected by regulatory authorities [38].

In summary, HCP analysis is a critical aspect of biopharmaceutical development and manufacturing, particularly for vaccines, due to the potential risks these impurities pose to patient safety and product efficacy. Various analytical methods are available, each with distinct strengths, limitations, cost implications, and required skill levels. ELISA remains widely used for routine screening and process monitoring due to

its high throughput and relative ease of use [37]. However, its dependence on antibody reactivity and inability to identify individual HCPs highlights the need for orthogonal methods to achieve a more comprehensive understanding of HCP impurities [38].

Western blot is valuable for assessing antibody coverage and detecting specific HCPs but suffers from lower sensitivity and throughput compared to ELISA and LC-MS/MS. LC-MS/MS offers the highest sensitivity and specificity for identifying and quantifying individual HCPs, making it indispensable for detailed characterization and as an orthogonal method for regulatory compliance, albeit at a high cost and requiring advanced expertise. AAE enhances the detection of low-abundance HCPs and provides a superior assessment of antibody coverage, but it is typically a specialized service. CE offers rapid, low-sample-volume analysis with charge-based separation capabilities and relatively lower instrumentation costs, making it a useful complementary technique for specific applications.

The selection of appropriate HCP analysis methods depends on the specific objectives, such as routine quality control, in-depth characterization, or meeting regulatory requirements. Often, a combination of methods is necessary to provide a comprehensive understanding of the HCP impurity profile. The regulatory landscape emphasizes the importance of minimizing and controlling HCP levels in vaccines, requiring the use of sensitive and validated analytical methods [38]. For vaccine production in pandemic response, even with some uncertainty regarding the final product, the platform process and the HCP profile should be well characterized using a combination of the methods discussed. However, for product release, ELISA currently remains the most viable choice for global technology transfer and consistency, due to its favorable balance of cost, ease of use, and reduced risk of human error compared to more complex methods.

As with all analytical methods, it is recommended that HCP analytical methods are developed and selected based on understanding of the product, process, and impurity profile using ICH Q14 as guidance to ensure phase appropriateness during the product development stages. Consistent with ICH principles, selection of HCP methods is science and risk-based, grounded in product and process understanding and the analytical target profile, rather than prescriptive or modality-based decision trees. ELISA remains the routine and release workhorse due to sensitivity/robustness and suitability for decentralized use, while orthogonal tools (particularly LC-MS/MS) are employed when deeper resolution is required (e.g., identification of persistent or productinteracting species, or to confirm ELISA antibody coverage). Advanced or information-rich tools (e.g., spectroscopic PAT, DIA-based MS) are integrated where enhanced monitoring or rapid in-process decisionmaking is needed. This approach prioritizes capability, fitnessforpurpose, and phase-appropriate control over any universal threshold. As per guidance, it is recommended to establish and maintain an Analytical Target Profile (ATP) for each method throughout the development of a product until such a point that assay/method validation is required, following ICH Q2(R2).

If the validated HCP method supports a platform manufacturing process, that will be used to manufacture multiple products (of the same modality), then the same method can be used with minimal qualification of its performance for each product, rather than a full revalidation study. A platform approach to HCP analytics could therefore improve consistency, speed, and comparability across vaccine programs.

HCP CLEARANCE STRATEGIES

HCP clearance is a critical aspect of vaccine manufacturing. Effective removal of these proteins is important for product safety,

efficacy, and regulatory compliance with the expectation of reducing them to levels as low as practically achievable through appropriate process design and control. Several HCPs persist because they are tightly associated with host cell DNA, RNA, or viral particles. Nuclease digestion, while designed for DNA clearance, also disrupts these complexes and thereby enhances HCP removal in subsequent purification steps. Thus, although host cell DNA is a distinct impurity, nuclease-related operations affect both impurity classes and may therefore be relevant to HCP control strategies. Beyond total HCP content, there is increasing emphasis on qualitative profiling to understand the identity, persistence, and potential risk of individual HCP species. The clearance process involves a combination of upstream and downstream strategies, robust analytical monitoring, and continuous process verification to minimize HCP levels in the final product [10].

Implementing a platform manufacturing approach, defined as a standardized, scalable, and transferable production process that uses the same antigen-agnostic expression system and largely common upstream and downstream unit operations across multiple vaccine candidates, can support a consistent HCP control strategy. Leveraging prior process knowledge, can allow reuse of key elements of the HCP risk assessment and control justification and can streamline the supporting content for regulatory submissions. However, candidate-specific attributes, such as antigen charge, hydrophobicity, and expression level, can influence HCP clearance. Therefore, the residual HCP profile and associated risk should still be assessed for each candidate, with targeted controls or additional optimization applied where needed. Given that HCP identity, abundance, and clearance are strongly modality-dependent, Box 1 presents schematic manufacturing flows representing the four vaccine platforms mainly discussed in this review. These process maps highlight the key unit operations that drive differences in HCP burden and clearance performance, including points where cell disruption, nuclease treatment, affinity capture, polishing chromatography, or viral clearance steps are introduced.

From a regulatory and licensure perspective, manufacturers must demonstrate that their purification processes are robust and reliable. This is typically achieved through process validation, particularly during process performance qualification batches, where the process must consistently clear HCPs to acceptable levels across multiple, consecutive production runs. Regulatory authorities expect manufacturers to demonstrate that purification processes remain under control, with consistent HCP clearance performance across batches. This expectation relates to overall process capability rather than to the replication of individual HCP profiles, which are not currently required to be demonstrated on a batchspecific basis. Defined log reduction values for HCPs should be established for key unit operations to demonstrate purification process capability and support validation, rather than to meet any predefined regulatory limits [21,22,23,24,26]. In addition, assessment of process variability, including factors such as resin lifetime and scale changes, is required to confirm that HCP clearance remains effective under different manufacturing conditions. A comprehensive risk assessment for residual HCPs is also mandated, evaluating the potential impact of any remaining impurities on product safety and efficacy. Finally, manufacturers must implement a control strategy that incorporates ongoing monitoring and trending analysis of HCP levels, enabling the detection of shifts in process performance and maintenance of stringent control over product quality [40,41]. Recent reviews and case studies [42,43,44] highlight that process-specific optimization, stepwise clearance data, and continuous process verification are important for achieving

▶ BOX 1

Schematic modality-specific manufacturing workflows for four vaccine platform examples.

Viral vector vaccine
Cell expansion (e.g., Vero/HEK293) → infection/transfection → virus amplification → harvest (lysis) → clarification → nuclease treatment → chromatography (AEX/CEX/MMC) → UF/DF → formulation → sterile filtration

Recombinant protein vaccine (stable cell line)
Fedbatch or perfusion cell culture (e.g., CHO) → harvest (lysis) → clarification → capture step (affinity) → polishing (AEX/CEX/HIC/MMC) → viral clearance → UF/DF → formulation → sterile filtration

Recombinant protein vaccine (BEVS)
Insect cell expansion (e.g., Sf9, Hi-5) → baculovirus infection → expression → harvest → clarification → nuclease treatment → capture step (AEX/CEX/affinity) → polishing (CEX/HIC/MMC) → viral clearance → UF/DF → formulation → sterile filtration

RNA vaccine (mRNA/LNP)
Plasmid DNA production (e.g., *E. coli*) → pDNA purification → linearization → IVT reaction → enzyme removal → RNA purification (chromatography/TFF) → LNP formulation → sterile filtration

It should be noted that there are variations for different platforms within a modality and the process flows above should be regarded as illustrative generic examples.

consistent HCP removal across production batches to support regulatory compliance.

UPSTREAM PROCESSING CONSIDERATIONS FOR HCP MINIMIZATION

First 'defense' in reducing HCP burden is upstream processing optimization. While substantial HCP clearance occurs during downstream processes, upstream parameters can significantly affect the overall HCP profile, including its composition, concentration, and variability. Decisions regarding cell line selection, genetic modification, media formulation, and bioreactor operation can all contribute to reducing HCP load, thereby facilitating downstream process development.

Host cell selection

Different host cell expression systems, such as Vero, *Escherichia coli*, *Saccharomyces cerevisiae*, HEK293 and CHO cells, are widely used in the production of various biopharmaceuticals, including vaccine products. Each of these expression systems inherently produces endogenous HCPs, which support essential cellular functions such as viability, protein synthesis, and metabolism. However, the HCP profile varies qualitatively and quantitatively depending on both the host cell system and the vaccine modality, e.g. viral vector, protein or nucleotide-based vaccines.

Table 3 provides an overview of expression systems commonly used in vaccine manufacturing, highlighting typical host organisms, application examples, advantages, challenges, and the specific HCP concerns associated with each system.

Engineering of vaccine production cell lines to reduce or eliminate high-risk HCPs may offer several advantages over relying solely on downstream purification. First, targeted removal of proteases, innate immune modulators, and highly immunogenic proteins that can interfere with antigen integrity, reduce stability, or alter potency during upstream production could help maintain antigen structure and functional epitopes.

TABLE 3

Expression systems used in vaccine manufacturing and associated host cell protein considerations.

Expression system	Typical host organism(s)	Vaccine application examples	Key advantages	Key challenges (incl. non-HCP)	Representative HCP/impurity concerns	Example residual HCP levels (based on prescription data)	References
Bacterial (recombinant proteins, Gram-negative)	*Escherichia coli*	Trumenba® (MenB fHbp); Bexsero® recombinant components (MenB)	High yield; fast growth; scalable; low cost of goods sold (COGS); simple media	No glycosylation; folding of complex proteins; endotoxin (LPS) control; inclusion bodies/refolding possible; high DNA burden	Chaperones (GroEL/DnaK); outer-membrane/membrane-anchored and periplasmic proteins; proteases; DNA-associated proteins	Numeric HCP limits not disclosed in public labels for most *E. coli*-derived vaccines; controlled via validated purification and release testing	[52,53,54]
Bacterial (glycoconjugate platform, Gram-positive)	*Streptococcus pneumoniae* (capsular polysaccharides) – *Corynebacterium diphtheriae* strain C7 (β197) (CRM197 carrier protein)	Prevnar 13®/ Prevenar 13® (pneumococcalconjugate vaccine; polysaccharides conjugated to CRM197)	High yield (though lower than *E. coli*); fast growth; scalable; low COGS; simple media; established conjugate platform; strong pediatric immunogenicity; mature manufacturing know-how	No glycosylation; limited post-translational modifications (PTMs); multi-stream manufacturing (each polysaccharide and carrier) and conjugation complexity; control of free saccharide/free protein; residual activation/conjugation reagents; polysaccharide heterogeneity	Membrane-anchored proteins (e.g. lipoproteins); fermentation-derived proteins from polysaccharide cultures; carrier-production host proteins; DNA-associated proteins	Public product information describes organisms and purification steps, but numeric residual HCP limits for Prevnar/PCVs are not publicly disclosed	[55,56]
Yeast	*Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha*	EngerixB®/ RecombivaxHB®(HBsAg; *S. cerevisiae*), Gardasil®/ Gardasil 9® (HPV L1 VLPs; *S. cerevisiae*)	Eukaryotic folding; robust scale-up; good safety history; supports virus-like particle (VLP) assembly	Yeast glycosylation patterns; cell-wall disruption; proteolysis; aggregation; process-related residues (e.g., formaldehyde in some processes)	Mannoproteins/cell-wall proteins; cell-wall remodeling enzymes; proteases (e.g., Yps1 and Pep4); stress-response proteins; DNA-associated proteins	EngerixB: ≤5% yeast protein. If 20 µg HBsAg adult dose → ≤1 µg = ≤1000 ng yeast protein/ dose (upper bound). Recombivax HB: <1% yeast protein. Gardasil HPV: contains <7 µg of yeast protein/dose	[54,57,58,59,60]
Insect cells – baculovirus expression vector system (BEVS)	*Spodoptera frugiperda* (Sf9/Sf21), *Trichoplusia ni* (Hi5) with baculovirus	Flublok® (recombinant influenza HA; Sf9), Cervarix® (HPV16/18 L1 VLPs; Hi-5)	High expression; some glycosylation and disulfide bond formation; supports large proteins/ VLPs; scalable	Baculovirus-related impurities; lacks sialylation; batch variability; high background protein content; potential proteolysis; higher COGS than bacterial systems	High-abundance soluble proteins; proteases; stress-response proteins; membrane-associated proteins; baculoviral proteins; DNA-associated proteins	Flublok: residual baculovirus + insect cell proteins ≤14.3 µg per 0.5 mL dose; Cervarix: Each dose contains residual baculovirus + insect cell protein <40 ng and bacterial cell protein <150 ng	[61,62,63,64,65,66]
Mammalian cells (recombinant proteins)	CHO, HEK293 (under development)	Shingrix® (recombinant VZV gE;CHO) ABRYSVO® (recombinant pre-F RSV GPs;CHO)	Human-like folding/PTMs; strong track record; well-developed analytics	Higher COGS; longer cell line development timelines; slower growth; sensitive to process variability; viral safety controls; glycan heterogeneity; 'sticky' HCP carryover possible	Persistent HCPs (stress proteins/chaperones); proteases (e.g., cathepsins); host cell–surface/membrane proteins; DNA-associated proteins; glycosylation-related enzymes	Shingrix: residual host cell proteins ≤3.0%; ABRYSVO: residual host cell proteins ≤0.1%w/w	[67,68,69]
Mammalian cells (viral vectors/live viral vaccines)	PER.C6/HEK293 (adenoviral vectors); Vero (some live viral vaccines)	Jcovden (Ad26.COV2-S; PER.C6); Vaxzevria (ChAdOX1 nCoV-19; HEK293), ERVEBO® (rVSV-ZEBOV; Vero)	Authentic particle assembly; Human-like PTMs; relevant biology for viral antigens/vectors; scalable	Higher COGS; impurity profile highly process-dependent (lysis/harvest timing, nuclease, formulation); viral safety and adventitious agent controls; DNA/HCP association with particles	HCPs that co-purify with particles; DNA-associated proteins; proteases; cytoskeletal and high-abundance cellular proteins; stress-response proteins	Jcovden: residual host cell proteins ≤0.15 µg/dose; Ervebo: vaccine may contain trace amounts of rice proteins	[70,71]
Egg-based (embryonated eggs)	SPF embryonated chicken eggs	Egg-derived influenza vaccines (various seasonal products)	Legacy platform with long clinical history (>70 years); large manufacturing capacity; high yields for specific viruses; clear regulatory frameworks	Limited platform versatility; egg-adaptation risk; allergen control; supply constraints (egg availability); batch-to-batch variability	Ovalbumin/ovomucoid/ ovotransferrin and other egg proteins (often treated as allergen specs rather than 'HCP' in biotech sense)	Vaxigrip Tetra lists trace ovalbumin ≤0.05 µg (50 ng) per 0.5 mL dose	[72,73]
Avian cells (cell culture; not eggs)	Primary chicken embryo fibroblasts (CEF); avian cell lines (DF-1, EB66, QOR2/2E11, AGE1.CR)	IMVANEX®/JYNNEOS® (MVABN®; CEF), cell-cultured influenza vaccine (H5N1; EB66)	Lower HCP complexity (compared to mammalian); can be serum-free; replication-competent for selected viruses; generally considered non-tumorigenic	Limited platform versatility; variability with primary cells; avian protein impurity profile and PTMs; adventitious agent controls; limited scale-up experience	Avian cell-surface membrane and cellular proteins; DNA-associated proteins; proteases; glycosidases; stress-response proteins	IMVANEX EPAR RMP notes the vaccine contains trace amounts of protein. USPI states <500 µg total protein	[74,75,76,77]

▶TABLE 3 (CONT.)

Expression systems used in vaccine manufacturing and associated host cell protein considerations (cont.).

Expression system	Typical host organism(s)	Vaccine application examples	Key advantages	Key challenges (incl. non-HCP)	Representative HCP/impurity concerns	Example residual HCP levels (based on prescription data)	References
Plant-based	*Nicotiana benthamiana* (leaf cells via agroinfiltration)	COVIFENZ® (CoVLP; *N. benthamiana*)	Low COGS, low risk of mammalian-derived pathogens; rapid scale-up; plant expression flexibility; capability of complex antigens, e.g. VLPs; improved thermostability	Plant glycans (potential xenoantigens); biomass clarification complexity; downstream processing complexity; regulatory familiarity varies	High HCP burden (e.g., RuBisCO) and plant-specific glycans; plant lectins/proteases, DNA-associated proteins	COVIFENZ monograph describes plant-based VLP production and notes possible trace antibiotics, but does not provide numeric residual plant-protein/HCP limits	[78,79]
Cell-free systems	*E. coli* lysate; wheat germ lysate; other extract-based systems	No licensed prophylactic human vaccines yet	Speed; no cell growth phase; simplified biosafety; high process control and reproducibility; platform flexibility	High COGS, scalability; PTM limitations; lysate complexity; cost at scale; regulatory familiarity varies	Lysate proteins/ribosomes; nucleases; membrane-associated proteins, chaperons	No standardized vaccine-spec examples; residuals are system- and process-dependent	[80,81]
RNA (IVT from plasmid DNA template; plasmid produced in *E. coli*)	*E. coli* (plasmid DNA template production)	Comirnaty® (BNT162b2 and variants); Spikevax® (mRNA-1273)	Well-established and high-yield DNA template production, rapid response capability (rapid sequence update); no cell culture during RNA synthesis; scalable enzymatic process; platform consistency; regulatory familiarity	Endotoxin (LPS) control; host-derived impurity impact on IVT performance; control of dsRNA/truncated RNA species; residual DNA template; residual enzymes (e.g., T7 polymerase, DNase); LNP CQAs (size, encapsulation, lipid impurities); raw-material supply chain	Residual *E. coli* proteins from plasmid DNA (starting material); DNA–protein complexes, host-derived proteins from enzyme manufacturing	Public sources describe plasmid DNA from transformed *E. coli*. Numeric residual *E. coli* HCP limits in final mRNA-LNP drug product are not publicly disclosed; for risk-ranking, a conservative assumption is ≤10 ng/dose bacterial proteins attributable to plasmid manufacture (expected below quantification after IVT purification + DNase treatment).	[82,83,84,85]

Furthermore, engineered reduction of problematic HCPs may decrease the risk that residual impurities provoke unintended immune responses or compromise safety. By lowering the burden of persistent HCPs entering downstream purification, such cell lines may also enable more streamlined, robust, and scalable processes, which are critical advantages when vaccines must be produced quickly and consistently during outbreak response. Overall, host-cell engineering may provide a proactive strategy that could complement traditional HCP monitoring and clearance approaches in vaccine manufacturing. However, use of targeted host cell engineering remains limited in vaccine development [45,46], though several research-stage examples exist in CHO-based biopharmaceutical production that demonstrate the feasibility of reducing or eliminating specific problematic HCPs [47,48,49,50]. CHO cells are increasingly used in vaccine manufacturing [51], further supporting the relevance of these engineering approaches even though they have not yet been applied in licensed products.

Culture conditions and feeding strategies

Optimizing culture conditions and feeding strategies is essential for controlling HCP levels during upstream bioprocessing [86,87,46]. Media composition plays a central role as the selection of chemically defined media, non-chemically defined or complex media can significantly affect cell growth, productivity, and the HCP profile. Chemically defined media offer improved consistency and reduce the risk of introducing undefined impurities, while complex media may support higher yields but can increase process variability and HCP diversity [88]. Feeding strategy and process control are also pivotal. Fed-batch processes often result in increased cellular stress in the later stages of culture, which can cause elevated HCP release as cells undergo apoptosis or lysis. In contrast, perfusion cultures provide better control over nutrient and metabolite concentrations such as glucose, lactate, and ammonia, thereby supporting cell viability and productivity. This results in a more consistent HCP profile, which may help mitigate HCP accumulation in the harvest [86,89]. Other upstream factors, such as osmolality, pH, and shear stress, also affect HCP concentrations. Elevated osmolality and agitation rates can increase cellular stress, promoting HCP release. Bioreactor geometry, impeller design, and sparger configuration all influence the degree of shear stress experienced by cells, which in turn affects both cell viability and the HCP profile in the culture supernatant [90]. Finally, harvest timing is a critical factor influencing HCP levels. Delayed harvesting increases the risk of HCP accumulation due to cell death and lysis, underscoring the importance of monitoring cell viability to define the optimal harvest time, ensuring that HCP levels are minimized before downstream processing [91,92].

DOWNSTREAM PROCESSING STRATEGIES FOR HCP CLEARANCE

Downstream processing (DSP) is the primary mechanism for controlling HCPs and other impurities in vaccine manufacturing, complementing upstream efforts to reduce the initial impurity burden. Vaccine purification design requires careful selection and sequencing of unit operations, definition of operating conditions and cost-performance targets, as well as optimization of the integrated purification train, since even minor variations in one step can impact the performance of subsequent steps. One of the key challenges in developing purification processes for HCP clearance is removing problematic HCPs that closely associate with the product and therefore behave similarly during downstream separation (particularly relevant for recombinant protein or subunit vaccines). Another challenge is preserving the antigen structure/stability and potency-critical attributes throughout purification under required process conditions, e.g., pH, temperature, shear, ionic strength [56].

Across vaccine platforms, robust impurity control is typically achieved through a platform-adapted sequence of orthogonal unit operations, selected to protect

potency-critical attributes while delivering consistent purity and yield at scale [54,93]. The unit operations listed in Table 4 illustrate downstream processing operations commonly used for HCP reduction, describing how each technique contributes to effective clearance of HCPs during vaccine and biologics manufacturing.

For viral vaccines and viral-vector platforms, the practical emphasis is on scalable chromatography and membrane operations that can handle large, fragile particles while reducing HCPs, host cell DNA, and other impurities. These large virus products can create aseptic challenges, i.e. 0.22 µm sterile filtration may be constrained by particle size, so manufacturing relies on robust aseptic processing with strong contamination and viral safety controls, ideally via closed processing. Recent examples in vaccine development show that combining orthogonal methods, such as multimodal chromatography with UF/DF, improves impurity removal and manufacturability for live virus vaccine candidates in Vero cells, achieving significant log-reductions in HCP and host cell DNA [54,93,94].

For protein vaccines (subunit and virus like particles (VLPs)), downstream processing more closely resembles classical biologics purification, with HCP clearance typically driven by a capture step followed by orthogonal polishing (AEX/CEX/MMC/HIC as appropriate) and UF/DF to remove residual soluble HCPs while maintaining antigen structure and potency-critical attributes [95]. Subunit proteins are generally compatible with sterile filtration, whereas VLPs can be more sensitive to shear and prone to aggregation, shifting the emphasis toward low-shear clarification, flow-through and/or membrane-based chromatography [54,96].

Looking ahead, vaccine development is focusing more on data- and knowledge-driven control strategies, backed by stronger process understanding of when and why impurities are co-purified, how clearance varies with process conditions, and ways to enhance resilience against drift and scale or site transfer effects. In parallel, downstream purification continues to evolve toward more standardized and intensified workflows thanks to advances in filtration and membrane design, expanded affinity capture options using engineered ligands, and wider adoption of convective chromatography formats like membranes and monoliths, alongside multicolumn continuous processing. Such technologies can help improve yield, speed up production, and improve impurity clearance while reducing buffer consumption, footprint, and operational steps [97,98]. Additionally, process analytical technology (PAT) provides real-time measurements that support faster development and more consistent manufacturing [99]. Recent literature on vaccines highlights the growing adoption of analytical methods as PAT tools for monitoring manufacturing (including impurity clearance and CQA), as well as the increasing use of model-based and artificial intelligence-supported approaches that integrate multivariate process and analytical data to inform process decisions and strengthen control [54,100,101].

For HCP monitoring in vaccines, the focus is shifting beyond reliance on a single "total HCP" value toward more identity-aware, risk-based impurity surveillance, while keeping fit-for-purpose immunoassays as core release and control tools. Recent examples include quantitative proteomics approaches that enable the simultaneous assessment of antigen and HCP content in COVID-19 vaccine samples, and alternative quantitative workflows (e.g., capillary western methods) evaluated for HCP analysis in Vero cell derived vaccine products, supporting a trend toward complementary, information-rich methods alongside traditional assays [102,103,104].

Overall, downstream processing is the primary mechanism for HCP and impurity control in vaccine manufacturing,

▶TABLE 4

Key downstream process unit operations used for host cell protein removal.

DSP unit operation	HCP removal principle
Anion exchange chromatography (AEX)	Exploits charge differences; often run in flow-through where acidic/negatively charged HCPs bind and product flows through (or as bind–elute if product is net negative)
Cation exchange chromatography (CEX)	Charge-based separation complementary to AEX; useful to remove basic/positively charged HCPs or variants, and can be used as capture if product is net positive at the chosen pH
Hydrophobic interaction chromatography (HIC)	Separates by surface hydrophobicity and can help with hydrophobic impurities/variants; can separate misfolded or sticky HCPs
Multimodal chromatography (MMC)	Targets multiple interaction mechanisms (e.g. electrostatic, hydrophobic, ionic, hydrogen bonding), enabling enhanced impurity removal beyond single-mode chromatography
Affinity chromatography	Specific binding of the product to an immobilized ligand; provides strong capture selectivity and typically achieves significant HCP reduction
Size exclusion chromatography (SEC)	Helps remove large aggregates or residual HCPs differing in size
Nuclease treatment	Degrading host cell DNA/RNA; may reduce co-purifying nucleic-acid–binding proteins
Ultrafiltration/diafiltration (UF/DF)	Concentration and buffer-exchanges; can help remove soluble HCPs by size discrimination

delivering clearance through orthogonal, platform-adapted purification trains that progressively reduce impurities while protecting yield and potency. As DSP strategies mature, they are increasingly strengthened by fit-for-purpose monitoring and lifecycle trending, with a push toward faster, more informative impurity monitoring (including HCP characterization) to support robustness, speed, and consistency across modern vaccine platforms [54,100,101].

HCP TRENDING, CONTROL STRATEGY, & LIFECYCLE MANAGEMENT

In process monitoring forms the backbone of a lifecycle HCP control strategy. Trending of HCP levels across batches allows manufacturers to detect shifts in process performance, identify emerging clearance challenges, and proactively implement corrective actions. Trends help determine whether HCP levels remain within justified specifications and whether unit operations continue to achieve expected log reduction values. HCP levels should be assessed at defined points across upstream and downstream operations to detect shifts in cell health, impurity burden, or purification performance early. Routine monitoring with a validated ELISA provides rapid, quantitative trending, while orthogonal tools such as LC-MS/MS or spectroscopic methods support deeper characterization when required. In practice, manufacturers use statistical processcontrol principles to establish phase-appropriate alert and action limits for HCP levels; however, these thresholds and trigger criteria are inherently process and product-specific, and therefore not generalizable across platforms. Spectroscopic tools such as Raman and Fourier-transform infrared spectroscopy enable non-destructive, rapid fingerprinting of HCPs and other impurities, and are increasingly applied for trending and deviation detection, particularly in continuous or intensified DSP formats [105,106,107,41]. Capillary Western and immunoblotting can supplement these methods where specific HCP risks are known.

Regulators expect manufacturers to

integrate HCP trends into continuous process verification, ensuring that purification processes remain in control over time. Trending also provides critical support during process changes, scale-up, technology transfer, and comparability assessments, especially in modalities where persistent HCPs may pose safety or analytical risks (e.g., viral vectors, insectcell products).

A robust control strategy for HCPs in vaccines should integrate upstream understanding, downstream clearance capability, analytical characterization, and lifecycle monitoring. Central to this approach is structured risk assessment, which drives control decisions and the definition of acceptance criteria. The strategy should focus not only on total HCP levels but on identifying specific HCPs that are persistent, product-interacting, biologically active, or safety-relevant. Understanding their nature, function, and potential clinical impact is important. For HCPs identified as potentially concerning, structured risk assessment should evaluate toxicity, similarity to human homologues, and T-cell epitope predictions to assess immunogenicity or autoimmunity risks. Structured risk assessment tools, such as those developed by the BioPhorum Development Group [43], help identify and prioritize process parameters that impact residual HCP levels and their potential safety implications.

CONCLUSION & FUTURE PERSPECTIVES

HCPs remain a central consideration in vaccine manufacturing, shaping product quality, safety, and regulatory confidence across diverse vaccine modalities. Despite significant advances in analytical technologies and downstream purification strategies, persistent challenges remain, including variable HCP profiles across platforms, inconsistent analytical coverage, gaps in global regulatory guidance, and disparities in regional testing capacity. As the field evolves, a harmonized, risk-based, approach to HCP control may be important for ensuring both manufacturing robustness and patient safety.

A key insight is that not all HCPs pose equal risk, and the clinical and mechanistic risk profiles for vaccines differ fundamentally from those of biotherapeutics. Since vaccines are typically administered at low doses, on an infrequent schedule, and are designed to be intrinsically immunogenic, decades of clinical experience demonstrate that most licensed vaccines contain residual HCPs at levels unlikely to cause harm. In some cases, HCPs may even provide stabilizing or adjuvant-like benefits. Nevertheless, certain HCP classes, such as proteases, cytokine-like molecules, or product-interacting proteins, can influence antigen integrity, analytical performance, batch consistency, or immune responses, which justifies targeted characterization and control.

A universal, harmonized numeric HCP limit is neither scientifically justified across vaccine modalities nor supported by current regulatory frameworks; instead, the field should converge on harmonized risk-based principles that enable consistent, transparent, and phase-appropriate control (assay capability, orthogonal analytics, and lifecycle trending). This stance reflects the explicit absence of prescriptive limits, the case-by-case expectations of agencies, and the growing role of MS for identity-aware risk assessment. Such principle-level harmonization offers a practical route to global alignment, without imposing a one-size-fits-all threshold. Looking forward, several areas will shape the future of HCP management in vaccines:

Risk-based control strategies

A shift from numerical specification setting toward holistic control strategies rooted in process understanding, orthogonal analytics, and platform and product-specific risk assessment is both scientifically justified

and aligned with emerging regulatory expectations. As highlighted across multiple studies, case-by-case evaluation of persistent or safety-relevant HCPs provides greater value than universal limits. Development of standardized platforms may enable the establishment of specifications for residual HCP in new vaccine candidates, leveraging prior knowledge gained from previous candidates using the same technologies.

Next-generation analytical technologies

Mass spectrometry, improved DIA workflows, antibody affinity extraction, and evolving spectroscopic tools are transforming the resolution and consistency of HCP profiling and in-process monitoring. These tools also strengthen safety oversight by enabling identification of high-risk species such as proteases, cytokine-like proteins, or product-interacting HCPs that may alter potency or affect immunogenicity. As analytical complexity rises, the challenge will be not only how comprehensively we can detect HCPs, but how meaningfully we interpret them and integrate them into regulatory risk frameworks. It is also envisioned that AI will evolve the field further and support residual HCP evaluation.

Process engineering and predictive design

Progress in host-cell engineering, including knockouts of problematic HCPs and modulation of secretion pathways, along with stress reduction strategies, offers the potential to reduce HCP burden at the source. Although their adoption in vaccine manufacturing is still limited, these technologies may become platform enablers for next-generation viral vectors and recombinant protein technologies with improved safety profiles. To complement these upstream innovations, downstream purification is expected to become increasingly standardized and intensified, with advances in membrane and chromatography technologies, expanded affinity options, and real-time PAT tools enabling faster, more robust, and more informative impurity clearance across vaccine platforms.

Strengthening regional manufacturing and global equity

A lack of standardized HCP guidance disproportionately affects LMIC manufacturers. Clearer global expectations, paired with decentralized, affordable analytical solutions, are critical for enabling rapid and equitable vaccine production, particularly under outbreak response scenarios. HCP analytics should not become a barrier to global access; rather, appropriately scaled approaches, i.e. analytical strategies and risk assessment tools tailored to the technical capacity, infrastructure, and risk profile of the manufacturing setting, can support both regulatory scrutiny and equitable deployment.

Toward lifecycle driven, data rich monitoring

The future of HCP control lies in continuous monitoring that integrates upstream signals, downstream clearance performance, and manufacturing trends. More sophisticated data analytics, process modelling, and digital quality systems will allow for early drift detection, faster comparability assessments, and more robust process comparability across scales and geographies.

In summary, the field is moving toward a more nuanced and scientifically grounded understanding of HCPs, one that acknowledges their diverse biological roles, varying risks across vaccine platforms, and the practical realities of globalized manufacturing. A HCP strategy should balance scientific rigor with regulatory pragmatism by targeting high-risk species, using orthogonal analytics, integrating process controls, and aligning expectations. Through such

an approach, vaccine manufacturers can ensure that HCPs remain controlled to levels that are as low as reasonably achievable while maintaining the high safety standards foundational to public trust and global vaccine deployment.

TRANSLATION INSIGHT

Effective pandemic response requires rapid, scalable, and safe vaccine production across diverse platforms and global regions. This review highlights actionable strategies for HCP management that support accelerated vaccine deployment without compromising quality. Risk-based HCP control strategies enable developers to tailor purification and analytical approaches to the unique challenges of each vaccine modality, while advanced analytical technologies, including mass spectrometry and orthogonal methods, allow precise impurity identification and monitoring, supporting safety risk assessments. Importantly, harmonized global guidelines and decentralized analytical capacity, particularly in low- and middle-income countries, can accelerate vaccine release timelines and promote equitable access during outbreaks. Integrating upstream cell engineering, robust downstream purification, and lifecycle monitoring provides a framework for continuous improvement and comparability, supporting the rapid deployment of safe and effective vaccines worldwide.

REFERENCES

1. Chiu J, Valente KN, Levy NE, Min L, Lenhoff AM, Lee KH. Knockout of a difficult-to-remove CHO host cell protein, lipoprotein lipase, for improved polysorbate stability in monoclonal antibody formulations. *Biotechnol. Bioeng.* 2017; 114,1006–1015.
2. Solá RJ, Griebenow K. Effects of glycosylation on the stability of protein pharmaceuticals. *J. Pharm. Sci.* 2009; 98,1223–1245.
3. Oh YH, Becker ML, Mendola KM, *et al.* Characterization and implications of host-cell protein aggregates in biopharmaceutical processing. *Biotechnol. Bioeng.* 2023; 120, 1068–1080.
4. Tait AS, Hogwood CE, Smales CM, Bracewell DG. Host cell protein dynamics in the supernatant of a mAb producing CHO cell line. *Biotechnol. Bioeng.* 2012; 109(4), 971–982.
5. Wilson LJ, Lewis W, Kucia-Tran R, Bracewell DG. Identification and classification of host cell proteins during biopharmaceutical process development. *Biotechnol. Prog.* 2022; 38(1), e3224.
6. Gupta SK, Shukla P. Advanced technologies for improved expression of recombinant proteins in bacteria: perspectives and applications. *Crit. Rev. Biotechnol.* 2016; 36(6), 1089–1098.
7. Zhu J. Mammalian cell protein expression for biopharmaceutical production. *Biotechnol. Adv.* 2012; 30(5), 1158–1170.
8. Kornecki M, Mestmäcker F, Zobel-Roos S, Heikaus de Figueiredo L, Schlüter H, Strube J. Host cell proteins in biologics manufacturing: the good, the bad, and the ugly. *Antibodies* 2017; 6(3), 13.
9. Wang X, Hunter AK, Mozier NM. Host cell proteins in biologics development: identification, quantitation and risk assessment. *Biotechnol. Bioeng.* 2009; 103(3), 446–458.
10. Baik JY, Guo J, Lee KH. Host cell proteins during biomanufacturing: challenges and risk considerations. In: *Cell Culture Engineering: Recombinant Protein Production,* 1st Edition (Editors: Lee GM, Kildegaard HF). 2020; Wiley-VCH, 295–312.
11. Jones M, Palackal N, Wang F, *et al.* 'High-risk' host cell proteins (HCPs): a multi-company collaborative view. *Biotechnol. Bioeng.* 2021;118(8), 2870–2885.
12. Panikulam S, Morgan H, Gutknecht M, Villiger TK, Lebesgue N, Karle AC. Host cell protein-mediated adjuvanticity and immunogenicity risks of biotherapeutics. *Biotechnol. Adv.* 2025; 81, 108575.

13. Pérez Medina Martínez V, Espinosa-de la Garza CE, Pérez NO. Opportunities in the field of host cell proteins: part 4: the future of immunogenicity prediction. *BioProcess Int.* Feb 9, 2023. https://www.bioprocessintl.com/qa-qc/opportunities-in-the-field-of-host-cell-proteins-part-4-the-future-of-immunogenicity-prediction (accessed Dec 26, 2025).

14. Goey CH, Alhuthali S, Kontoravdi C. Host cell protein removal from biopharmaceutical preparations: towards the implementation of quality by design. *Biotechnol. Adv.* 2018; 36(4), 1223–1237.

15. Li Y. Effective strategies for host cell protein clearance in downstream processing of monoclonal antibodies and Fc-fusion proteins. *Protein. Expr. Purif.* 2017; 134, 96–103.

16. Pérez Medina Martínez V, Espinosa-de la Garza CE, Pérez NO. Opportunities in the field of host cell proteins: part 2: ensuring patient safety. *BioProcess Int.* Oct 20, 2022. https://www.bioprocessintl.com/separation-purification/opportunities-in-the-field-of-host-cell-proteins-part-2-ensuring-patient-safety (accessed Dec 26, 2025).

17. Kanwal F, Henry M, Meleady P. Analytical tools for HCP detection, identification, and quantitation. *Methods Mol. Biol.* 2025; 2853, 235–248.

18. Pérez Medina Martínez V, Espinosa-de la Garza CE, Pérez NO. Opportunities in the field of host-cell proteins: part 1: their sources and implications for protein-drug efficacy. *BioProcess Int.* Sep 28, 2022. https://www.bioprocessintl.com/qa-qc/opportunities-in-the-field-of-host-cell-proteins-part-1-their-sources-and-implications-for-protein-drug-efficacy (accessed Dec 26, 2022).

19. Saville M, Cramer JP, Downham M, *et al.* Delivering pandemic vaccines in 100 days – what will it take? *N. Engl. J. Med.* 2022; 387(2), e3.

20. ICH Q6B. Specifications: test procedures and acceptance criteria for biotechnological/biological products. *European Medicines Agency* Sep 1999. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf (accessed Jan 8, 2026).

21. US Food and Drug Administration. Code of Federal Regulations, 21 CFR Part <610.13>: Purity. 2018.

22. US Pharmacopeia. Residual host cell protein measurement in biopharmaceuticals by liquid chromatography–mass spectrometry. USP–NF <1132.1> 2024. https://doi.usp.org/USPNF/USPNF_M17756_02_01.html (accessed Dec 26, 2025).

23. European Medicines Agency. Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission. Report No.: EMA/CHMP/BWP/187338/2014. 2016.

24. European Medicines Agency. CPMP position statement on DNA and host cell proteins (HCP) impurities, routine testing versus validation studies. Report No.: CPMP/BWP/382/97. 1997.

25. European Medicines Agency. Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines. Report No.: EMA/CHMP/VWP/141697/2009. Jun 24, 2010. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-and-clinical-aspects-live-recombinant-viral-vectored-vaccines_en.pdf (accessed Jan 8, 2026).

26. World Health Organization. Annex 4: guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology. Jun 18, 2014. https://www.who.int/publications/m/item/recombinant-dna-annex-4-trs-no-987 (accessed Jan 5, 2026).

27. US Pharmacopeia. General chapter <1132>: residual host cell protein measurement in biopharmaceuticals. 2016.

28. World Health Organization. WHO guideline for the safe production and quality control of monoclonal antibodies for use in humans. Oct 12, 2021. https://cdn.who.int/media/docs/default-source/biologicals/mabs-manufacture-guideline-draft-for-1st-public-comment.pdf (accessed Jan 8, 2026).

29. Krutzke L, Rösler R, Allmendinger E, Engler T, Wiese S, Kochanek S. Process- and product-related impurities in the ChAdOx1 nCov-19 vaccine. *Elife* 2022; 11, e78513.

30. Dagouassat N, Haeuw JF, Robillard V, *et al.* Development of a quantitative assay for residual host cell proteins in a recombinant subunit vaccine against human respiratory syncytial virus. *J. Immunol. Methods* 2001; 251(1–2), 151–159.

31. Champion K, Madden H, Dougherty J, Shacter E. Defining your product profile and maintaining control over it, part 2. *Bioprocess Int.* 2005; 52–7.

32. European Medicines Agency. Draft guideline on the quality aspects of mRNA vaccines. *London (UK): EMA;* 2025. Report No.: EMA/CHMP/BWP/82416/2025.

33. European Medicines Agency; Committee for Medicinal Products for Human Use (CHMP). Assessment report: Kostaive (international non-proprietary name: zapomeran). *London (UK): EMA;* 2024. Report No.: EMA/18711/2025 corr.11. Procedure No.: EMEA/H/C/006207/0000.

34. Baker MP, Reynolds HM, Lumicisi B, Bryson CJ. Immunogenicity of biotechnology products: the key causes, consequences and challenges. *Self/Nonself.* 2010; 1(4), 314–322.

35. De Groot AS, Scott DW. Immunogenicity of protein therapeutics. *Trends Immunol.* 2007; 28(11), 482–490.

36. Therapeutic Goods Administration. Overview of vaccine regulation and safety monitoring and investigation into adverse events following 2010 seasonal influenza vaccination in young children. *Canberra (Australia): Australian Government Department of Health/Aged Care & TGA;* 2010. Available from: https://www.tga.gov.au/sites/default/files/alerts-medicine-seasonal-flu-101008.pdf (accessed Jan 8, 2026).

37. Anaquant. HCP analysis: ELISA or mass spectrometry. https://www.anaquant.com/hcp-analysis-elisa-or-mass-spectrometry (accessed 15 May 2025).

38. Doneanu CE, Lennon S, Anderson M, *et al.* A comprehensive approach for HCP identification. *Waters Corporation;* 2018. https://www.waters.com/nextgen/us/en/library/application-notes/2018/hcp-identification-quantification-monitoring-based-single-dimension-separation.html (accessed May 15, 2025).

39. Dzau V, Hatchett R, Bishen S, *et al.* RVMC Secretariat. The time for regionalised vaccine manufacturing has arrived. *Lancet* 2025; 405(10475), 280–282.

40. Fang Z, Lyu J, Li J, *et al.* Application of bioreactor technology for cell culture-based viral vaccine production: present status and future prospects. *Front. Bioeng. Biotechnol.* 2022; 10, 921755.

41. Ito T, Lutz H, Tan L, *et al.* Host cell proteins in monoclonal antibody processing: control, detection, and removal. *Biotechnol. Prog.* 2024; 40(4), e3448.

42. Pilely K, Johansen MR, Lund RR, *et al.* Monitoring process-related impurities in biologics-host cell protein analysis. *Anal. Bioanal. Chem.* 2022; 414(2), 747–758.

43. Coye L, Jones MA, Gaza-Bulseco G, *et al.* Host cell protein clinical safety risk assessment - an updated industry review. *Biotechnol. Bioeng.* 2025; 122(11), 3229–3248.

44. Pérez Medina Martínez V, Espinosa-de la Garza CE, Pérez NO. Opportunities in the field of host cell proteins: part 3: case studies in impurity detection and identification. *BioProcess Int.* Dec 6, 2022. https://www.bioprocessintl.com/separation-purification/opportunities-in-the-field-of-host-cell-proteins-part-3-case-studies-in-impurity-detection-and-identification (accessed Dec 7, 2025).

45. Li SW, Wright M, Healey JF, *et al.* Gene editing in CHO cells to prevent proteolysis and enhance glycosylation: production of HIV envelope proteins as vaccine immunogens. *PLoS One* 2020; 15(5), e0233866.

46. Kiesslich S, Kamen AA. Vero cell upstream bioprocess development for the production of viral vectors and vaccines. *Biotechnol. Adv.* 2020; 44, 107608.

47. Kol S, Ley D, Wulff T, *et al.* Multiplex secretome engineering enhances recombinant protein production and purity. *Nat. Commun.* 2020; 11(1), 1908.

48. Dovgan T, Golghalyani V, Zurlo F, *et al.* Targeted CHO cell engineering approach reduces HCP-related enzymatic degradation and improves mAb product quality. *Biotechnol. Bioeng.* 2021; 118(10), 3821-3831. doi:10.1002/bit.27857.

49. Glinšek K, Bozovičar K, Bratkovič T. CRISPR technologies in Chinese hamster ovary cell line engineering. *Int. J. Mol. Sci.* 2023; 24(9), 8144.

50. Yuk IH, Ko P, Ahyow P, *et al.* Engineering Chinese hamster ovary cells to mitigate polysorbate degradation in biotherapeutics. *Biotechnol. Bioeng.* 2025; 122(11), 3139–3159.

51. Sanchez-Martinez ZV, Alpuche-Lazcano SP, Stuible M, Durocher Y. CHO cells for virus-like particle and subunit vaccine manufacturing. *Vaccine* 2024; 42(10), 2530–2542.

52. Pfizer Inc. COMIRNATY® (COVID-19 mRNA vaccine) prescribing information. 2024. https://labeling.pfizer.com/showlabeling.aspx?id=1796 (accessed Jan 8, 2026).

53. GlaxoSmithKline Biologicals SA. BEXSERO® (meningococcal group B vaccine) prescribing information. 2023. https://gskpro.com/content/dam/global/hcpportal/en_US/Prescribing_Information/Bexsero/pdf/BEXSERO.PDF (accessed Jan 8, 2026).

54. Buckland B, Sanyal G, Ranheim T, *et al.* Vaccine process technology-A decade of progress. *Biotechnol. Bioeng.* 2024; 121(9), 2604–2635.

55. US Food and Drug Administration. PREVNAR 13® (pneumococcal 13-valent conjugate vaccine) package insert. 2021. https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Package-Insert------Prevnar-13.pdf (accessed Jan 8, 2026).

56. Keulen D, Geldhof G, Le Bussy O, Pabst M, Ottens M. Recent advances to accelerate purification process development: a review with a focus on vaccines. *J. Chromatogr. A* 2022; 1676, 463195.

57. Baindara P, Dinata R, Kumar R. Yeast-based vaccine platforms: applications and key insights from the COVID-19 era. *Biomolecules* 2026; 16(1), 116.

58. US Food and Drug Administration. ENGERIX-B (hepatitis B vaccine (recombinant)) package insert. 1989. https://www.fda.gov/media/119403/download (accessed Feb 27, 2026).

59. US Food and Drug Administration. GARDASIL 9® (human papillomavirus 9-valent vaccine) package insert. 2022. https://www.fda.gov/media/90064/download (accessed Jan 8, 2026).

60. US Food and Drug Administration. RECOMBIVAX HB® (hepatitis B vaccine (recombinant)) package Insert. 2021. https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/package-insert-recombivax-hb.pdf (accessed Jan 8, 2026).

61. Felberbaum RS. The baculovirus expression vector system: a commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnol. J.* 2015; 10(5), 702–714.

62. Cox MM. Recombinant protein vaccines produced in insect cells. *Vaccine* 2012; 30(10), 1759–1766.

63. US Food and Drug Administration. FLUZONE® quadrivalent influenza vaccine package insert. 2023. https://www.fda.gov/media/179778/download (accessed Jan 8, 2026).

64. European Medicines Agency. CERVARIX® EPAR – product information. 2023. https://www.ema.europa.eu/en/documents/product-information/cervarix-epar-product-information_en.pdf (accessed Jan 8, 2026).

65. US Food and Drug Administration. Flublok (influenza vaccine) package insert. 2013. https://www.fda.gov/media/179778/download (accessed Feb 27, 2026).

66. US Food and Drug Administration. CERVARIX (human papillomavirus bivalent (types 16 and 18) vaccine, recombinant) package insert. 2009. https://www.fda.gov/media/78013/download (accessed Feb 27, 2026).

67. Walsh G, Walsh E. Biopharmaceutical benchmarks 2022. *Nat. Biotechnol.* 2022; 40(12), 1722–1760.

68. US Food and Drug Administration. SHINGRIX® (zoster vaccine recombinant, adjuvanted) package insert. 2021. https://www.fda.gov/media/108597/download (accessed Feb 27, 2026).

69. US Food and Drug Administration. ABRYSVO® (respiratory syncytial virus vaccine) package insert. 2022. https://www.fda.gov/media/168889/download (accessed Jan 8, 2026).

70. US Food and Drug Administration. Janssen COVID-19 vaccine (Ad26.COV2.S) fact sheet for healthcare providers administering vaccine. 2021. https://www.fda.gov/media/146304/download (accessed Jan 8, 2026).

71. US Food and Drug Administration. ERVEBO® (ebola Zaire vaccine, live) package insert. 2019. https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Package-Insert-ERVEBO_1.pdf (accessed Jan 8, 2026).

72. Rajaram S, Wojcik R, Moore C, *et al.* The impact of candidate influenza virus and egg-based manufacture on vaccine effectiveness: literature review and expert consensus. *Vaccine* 2020; 38(38), 6047–6056.

73. Australian Government Department of Health. Vaxigrip Tetra. *Australian Immunisation Handbook.* 2024. https://immunisationhandbook.health.gov.au/vaccines/vaxigrip-tetra (accessed Jan 8, 2026).

74. Göbel S, Jaén KE, Fernandes RP, *et al.* Characterization of a quail suspension cell line for production of a fusogenic oncolytic virus. *Biotechnol. Bioeng.* 2023; 120(11), 3335–3346.

75. Jordan I, Vos A, Beilfuss S, Neubert A, Breul S, Sandig V. An avian cell line designed for production of highly attenuated viruses. *Vaccine.* 2009; 27(5), 748–756.

76. European Medicines Agency. IMVANEX® EPAR – risk management plan. 2023. https://www.ema.europa.eu/en/documents/rmp/imvanex-epar-risk-management-plan_en.pdf (accessed Jan 8, 2026).

77. US Food and Drug Administration. JYNNEOS® (smallpox and monkeypox vaccine) package insert. 2021. https://www.fda.gov/media/131078/download (accessed Jan 8, 2026).

78. Rosales-Mendoza S, Márquez-Escobar VA, González-Ortega O. Plant-based vaccines: current status and future perspectives. *Front. Plant Sci.* 2023; 14, 1273958.

79. Health Canada. COVIFENZ® (COVID-19 plant-based vaccine) product monograph. 2022. https://covid-vaccine.canada.ca/info/pdf/covifenz-pm-en.pdf (accessed Jan 8, 2026).

80. Dondapati SK, Stech M, Zemella A, Kubick S. Cell-free protein synthesis: a promising option for future drug development. *BioDrugs* 2020; 34(3), 327–348.

81. Das Gupta M, Flaskamp Y, Roentgen R, *et al.* ALiCE®: a versatile, high yielding and scalable eukaryotic cell-free protein synthesis (CFPS) system. *bioRxiv* 2022. doi:10.1101/2022.11.10.515920. Update: *Biotechnol. Bioeng*. 2023; 120(10), 2890–2906. doi:10.1002/bit.28461.

82. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines – a new era in vaccinology. *Nat. Rev. Drug Discov.* 2018; 17(4), 261–279.

83. Kis Z, Kontoravdi C, Shattock R, Shah N. Resources, production scales and time required for producing RNA vaccines for the global pandemic demand. *Vaccines* 2020; 9(1), 3. Erratum in: *Vaccines* 2021; 9(3), 205.

84. European Medicines Agency. COMIRNATY® EPAR – public assessment report. 2023. https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf (accessed Jan 8, 2026).

85. US Pharmacopeia. Draft general chapter: mRNA vaccine analysis. USP–NF. 2024. https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/usp-nf-notices/mrna-vaccine-chapter.pdf (accessed Jan 8, 2026).

86. Sahoo A, Devenney K, Nicolas MA, Zill N, Gupta B, Madabhushi SR. Beyond viability: advancing CHO cell culture process strategies to modulate host cell protein levels. *N. Biotechnol.* 2024; 84, 128–139.

87. Gilbert A, Huang YM, Ryll T. Identifying and eliminating cell culture process variability. *Pharm. Bioprocess.* 2014; 2(6), 519–534.

88. Reinhart D, Damjanovic L, Kaisermayer C, Kunert R. Benchmarking of commercially available CHO cell culture media for antibody production. *Appl. Microbiol. Biotechnol.* 2015; 99(11), 4645–4657.

89. Sahoo A, Tsukiadate T, Lin BR, *et al.* Proteomics reveals distinctive host cell protein expression patterns in fed-batch and perfusion cell culture processes. *Biotechnol. J.* 2025; 20(1), e202400567.

90. Grein TA, Loewe D, Dieken H, Weidner T, Salzig D, Czermak P. Aeration and shear stress are critical process parameters for the production of oncolytic measles virus. *Front. Bioeng. Biotechnol.* 2019; 7, 78.

91. Wilson LJ, Lewis W, Kucia-Tran R, Bracewell DG. Identification of upstream culture conditions and harvest time parameters that affect host cell protein clearance. *Biotechnol. Prog.* 2019; 35(3), e2805.

92. Achleitner L, Winter M, Aguilar P, *et al.* Robust and resource-efficient production process suitable for large-scale production of baculovirus through high cell density seed train and optimized infection strategy. *New Biotechnol.* 2024; 80, 46–55.

93. Konstantinidis S, Poplyk MR, Ma WJ, *et al.* Purification processes of live virus vaccine candidates expressed in adherent Vero cell lines via multimodal chromatography in flowthrough mode. *Biotechnol. Bioeng.* 2024; 121(8), 2482–2499.

94. Makovitzki A, Harel C, Mendelson E, Lev D, Gal-Tanamy M. In-line monitoring of downstream purification processes for VSV-based SARS-CoV-2 vaccine using a novel technique. *BioTech.* 2021; 10(4), 25.

95. Hołubowicz R, Gao F, Du SW, *et al.* Scalable purification enables high-quality virus-like particles for therapeutic translation. *J. Biol. Chem.* 2025; 301(12), 110946.

96. Goodarzi MM, Jalalirad R, Doroud D, Hozouri H, Aghasadeghi M, Paryan M. Determining buffer conditions for downstream processing of VLP-based recombinant hepatitis B surface antigen using multimodal resins in bind-elute and flow-through purification modes. *Sci. Rep.* 2023; 13(1), 10745.

97. Kilgore R, Minzoni A, Shastry S, *et al.* The downstream bioprocess toolbox for therapeutic viral vectors. *J. Chromatogr. A* 2023; 1709, 464337.

98. Matos T, Hoying D, Kristopeit A, Wenger M, Joyce J. Continuous multi-membrane chromatography of large viral particles. *J. Chromatogr. A* 2023; 1705, 464194.

99. Sathiyapriyan P, Mukherjee S, Vogel T, Essen LO, Boerema D, Vey M, Kalina U. Current PAT landscape in the downstream processing of biopharmaceuticals. *Anal. Sci. Adv.* 2025; 6(1), e70013.

100. Sripada S, Hosseini Ramesh N, Menegatti S, Daniele S. Advances and opportunities in process analytical technologies for viral vector manufacturing. *Biotechnol. Adv.* 2024; 108391.

101. Yi J, McCracken C, Davide J, *et al.* Development of process analytical tools for rapid monitoring of live virus vaccines in manufacturing. *Sci. Rep.* 2022; 12, 15494.

102. Gillespie PF, Wang Y, Yin K, *et al.* Automated, quantitative capillary Western blots to analyze host cell proteins in COVID-19 vaccine produced in Vero cell line. *Vaccines* 2024; 12(12), 1373.

103. Zhao H, Li W, Liu J, *et al.* Label-free quantitative proteomics analysis of COVID-19 vaccines by nano LC-HRMS. *Vaccines* 2024; 12(9), 1055.

104. Pearson C, Wang Y, Alkurdi E, *et al.* Quantitation of host cell proteins in biopharmaceuticals from Chinese hamster ovarian and Vero cell lines using capillary electrophoresis western blots. *J. Pharm. Biomed. Anal.* 2023; 233, 115420.

105. Rolinger L, Rüdt M, Hubbuch J. A critical review of recent trends, and a future perspective of optical spectroscopy as PAT in biopharmaceutical downstream processing. *Anal. Bioanal. Chem.* 2020; 412, 2047–2064.

106. Athalye SM, Maruthamuthu MK, Esmaili E, *et al.* Real-time monitoring of attenuated cytomegalovirus using Raman spectroscopy allows non-destructive characterization during flow. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 2026; 345, 126761.

107. Capito F, Skudas R, Kolmar H, Stanislawski B. Host cell protein quantification by Fourier transform mid infrared spectroscopy (FT-MIR). *Biotechnol. Bioeng.* 2013; 110(1), 252–259.

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VACCINE INSIGHTS

INTERVIEW

TOPIC FOCUS • Therapeutic Vaccines

Modulation of antitumor immunity by COVID-19 mRNA vaccination: retrospective association, mechanistic evidence, and need for prospective validation

Adam Grippin

INTERVIEW

"Enthusiasm is warranted, but it must be matched with careful validation."

Ashling Cannon, Editor, *Vaccine Insights*, speaks with Adam Grippin, Resident, Division of Radiation Oncology, MD Anderson Cancer Center, USA, about his team's retrospective clinical analysis and complementary preclinical studies suggesting that COVID-19 mRNA vaccination may influence outcomes in patients receiving immune checkpoint blockade. He discusses the mechanistic work underpinning this signal, the biologic implications of treatment timing, and why randomized Phase 3 validation is essential before clinical integration.

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"We were able to track that response within the tumor microenvironment and demonstrate that it can be augmented with immune checkpoint blockade."

Q To what extent can residual confounding, including a potential healthy vaccine bias, account for the observed association? What features of the data argue for a mechanistic explanation?

AG It is an important question. Any retrospective analysis is mired in biases that we cannot fully control. Our goal was not to claim definitive causality, but rather to determine whether a reproducible signal could be identified and supported by biological evidence.

The clinical data suggest an association between mRNA vaccination and improved outcomes in patients receiving immunotherapy [1]. However, the core of our work focuses on mechanism. The majority of the study centers on mouse models designed to dissect how an mRNA vaccine targeting a non-tumor antigen can initiate an antitumor immune response. We were able to track that response within the tumor microenvironment and demonstrate that it can be augmented with immune checkpoint blockade.

Importantly, we also show that immune correlates observed in preclinical models are present in vaccinated patients. Taken together, these data support the hypothesis that mRNA vaccination may confer unexpected immunologic benefits in oncology settings. That said, randomized validation is required to determine whether these observations translate into meaningful clinical benefit.

Q If mRNA vaccination can positively modulate immunotherapy outcomes, why has this signal not been more widely recognized?

AG In biomedical research, insights that later seem intuitive are often far from apparent when first observed. After nearly a decade of work on personalized mRNA cancer vaccines, one of the more surprising observations was that full personalization was not strictly required to achieve meaningful benefit. Even in early clinical studies, when vaccines were not personalized and more generic messenger RNA constructs were used, approximately half the benefit of the fully individualized approach was still observed.

When it was announced that an mRNA vaccine would be deployed globally against COVID-19, a natural question was whether such vaccines might also influence cancer outcomes. That question was informed by prior preclinical work examining how mRNA nanoparticles modulate immune responses in tumor-bearing models. At the time, relatively few groups working in oncology had that specific mechanistic background in mRNA immunobiology, which likely contributed to the signal going largely unrecognized.

The unprecedented global rollout of mRNA vaccines, moving from no oncology exposure to billions of administered doses within two years, created a unique opportunity to evaluate this interaction at scale. Now that both mechanistic data and retrospective clinical signals have been demonstrated, broader investigation seems increasingly warranted.

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Q Your data indicates a survival benefit when vaccination occurs within 100 days of initiating immunotherapy. If biologically meaningful, what might this timing effect suggest about how modifiable the antitumor immune response remains after treatment begins?

AG The observation that mRNA vaccination may modulate the tumor microenvironment is central to the hypothesis. Immune checkpoint blockade is currently the most broadly applicable immunotherapy strategy, but its efficacy depends on the presence, or inducibility, of tumor-specific T cell responses.

If a safe intervention can provide an early priming signal within the tumor, effectively initiating immune activation at a critical window, it may enhance the subsequent activity of checkpoint inhibitors. In that context, timing becomes biologically relevant rather than incidental.

Should this effect be confirmed prospectively, the implications would be significant. Given the existing manufacturing and distribution infrastructure for mRNA vaccines, such an approach could be implemented rapidly and at scale. However, as with the broader findings, randomized validation will be essential before drawing definitive conclusions.

Q Should oncologists be thinking about vaccine timing as part of treatment optimization, similar to how steroid or antibiotic exposure is considered?

AG It is a reasonable question for oncologists to consider, but the field is not yet at a point where timing should influence routine decision-making. The evidence linking corticosteroid exposure to altered immunotherapy outcomes is supported by multiple analyses across clinical settings. In contrast, the present findings derive from a single retrospective study, albeit one strengthened by mechanistic data.

The biologic rationale is credible, and the signal warrants prospective evaluation. However, integration of vaccine timing into clinical algorithms would be premature without confirmation from randomized Phase 3 trials.

Q Safety concerns often dominate discussions about combining immune stimuli. Did your data challenge the assumption that increased immune activation inevitably leads to increased toxicity?

AG The working assumption prior to this study was that immune activation and toxicity would be closely linked, that increasing one would inevitably increase the other. Historically, efforts to maximize antitumor immunity have often involved treatments associated with significant toxicity. That experience shaped expectations going into this work. What the COVID-19 mRNA vaccine experience suggests, however, is that immune activation and toxicity may not be inseparable.

In preclinical models, meaningful antitumor immune responses were observed without exceeding acceptable toxicity thresholds. The clinical safety profile of COVID-19 mRNA vaccines further supports the possibility that a therapeutic 'Goldilocks' window may exist in which sufficient immune stimulation initiates tumor priming without triggering excessive systemic toxicity.

Substantial work remains to define where that balance lies and how best to optimize it. However, the combination of strong safety data and measurable antitumor immune activation provides a compelling rationale for further investigation.

Q Do these findings strengthen the case for mRNA platforms as programmable immune adjuvants rather than purely antigen delivery vehicles?

AG Much of the work in cancer vaccines has focused on programmable mRNA platforms as personalized therapeutics. These findings do not conflict with that approach. If anything, they suggest that different mRNA strategies may ultimately be paired in complementary ways.

Personalized mRNA vaccines require significant manufacturing time and expense. In practice, they are often deployed after surgery: patients receive initial therapy, undergo resection, and then wait several weeks for a tailored product to be generated. Depending on the platform, that interval can extend to two months or longer. During that period, treatment decisions are already being made.

That gap creates an opportunity for universal, off-the-shelf mRNA constructs. Administered immediately prior to initiation of immune checkpoint blockade, such agents could prime the tumor microenvironment and initiate immune activation while personalized vaccines are still in development.

Over time, a combinatorial strategy may prove most effective: early immune priming with a universal construct, followed by antigen-specific reinforcement once a personalized vaccine becomes available. Prospective trials will be necessary to determine how best to integrate these approaches in clinical practice.

Q Would you support prospective trials intentionally pairing vaccination with immunotherapy?

AG Absolutely. A national randomized Phase 3 study is now being launched in the United States to evaluate the addition of COVID-19 mRNA vaccination to standard-of-care immunotherapy in patients with lung cancer. The goal is to determine, under controlled conditions, whether the associations observed in retrospective analyses translate into a measurable clinical benefit.

Enrollment is being prioritized so that the data can be read out efficiently. Only through prospective validation will it be possible to define the magnitude of any effect and determine whether this approach should influence routine practice.

Q In many oncology studies conducted during the pandemic, COVID-19 vaccination was included as a covariate rather than a variable of mechanistic interest. Does your work suggest it warrants closer scrutiny?

AG It is important to note that many investigators appropriately included COVID-19 mRNA vaccination as a variable to control for during the

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pandemic. That was the correct instinct. What this study suggests, however, is that vaccination may not simply function as background noise. Its biological impact may be greater than initially appreciated.

The implications extend beyond viewing vaccination as a potential therapeutic adjunct. They also affect how clinical trial outcomes from the pandemic era are interpreted. In studies comparing immunotherapy to non-immunotherapy regimens, conducted during periods of widespread vaccination, it is reasonable to ask whether some portion of observed benefit could reflect interaction effects rather than monotherapy alone.

This does not imply that prior conclusions are invalid. It does suggest, however, that COVID-19 mRNA vaccination should be explicitly accounted for when interpreting oncology datasets from that period.

Q What is the most likely way this work could be misinterpreted by the field and what would you caution clinicians against concluding at this stage?

AG The primary risk is overinterpretation. Retrospective associations, even when supported by mechanistic data, do not establish causality. It would be premature to conclude that mRNA vaccination definitively improves cancer outcomes outside of a randomized clinical trial.

There has already been substantial interest from both clinicians and patients seeking to apply these findings immediately. While COVID-19 vaccination remains appropriate and recommended for many patients with cancer, it is important not to overstate its potential oncologic benefit. The magnitude, and even the existence, of any survival advantage must be confirmed prospectively.

At the same time, the data are encouraging. They suggest that mRNA platforms may be capable of modulating antitumor immunity in ways that were not originally anticipated. It is equally important to remember that COVID-19 vaccines were not designed as cancer therapeutics. In parallel laboratory work, next-generation RNA constructs optimized specifically for antitumor immune activation have demonstrated substantially greater potency in preclinical systems. Advancing those candidates into rigorous clinical testing is a priority.

Enthusiasm is warranted, but it must be matched with careful validation.

REFERENCE

1. Gripping AJ, *et al.* SARS-CoV-2 mRNA vaccines sensitize tumours to immune checkpoint blockade. *Nature* 2025; 647, 488–497.

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INTERVIEW

TOPIC FOCUS • Formulation and Delivery

Preclinical & clinical

Reimagining vaccine delivery with oral tablet immunization

Sean Tucker

INTERVIEW

"we built an adjuvant system where a double-stranded RNA hairpin is co-expressed along with the protein of interest to generate a strong immune response."

Charlotte Barker, Editor, *Vaccine Insights*, speaks with Sean Tucker, Founder and Chief Scientific Officer, Vaxart, about the scientific and translational journey behind the company's oral tablet vaccines and the use of controlled human infection models to refine immune correlates.

Vaccine Insights 2026; 5(2), 76–80 · DOI: 10.18609/vac.2026.016

Q What led you to focus your work on oral vaccines?

ST I started out working in cell and gene therapies. My group was working on delivering a gene therapy to the intestine in tablet form and one day a colleague

said, 'If you could do something like this for vaccines, it would be the holy grail.' Like most people, I don't enjoy standing in line or getting stuck with needles, so that struck an immediate chord. I wanted to build a technology where the vaccine could come to you. That is what we focused on at Vaxart – not just creating oral vaccines, but making them in tablet form.

Q What are the greatest roadblocks oral vaccines face on the path to the clinic and how does Vaxart's approach aim to overcome them?

ST The first problem is that if you put protein into your intestine, your body treats it as food. So we built an adjuvant system where a double-stranded RNA hairpin is co-expressed along with the protein of interest to generate a strong immune response.

The other issue is that animal models are not always predictive of how oral vaccines behave in humans, leading to many previous oral vaccines failing during clinical translation. It took us a while to overcome that barrier, but we have now been able to move our vaccine indications into the clinic, with a number of successful clinical trials completed.

At this stage, I think we have a pretty good handle on the platform. The challenges we are facing now are indication-specific rather than platform-related.

Q Have there been any surprises along the way?

ST We expected that protective immune responses from oral vaccines may not just be serum antibodies, but we were surprised by how strong a role mucosal immunity plays. In a human challenge study, we compared our oral influenza vaccine to a market-leading injected vaccine, as well as to placebo, and found that the oral vaccine did just as well or better than the injected vaccine. When we looked more closely at the immunological data, serum antibody response was much lower in the oral vaccine group; however, the oral vaccine was able to induce mucosal antibodies in the nose.

Subsequent modeling suggested that a modest mucosal response is just as good as a really strong serum response. We were surprised at just how much protection a small mucosal response could provide compared to very high serum antibodies.

Q What are the benefits and challenges of a controlled human infection study, compared with field efficacy trials?

ST The key thing about challenge studies is that when the virus is given to a person, we know exactly when they got it, and we can monitor the virus very closely thereafter. We can also study the immune responses before and after vaccination in detail.

We have carried out machine learning and other computational analyses to drill down into which immune responses correlate most strongly with protection. In our recent study on norovirus, we found that fecal antibodies were among the most important parameters for improved protection, alongside a functional serum response. For norovirus, which enters through the mouth and infects the intestine, it makes sense that having antibodies in the intestine would be protective.

"I think we will see advances where researchers can track cells moving from the intestine to the nose or throat, through lymphatic or circulatory routes."

We have had to develop robust and reproducible techniques to measure these mucosal responses, especially for samples like feces, which have a lot of variability. It took us a while to standardize these methods to ensure that we could get samples processed quickly and consistently. This has been important for generating reliable data.

Q Are there misconceptions you frequently encounter about your approach?

ST I think there are two main misconceptions. One is that serum antibodies tell you everything you need to know about protection. Our studies in norovirus and influenza suggest that mucosal responses can be just as important.

The second misconception is that, due to the uncleanliness of the intestine, oral vaccines will not get recognized properly and fewer people will respond to them. We have shown that our vaccine can be just as protective as an injected vaccine, which went a long way toward dispelling that notion.

Q What are some of the most pressing scientific questions for mucosal vaccines over the next 5 years?

ST One question is whether the mucosal memory population is different from systemic memory. For example, children are exposed to multiple coronaviruses over their lifetime. That creates a memory pool that may be different to that in the blood because the memory cells are not necessarily present outside the mucosa.

There is also growing recognition that immune cells are shared between mucosal sites, but it is not clear how that occurs. I think we will see advances where researchers can track cells moving from the intestine to the nose or throat, through lymphatic or circulatory routes. Understanding mucosal memory populations and cell trafficking will be important areas of focus.

Q What one technological or methodological advance would most accelerate vaccine development?

ST I would like to see safety testing technologies that allow us to detect contaminants in vaccine products within days rather than weeks.

It would make a huge difference if we could use technologies such as next-generation sequencing or nanopore sequencing to rapidly and confidently detect contaminants, not just for oral vaccines but for all vaccines. It takes a long time to make a vaccine, but it often takes even longer to test it.

Vaccine Insights 2026; 5(2), 76–80 · DOI: 10.18609/vac.2026.008

Q What is next for Vaxart?

ST We have announced a partnership with Dynavax, now part of Sanofi, to move our COVID-19 vaccine into Phase 3 and commercial development.

We are also participating in the BARDA Project NextGen program. While the program has been paused and enrollment ceased, we were able to recruit 5,000 subjects in a study comparing our vaccine to an mRNA vaccine for COVID-19, and we are looking forward to analyzing the results. If our efficacy is comparable and the product is delivered as a tablet, we're confident it will generate strong enthusiasm.

It is an exciting time for Vaxart, as we move steadily closer to commercialization. To date, we have conducted more than 15 clinical trials, and we have made significant improvements to our manufacturing processes in preparation for Phase 3.

BIOGRAPHY

Sean Tucker is the Founder of Vaxart and has served as its Chief Scientific Officer since February 2010. From March 2004 to February 2010, Dr Tucker served as Vice President of Research and Director of Immunology. He has held numerous scientific and engineering roles at various biotechnology companies. Dr Tucker has a BSc in Chemical Engineering from the University of Washington, an MSc in Chemical Engineering from the University of California, Berkeley, and a PhD in Immunology from the University of Washington.

Sean Tucker PhD, Founder, Vaxart, San Francisco, CA, USA

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INDUSTRY INSIGHTS ♦ MARCH 2026

mRNA milestones, industry restructuring, and regulatory upheavals in a turbulent month for vaccines

FORMULATION AND DELIVERY ♦ CLINICAL TRIALS ♦ MARKET TRENDS ♦ REGULATION AND POLICY

Ashling Cannon
Commissioning Editor, *Vaccine Insights*

Across mid-February to mid-March 2026, the vaccine landscape was marked by significant regulatory activity, major industry restructuring, and continued clinical progress across multiple platforms. The European Medicines Agency issued a positive recommendation for the world's first combination influenza and COVID-19 mRNA vaccine, while the US FDA's vaccine regulatory environment remained unsettled, with the resignation of the Center for Biologics Evaluation and Research director, ongoing legal challenges to childhood vaccine policy changes, and the launch of a new unified adverse event reporting platform. In clinical development, first-in-human and first-in-world trials advanced for a pan-influenza universal vaccine candidate and a personalised paediatric mRNA cancer vaccine, respectively. Market activity was headlined by Moderna's $2.25B settlement with Genevant Sciences and Arbutus Biopharma resolving long-running lipid nanoparticle patent litigation, and the announcement that BioNTech's co-founders will establish an independent mRNA-focused company.

FORMULATION AND DELIVERY

Thermostable fridge-free tetanus and diphtheria vaccine SPVX02 completed Phase 1 and advanced to next stage of clinical development [1]

The UK Health Security Agency (UKHSA) and Stablepharma Ltd announced the completion of Phase 1 trials of SPVX02, a thermostable, fridge-free tetanus and diphtheria vaccine. Evaluation of trial results at UKHSA's Vaccine Development and Evaluation Centre indicated that SPVX02 is effective without refrigeration after storage at 30 °C for 24 months. The vaccine will begin a larger clinical trial in healthy participants, comparing its effectiveness against a licensed European vaccine, with UKHSA continuing to assess immune responses. The program, supported by Innovate UK and the National Institute for Health and Care Research Southampton Clinical Research Facility, aims to address cold-chain barriers to global vaccine distribution, particularly in low-resource and emergency settings.

CLINICAL TRIALS

World-first personalized pediatric mRNA cancer vaccine trial initiated for children with high-grade brain tumors [2]

Providence Therapeutics announced the launch of PaedNEO-VAX, the first multi-site personalised mRNA cancer vaccine trial in children and adolescents with relapsed or refractory high-grade brain tumors, including high-grade glioma, diffuse midline glioma, medulloblastoma, and ependymoma. The Phase 1/2 trial, co-led by the University of Queensland and

World-first personalized pediatric mRNA cancer vaccine trial initiated; **Clinical Trials, page 133.** Credit: iStock.

Bavarian Nordic and Serum Institute of India expand chikungunya vaccine partnership; **Market Trends, page 135.** Credit: iStock.

the South Australian Health and Medical Research Institute, will be conducted across eight pediatric hospitals in Australia. Phase 1 will assess safety and optimal dosing; Phase 2 will evaluate clinical outcomes, including disease progression and survival. Tumor-specific mRNA vaccines will be designed using genome sequencing and manufactured by Southern RNA, with an expected turnaround of approximately 10 weeks from enrolment to dosing. Providence Therapeutics is contributing its INTENT™ lipid nanoparticle delivery platform.

Self-amplifying mRNA COVID-19 vaccine GRT-R910 demonstrated safety and immunogenicity as a booster in Phase 1 study [3]

Phase 1 data were published for GRT-R910, a self-amplifying mRNA (saRNA) vaccine expressing SARS-CoV-2 (D614G) spike protein and T cell epitopes, evaluated as a booster in 48 previously vaccinated or infected healthy adults. Single doses of 3–10 µg were administered across age groups (18–60 years and >60 years). Most participants experienced mild-to-moderate reactions; 17% had severe systemic reactions. Pseudovirus neutralizing antibody geometric mean fold rise responses were durable to Day 181 across most dose groups, with the 3 µg dose performing comparably to or better than higher doses, potentially reflecting higher baseline geometric mean titers in the 6 and 10 µg groups. Findings support saRNA as a viable, dose-efficient booster platform against COVID-19.

MARKET TRENDS

Moderna reached $2.25 billion settlement with Genevant Sciences and Arbutus Biopharma to resolve LNP patent dispute [4]

Moderna reached a global settlement with Genevant Sciences and Arbutus Biopharma on March 3, 2026, resolving all patent-infringement litigation arising from Moderna's use of LNP delivery technology in its COVID-19 vaccines. Under the terms, Moderna will pay $950M upfront in July 2026 and a further $1.3B contingent on the outcome of a pending appellate ruling regarding government contractor immunity under US law. Moderna has consented to a court judgment of infringement and no invalidity on four patents. In return, Genevant grants Moderna a global non-exclusive license to its LNP delivery technology for SM-102-containing mRNA vaccines for infectious disease. Separate Pfizer/BioNTech litigation over the same LNP patents remains ongoing in the USA.

BioNTech co-founders announced plans to establish independent mRNA-focused company, with BioNTech contributing technology in exchange for minority stake [5]

BioNTech announced on March 10, 2026 that co-founders Ugur Sahin and Özlem Türeci will establish an independent biotechnology company focused on next-generation mRNA innovations, with both transitioning out of their current roles at BioNTech by the end of 2026. BioNTech plans to contribute related mRNA rights and technologies to the new company on an arm's length basis in exchange for a minority stake, milestones, and royalties. A binding agreement is expected to be signed by the end of the first half of 2026. BioNTech stated that its existing clinical pipeline, including its COVID-19 vaccine franchise and 15 planned Phase 3 oncology trials, will remain unaffected. Further details

will be communicated following the signing of the binding agreement.

SK bioscience, IDT Biologika, and Vaxxas selected for EU-funded next-generation influenza vaccine development programme [6]

SK bioscience, IDT Biologika, and Vaxxas announced their selection for Phase 1 of a next-generation vaccine development initiative managed by the European Health and Digital Executive Agency on behalf of the European Commission's Health Preparedness and Response Authority. The consortium will develop a seasonal influenza vaccine for older adults and a pandemic influenza vaccine using Vaxxas' high-density microarray patch (HD-MAP) needle-free delivery technology. HaDEA will provide up to €12.9M to support Phase 1, including a Phase 1 clinical trial, as part of a broader framework valued at up to €225M across three contracts. HD-MAP vaccines are designed to achieve comparable immunogenicity with lower antigen doses and offer room-temperature stability. IDT Biologika will serve as project lead and European manufacturing hub.

Bavarian Nordic transferred chikungunya vaccine manufacturing to Serum Institute of India to scale supply for low- and middle-income countries [7]

Bavarian Nordic announced an expansion of its strategic partnership with Serum Institute of India (SII) to include a contract manufacturing agreement covering a full technology transfer of the manufacturing process for its chikungunya virus-like particle (VLP) vaccine, CHIKV VLP (Vimkunya®), to enable future supply to endemic low- and middle-income countries. The agreement replaces a previously entered arrangement with Biological E. Limited and builds on an existing mpox vaccine license and manufacturing agreement with SII. The expanded collaboration also provides for potential future co-development opportunities. CHIKV VLP is a single-dose, adjuvanted recombinant protein vaccine approved by the US FDA, European Commission, and Medicines and Healthcare products Regulatory Agency in 2025, with regulatory review ongoing in Switzerland and Canada.

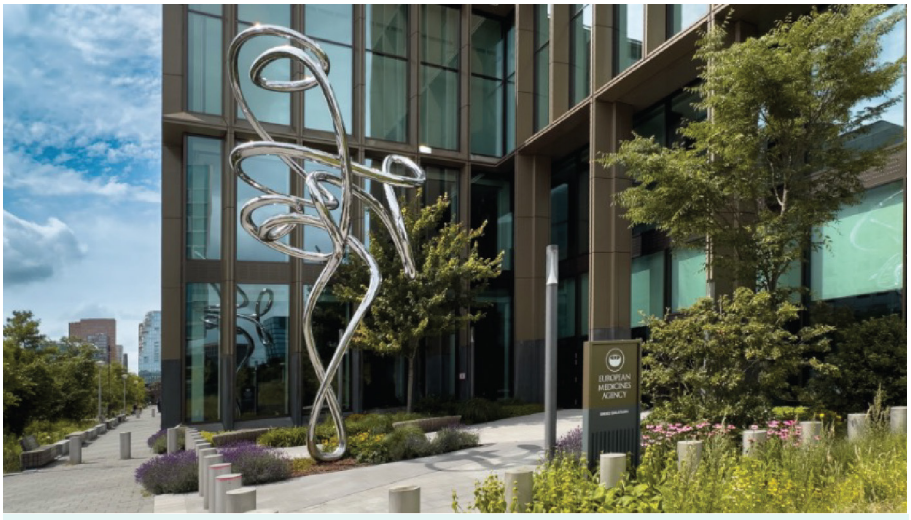
CK Life Sciences established Sequencio Therapeutics to consolidate therapeutic cancer vaccine portfolio [8]

CK Life Sciences announced the establishment of Sequencio Therapeutics Company Limited, a wholly-owned subsidiary dedicated to advancing its therapeutic cancer vaccine pipeline. Sequencio's preclinical portfolio includes vaccine candidates targeting Trophoblast Cell Surface Antigen 2 (TROP2), which demonstrated robust T cell immune responses and tumor growth inhibition in preclinical breast and colorectal cancer mouse studies, alongside candidates targeting preferentially expressed antigen in melanoma (PRAME), programmed cell death ligand 1 (PD-L1), B7 homolog 3 (B7-H3), and claudin 6. Vaccine discovery and design will be conducted in-house, with development advanced through internal capabilities and external collaborations. The subsidiary was established to consolidate CK Life Sciences' therapeutic cancer vaccine R&D under a dedicated organization.

REGULATION AND POLICY

European Medicines Agency issued positive recommendation for Moderna's combination mRNA flu/COVID-19 vaccine [9]

On February 27, 2026, the European Medicines Agency (EMA) issued a positive recommendation for



EMA issues positive recommendation for world-first combination flu/COVID-19 mRNA vaccine; **Regulation and Policy, page 135.** Credit: (European Medicines Agency).

Multistate lawsuit challenges removal of universal childhood vaccine recommendations; **Regulation and Policy, page 136.** Credit: iStock.

approval of Moderna's combination influenza and COVID-19 mRNA vaccine, mCombriax, for adults aged 50 years and older – making it the world's first combination flu/COVID vaccine to receive a regulatory recommendation. The EMA's decision diverges from the FDA's position; Moderna's application for the equivalent product (mRNA-1073) in the US has faced a more challenging regulatory path under the current administration. Official clearance for use in the EU is subject to ratification by the European Commission.

VRBPAC unanimously recommended viral strains for the 2026–27 influenza season at first meeting under current US administration [10]

The US FDA's Vaccine and Related Biological Products Advisory Committee (VRBPAC) unanimously recommended viral strains for the 2026–27 influenza season at a meeting held on March 12, 2026 – the first such meeting under the current administration. The meeting took place against a backdrop of disruption to the US vaccine regulatory advisory process, including the cancellation of an Advisory Committee on Immunization Practices meeting scheduled for March 18–19.

Coalition of 14 state attorneys general and Governor of Pennsylvania filed lawsuit challenging CDC Decision Memo that removed universal recommendation status from seven childhood vaccines [11]

A multistate lawsuit (State of Arizona et al. v Kennedy et al.) was filed on February 24, 2026 by 14 state attorneys general and the Governor of Pennsylvania in the US District Court for the Northern District of California, challenging the January 5, 2026 CDC Decision Memo that stripped seven childhood vaccines (protecting against rotavirus, meningococcal disease, hepatitis A, hepatitis B, influenza, COVID-19, and RSV) of their universally recommended status. The lawsuit also challenges Secretary Kennedy's replacement of Advisory Committee on Immunization Practices members. Plaintiffs argue both actions violated the Federal Advisory Committee Act, which requires advisory committees to be balanced in membership and to follow transparent appointment processes. The coalition is seeking declaratory and injunctive relief to have both the new schedule and appointments declared unlawful and set aside.

FDA CBER director Vinay Prasad resigned [12]

On 6 March 2026, Dr Vinay Prasad, Director of the FDA's Center for Biologics Evaluation and Research (CBER), the division responsible for approving vaccines, blood products, and cell and gene therapies, announced his resignation after a short tenure, with his departure effective at the end of April. Prasad's tenure had been marked by controversy, including disputes over CBER's handling of Moderna's mRNA influenza vaccine application and disagreements over the regulatory path for gene therapies and rare disease drugs. His departure adds to ongoing uncertainty surrounding the FDA's vaccine regulatory leadership.

US FDA launched unified Adverse Event Monitoring System to replace fragmented legacy reporting platforms [13]

The US FDA launched the Adverse Event Monitoring System (AEMS), a unified platform consolidating adverse event reporting across drugs, biologics, vaccines, cosmetics, and animal food into a single dashboard. AEMS replaces seven legacy databases, including the FDA Adverse Event Reporting System and the Vaccine Adverse Event Reporting System. By end of May 2026, AEMS will contain real-time adverse event reports for all US FDA-regulated products, replacing quarterly publication. The agency projects savings of approximately $120M over 5 years. Historical adverse event data will be migrated to AEMS, and enhanced application program interfaces and data analytics tools will be rolled out in the coming months.

EMA's Pharmacovigilance Risk Assessment Committee recommended update to Ixchiq product information following aseptic meningitis cases in healthy young adults [14]

The European Medicines Agency's Pharmacovigilance Risk Assessment Committee (PRAC) completed a

review of a safety signal for aseptic meningitis with Ixchiq, a live attenuated chikungunya vaccine, and recommended an update to the vaccine's product information. The review was triggered by a reported case of aseptic meningitis in a healthy young adult following vaccination. While aseptic meningitis is already listed as a known side effect of Ixchiq at unknown frequency, the product information will now be updated to reflect that serious neurological side effects have also been observed in healthy young adults, not only in individuals aged over 65 years or those with multiple long-term medical conditions. A 6-monthly periodic safety update report assessment is ongoing and will conclude in June 2026.

Ashling Cannon, Commissioning Editor of *Vaccine Insights*, works with leading scientists and industry experts to develop high-impact, open-access content across vaccine R&D and manufacturing. She holds a BSc (Hons) in Biological Sciences and an MSc in Wild Animal Biology, with experience in biotechnology and clinical research publishing across journals and books.

REFERENCES

1. Gov UK. UKHSA and Stablepharma highlight breakthrough with fridge-free tetanus and diphtheria vaccine. 2026; Gov UK Press Release.
2. Providence Therapeutics. Providence Therapeutics Announces World-First Personalized Pediatric mRNA Cancer Vaccine Trial. 2026; Providence Therapeutics Press Release.
3. Whitaker JA, Rebolledo PA, Abate G, *et al*. The safety, reactogenicity, and immunogenicity of the self-amplifying mRNA COVID-19 vaccine GRT-R910 as a booster in healthy adults. *Elsevier* 2026; 77, 128358.
4. Moderna. Moderna Resolves Global Patent Litigation with Arbutus/Genevant. March 3, 2026.
5. BioNTech. BioNTech and Co-Founders Announce Plan to Pursue Next-Generation mRNA Innovations in Co-Founders-Led New Company as BioNTech Advances Toward Becoming a Multi-Product Company by 2030. March 10, 2026.
6. SK Bioscience. SK bioscience, IDT Biologika and Vaxxas Selected for Next-Generation Influenza Vaccine development Financed by the European Union. February 26, 2026.
7. Bavarian Nordic. Bavarian Nordic and Serum Institute of India expand strategic partnership with chikungunya vaccine manufacturing agreement. March 11, 2026.
8. CK Life Sciences. Voluntary announcement relating to establishment of Sequencio Therapeutics Company Limited to advance therapeutic cancer vaccines development. March 10, 2026.
9. European Medicines Agency. mCombriax. February 27, 2026.
10. FDA. Influenza Vaccine Composition for the 2026-2027 U*.S. Influenza Season.* March 12, 2026.
11. State of California Department of Justice. Attorney General Bonta Co-Leads Multistate Lawsuit to Block Trump Administration's Unlawful Overhaul to Childhood Vaccine Schedule. March 5, 2026.
12. Choudhury K and Erman M. FDA vaccines chief Vinay Prasad to leave regulator in April. *Reuters.* March 6, 2026.
13. FDA. FDA Launches New Adverse Event Look-Up Tool. March 11, 2026.
14. European Medicine Agency. PRAC warns about known risk of aseptic meningitis with chikungunya vaccine Ixchiq. March 13, 2026.

INTERVIEW

Meet the *Vaccine Insights* Senior Editor: Ingrid Kromann

The Coalition for Epidemic Preparedness Innovations (CEPI) Process Development and Manufacturing Division supports vaccine development, advances innovative technologies, and strengthens manufacturing and supply chain capacity to enable rapid and equitable vaccine access. Ingrid is a chemical engineer with more than 30 years of vaccine development experience from her tenure at Statens Serum Institut in Denmark, where she led the Vaccine Development Department. She has overseen the development of more than ten vaccine candidates from research through clinical trials, primarily in Tuberculosis, Polio, and Chlamydia, with two products reaching the market.

INTERVIEW

Ingrid Kromann, Senior Advisor, Process Development and Manufacturing, CEPI

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, speaks with *Vaccine Insights* Senior Editor Ingrid Kromann, Senior Advisor, Process Development and Manufacturing, CEPI, to gain insights on the scientific, operational, and policy challenges shaping the future of outbreak preparedness.

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Q How did you first get involved in vaccine development?

IK I am a chemical engineer by background. It was serendipity that I ended up working with vaccines. My focus was protein chemistry, particularly how to purify an antigen, and by coincidence that antigen happened to be a vaccine antigen. But once I began working on vaccines, I found it extremely meaningful and a great way to combine

"Standardized manufacturing platforms can bring us closer to meeting the 100 Days Mission, but regulatory approval remains a major barrier."

technical protein chemistry with health solutions. My first job was in a research unit, developing downstream solutions to produce an acellular pertussis vaccine. The job came with a lot of freedom to operate and a lot of trust in the engineers, biochemists and biologics to come up with proposals that were operational and kept the product in sight.

Q What motivates you in your current role at CEPI?

IK I still find vaccines fascinating, especially how the immune system works together. For example, there is currently a lot of exciting research on how the gut and its microbiome interact with the immune system.

Then there is the question of how you can influence these processes using relatively simple molecules. You realize that by formulating something slightly differently, you can influence the immune response in another direction. I find that interconnection very interesting.

I also appreciate the immense impact vaccines have. Vaccines are widely recognized as one of the medical advances that have had the greatest impact on global health and it is important to me to be part of that.

Over time, I have also realized that it matters to me to work in environments where no one earns money directly from my work. I appreciated working in a governmental institute and now in a non-profit organization. Companies have an important part to play, but I find it motivating to work somewhere where profit is not the main driver.

Q Looking back across your career, what have been the biggest inflection points for you?

IK When I look back across my career, a few moments stand out as true inflection points. One of the most formative came during the restructuring of the Statens Serum Institut. The organization was split in two – one part transitioning to a private manufacturing company, and a more R&D oriented part remaining within the Danish government, where I stayed. Having worked across both areas, I suddenly became a bridge between two newly separated entities. It was a demanding period, but also an eyeopening one.

That experience pushed me to step forward in ways I hadn't before. I realized that the knowledge I carried mattered – and that I had a responsibility to use my voice. There is a moment in your career when you understand that there is no 'grownup in the room' who will magically appear to make decisions. You are that person. That shift in mindset changed how I worked and how I saw myself professionally.

Another major turning point was joining CEPI. Overnight, I found myself collaborating with colleagues from 54 different nationalities. The exposure to such a diverse range of perspectives profoundly shaped my understanding of politics and global health. It broadened my worldview in a way that would have been impossible in a more homogeneous environment.

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Q How much experience of the political dimension of global health did you have when you moved to CEPI?

IK Before CEPI, I had spent my career in government, where – at least earlier on – the political layer rarely touched my daytoday work. You answered to a ministry, of course, but the political dimension felt distant. At CEPI, it became much more visible, also because of the level at which I now operate. You quickly learn that science and policy are inseparable; decisions in one domain reverberate across the other.

Even so, it is the science that continues to energize me. A scientific meeting leaves me more inspired and motivated than any political driven meeting ever will. That remains a constant throughout all those inflection points: the science is what brought me into this field, and it is still what drives me today.

Q Given CEPI's role as a funder, convener, and strategic partner, what insights have you gained about collaboration across industry, academia, and global health organizations?

IK Collaboration across sectors is strong, which is encouraging. What has stood out most is the rapid pace and volatility of the biotechnology landscape – companies emerge and disappear quickly, and academic institutions are also facing increasing pressures.

Strong scientific foundations are essential, but they are not sufficient on their own. Effective global networks are critical for translating scientific advances into realworld products. I have also seen how impactful CEPI funding can be for smaller companies – not only through financial support but through the credibility and visibility it provides, which often helps them attract further investment.

Equity and access remain central to CEPI's mission. Our teams engage directly with regions around the world to understand their needs rather than relying on assumptions.

Q What are the most persistent bottlenecks in development and manufacturing for outbreak vaccines in the context of the 100 Days Mission?

IK From CEPI's perspective, the challenge is not only technical – the financial sustainability of manufacturing facilities is of essence. These facilities must remain viable between outbreaks, and atrisk investments are needed to advance technologies and ensure readiness when crises arise.

Platforms are essential to achieving the 100 Days Mission. We need a more standardized approach to developing and prioritizing the platforms that can deliver this. Researchers will always introduce new technologies – that is their role – but in product development we must prioritize and invest in flexible platforms capable of supporting multiple products, even as new innovations emerge.

Standardized manufacturing platforms can bring us closer to meeting the 100 Days Mission, but regulatory approval remains a major barrier. Without regulatory endorsement of platform technologies, the target will be difficult to meet.

I also continue to emphasize the importance of release assays. Without robust assays, a final product cannot be delivered. We have seen vaccine candidates produced rapidly,

only to face delays of several weeks because release assays were not ready. Assay development must be integrated into plans from the outset.

Q What other technological developments do you see as important for outbreak preparedness?

IK AI has significant potential to strengthen outbreak preparedness. While machine learning tools have existed for decades, they remain underutilized in vaccine development and manufacturing. Integrating these capabilities into development and production workflows could accelerate timelines and improve efficiency. However, with AI becoming a buzzword, focused and purposeful application is essential.

Some companies are also exploring whether personalized medicine approaches could inform future vaccine production. One emerging concept is transportable manufacturing systems that could operate at or near vaccination sites. Although still conceptual, such technologies could ultimately expand global access.

Q In outbreak response, which operational challenges remain underestimated?

IK Agreements are critical. This includes developer–manufacturer agreements, import licenses, and sponsor agreements. These processes can take considerable time.

There is a useful case study from Rwanda that is often discussed within CEPI. Teams conducted an exercise in the country to identify gaps in implementing the 100 Days Mission. Two weeks later, Rwanda experienced an outbreak of Marburg virus. They were able to respond within 4 days, not due to any technological advance, but because the agreements and procedures had already been discussed.

Establishing agreements before an outbreak occurs is essential. If those agreements do not exist, it becomes extremely difficult to respond quickly.

Q What is top of your wish list for the next decade of vaccine policy?

IK Development of platform technologies and raw materials that support locally based, lowcost vaccine production is critical. I would like to see more independent vaccine manufacturers in Africa, backed by strong supply chains and sustainable production capacity. For longterm viability, these facilities must produce routine immunizations in addition to outbreak vaccines; otherwise, they will not remain economically sustainable.

Q Looking back on your career, what are you most proud of?

IK I contributed to the development of two products that reached the market: a diagnostic skin test for TB that recently received European approval, and a reduceddose polio vaccine adopted as a UNICEFlisted product.

I am also proud of my role in COVAX during the COVID-19 pandemic, where I cochaired the manufacturing team with David Robinson of the Gates Foundation. Together, we

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convened global workshops for vaccine developers, creating a forum to surface challenges and address them collaboratively.

Q Finally, what advice would you give to people entering the vaccine development field today?

IK I encourage younger professionals to seek international experience early in their careers, particularly if they come from smaller countries. I also advise against overly rigid career planning. Rather than setting fixed expectations for what you should be doing in a few years, focus on what you do well. Let your strengths guide the direction of your career.

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INTERVIEW

Meet the

Vaccine Insights Editorial Board

Advances in mass spectrometry, glycomics, and structural analysis are increasingly informing vaccine antigen characterization and assessment. Working at the interface of regulatory science, analytical characterization, and immunology, John Cipollo explores how antigen structure and glycosylation relate to immune recognition, viral evolution, and vaccine performance.

INTERVIEW

John Cipollo, Research Chemist & Principal Investigator, Vaccine Structure Group, FDA CBER DBPAP

Vaccine Insights 2026; 5(1), 31–33 · DOI: 10.18609/vac.2026.007

Q What inspired your journey into the vaccines field?

A defining point in my career came when I was recruited to the US FDA to establish a laboratory dedicated to mass spectrometry–based analysis of vaccine antigen structure. Given my background in glycomics, carbohydrate chemistry, proteomics, and glycosylation processes, I was already familiar with the types of molecular questions that sit at the core of vaccinology.

Once I began working at the intersection of antigen structure, host–pathogen interactions, and immune recognition, it quickly became clear that vaccines offered a uniquely rich scientific environment. The inherent complexity of these systems, combined with their direct relevance to public health, drew me in and has kept me engaged throughout my career.

Q What project are you currently most excited about?

At present, a major focus of my work is the study of influenza antigen structure and glycosylation and their roles in viral evolution and immune escape. By studying the structural

and glycan-mediated mechanisms through which influenza viruses adapt, we can gain important insights into how to design vaccines with greater breadth and durability.

This work remains highly relevant given the continued global burden of influenza and the constant need to anticipate and respond to viral change. Understanding these molecular dynamics is essential if we are to improve the long-term effectiveness of influenza vaccines.

Q What developments do you expect to shape the vaccines field in the next 5 years?

Several developments are likely to shape the vaccines field over the next 5 years. Among these, AI will increasingly support decision-making throughout vaccine development, from interpreting complex clinical datasets to informing antigen selection and structural design. At the same time, continued refinement of adjuvants will enable more precise modulation of immune responses, offering new opportunities to optimize both efficacy and safety.

mRNA technologies are also likely to expand beyond infectious diseases, particularly in oncology, where rapid design and customization are critical. In parallel, polysaccharide conjugate vaccines continue to evolve in both scope and complexity. A decade ago, the largest of these vaccines contained 13 antigens; today, candidates with more than 30 antigens are under development, reflecting substantial advances in conjugation chemistry and analytical characterization.

Q Why did you join the *Vaccine Insights* Editorial Advisory Board?

Vaccine Insights appealed to me because it consistently engages with the practical scientific questions that sit between discovery, manufacturing, and regulation. The journal provides a space where analytical rigor, CMC considerations, and clinical relevance can be discussed together, rather than in isolation.

Given my experience working across research, regulatory review, and manufacturing oversight, joining the Editorial Advisory Board offered an opportunity to contribute to those conversations and help ensure that complex vaccine science is communicated in a way that is both technically sound and directly relevant to decision-making across the field.

BIOGRAPHY

John Cipollo earned his PhD at State University of New York at Albany in 2000. He served as Research Assistant Professor at Boston University from 2005–2007 and as a Principal Investigator and CMC reviewer for the US FDA 17 years, primarily focused on bacterial and viral vaccines with major focus on polysaccharide conjugate, subunit and combination vaccines. He has also served as Team Lead and Senior Principal Scientist for US Pharmacopeia's Vaccine Pipeline. His experience in vaccine CMC spans a range of vaccine types including mRNA, viral vector, attenuated, split virus, polysaccharide and polysaccharide conjugate, combination, and subunit types. Dr Cipollo's research efforts have focused on mass

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spectrometry and related chemistries-based approaches for the study of polysaccharide conjugates, viral antigens and their interaction with the innate immune system. He takes a structure–function approach to investigate how antigens interact with the host immune system, especially where glycosylation is a major component of these interactions. His group also focuses on development of in-house informatics for mass spectrometry data analysis and structural assignment. Dr Cipollo has published more than 70 peer reviewed manuscripts, the majority of which focus on vaccines and related topics. He has served on editorial boards and guidelines panels for WHO and as government liaison for US Pharmacopeia

John Cipollo PhD, Research Chemist & Principal Investigator, Vaccine Structure Group, FDA CBER DBPAP, USA

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INTERVIEW

Meet the *Vaccine Insights* Editorial Board

Cristiana Campa examines how advances in analytical characterization and risk-based regulatory frameworks are redefining vaccine development and manufacturing. Drawing on her work revising the ICH Q6 guideline on specifications, she discusses the role of harmonized, science-driven standards in supporting vaccine quality, global development pathways, and emerging modalities such as therapeutic and mRNA-based vaccines.

INTERVIEW

Cristiana Campa, CMC External Intelligence Lead, Vaccines Technical R&D, MDS, GSK, Italy

Vaccine Insights 2026; 5(1), 35–37 · DOI: 10.18609/vac.2026.008

Q What inspired your journey into the vaccines field?

Exposure to glycobiology research during my postdoctoral training highlighted the transformative impact of glycoconjugate vaccines on bacterial infectious diseases. Seeing how advances in polysaccharide chemistry and conjugation science translated into tangible public health gains highlighted the role that analytical characterization could play in supporting vaccine quality and development.

A few years later, this realization led me to join Novartis Vaccines, marking the beginning of a career focused on strengthening the analytical and quality frameworks that underpin safe, effective vaccine manufacturing.

Q What project are you currently most excited about?

As a member of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Expert Working Group, a major focus of my current

work is the revision of the ICH Q6 guideline on specifications. This initiative aims to modernize the guideline by reinforcing science- and risk-based decision-making and by explicitly expanding its scope to include vaccines.

As outlined in the Concept Paper, the revision is intended to harmonize global regulatory expectations, improve transparency, and provide clearer scientific justification for specifications. It is a substantial and complex undertaking, but one with the potential to have a lasting impact on how vaccines are developed, assessed, and regulated worldwide.

Q What developments do you expect to shape the field in the coming years?

I predict continued progress in the adoption of science- and risk-based approaches to vaccine specifications, particularly as part of the ICH Q6 modernization. Model-informed development is likely to play an increasingly important role by strengthening the links between nonclinical data, clinical outcomes, and CMC justifications.

The expanded use of platform knowledge and platform analytics should also help accelerate development and streamline regulatory pathways. In addition, I anticipate broader acceptance of stability modelling to support shelf-life determination and temperature-excursion strategies. Beyond these methodological advances, I am encouraged by the growing momentum behind therapeutic vaccines, including mRNA-based approaches, which are opening new possibilities for both disease prevention and treatment.

Q Why did you join the *Vaccine Insights* Editorial Advisory Board?

I was drawn to *Vaccine Insights* because of its multidisciplinary scope and its commitment to addressing topics that span research, manufacturing, and regulatory science. The journal has consistently demonstrated an ability to identify emerging trends and present them in a way that resonates across the vaccine community.

I value the opportunity to contribute to a publication that allows complex scientific and regulatory topics to be explored in depth, while still being readable and relevant to a broad vaccine audience, and I am honored to support the journal's mission by helping to guide its content and direction.

BIOGRAPHY

Cristiana Campa has more than 20 years' experience in Chemistry, Manufacturing and Control for biologics research and development. She is actively promoting dialogue across industry and with Regulatory Agencies on several topics, including innovative technologies, specifications setting, stability, accelerated development strategies, and pandemic preparedness. Since 2023, she is a member of the PDA Board of Directors, and, since 2024, she is the EFPIA lead in the ICH Expert Working Group for ICH Q6 (specifications) Guideline revision, co-chair of the PDA Vaccine Interest Group, and chair of the Vaccines Europe/ IFPMA CMC Adaptive Pathways team (former COVAX support team). In 2025, she joined the US Pharmacopoeia Vaccines Expert Committee (2025–2030 cycle). After her PhD and Post-Doc in Chemistry, Cristiana worked at Bracco Imaging SpA, first as a senior

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researcher and then as head of the research laboratory in Trieste, Italy. She joined Novartis Vaccines in 2006, first as Analytical Senior Manager and subsequently as Head of Analytical Development, Italy. After acquisition of Novartis Vaccines by GSK in 2015, she has been the Head of Quality by Design Integration and, until June 2018, the Head of Science and Development Practices in Global Technical R&D, covering Quality by Design, Knowledge Management and Development roadmaps; until February 2025, she worked as a Global Vaccines Technical R&D Advisor, GSK.

Cristiana Campa, CMC External Intelligence Lead, Vaccines Technical R&D, GSK, Tuscany, Italy

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INTERVIEW

Meet the *Vaccine Insights* Editorial Board

Matthew Laurens reflects on how early exposure to vaccine-preventable disease in resource-limited settings guided his career in vaccinology, and the ways in which that perspective informs his work on whole-organism malaria vaccines. He discusses the clinical potential of these approaches, the realities of large-scale deployment, and the role of implementation science in advancing global vaccine impact.

INTERVIEW

Matthew Laurens, Professor, University of Maryland School of Medicine, USA

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Q What inspired your journey into the vaccines field?

My interest in vaccines really began during my time with the Peace Corps in Benin. Working in a resource-limited setting, I saw firsthand the devastating impact of diseases that are entirely preventable. What struck me most was the contrast between prevention and treatment. In many of these settings, access to effective care is limited, delayed, or simply unavailable, whereas vaccines offer a proactive way to prevent illness altogether.

That experience fundamentally shaped how I think about medicine and public health. Vaccines represent one of the most effective and equitable interventions available, particularly for populations with limited healthcare infrastructure. That realization ultimately guided my decision to pursue a career focused on vaccine development and global health.

What project are you currently most excited about?

A major focus of my current work is the development of whole-organism malaria vaccines, particularly the PfSPZ-LARC2 approach. Malaria remains a significant global health challenge, and while existing tools have reduced disease burden, they are not sufficient to achieve long-term control or elimination.

Whole-organism vaccines offer a fundamentally different strategy by exposing the immune system to the full breadth of antigens present in the parasite. Clinically, this approach has the potential to induce more durable and comprehensive protection than subunit vaccines. At the same time, it raises important programmatic considerations around delivery, dosing, and implementation. Ensuring that such vaccines can be deployed at scale is central to realizing their public health impact.

Another project that I am working on is to advance typhoid conjugate vaccine (TCV) use in endemic settings. Typhoid fever continues to cause significant morbidity and mortality, particularly in areas with limited water and sanitation infrastructure. TCVs provide single-dose protection to infants, adolescents, and adults. Four countries conducted nationwide TCV campaigns in 2025, vaccinating over 85 million children who are now protected against typhoid.

What developments do you expect to shape the field in the coming years?

I hope to see continued progress toward vaccines that are not only more effective, but also more durable and easier to deliver. Combination vaccines, longer-lasting immunity, and formulations that reduce cold-chain dependence could all have a significant impact on global vaccination efforts.

Equally important is the growing role of implementation science. Understanding how vaccines perform in real-world settings, and how best to integrate them into existing health systems, will be critical. Innovation should not stop at the laboratory or the clinic; it must extend into how vaccines are deployed and sustained over time.

Why did you join the *Vaccine Insights* Editorial Advisory Board?

I joined the Editorial Advisory Board because I value opportunities that bring together perspectives from academia, industry, policy, and global health practice. Vaccine development is inherently multidisciplinary, and progress depends on meaningful dialogue across these sectors.

Vaccine Insights provides a platform for that kind of exchange, helping to bridge scientific discovery with translational and policy considerations. Contributing to those conversations, and supporting the dissemination of high-quality, thoughtful content, aligns closely with my own goals as a clinician–scientist working at the intersection of research and public health.

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BIOGRAPHY

Matthew Laurens is a pediatric infectious disease specialist with a primary research interest in malaria, typhoid fever, and other diseases that disproportionately affect people who live in resource-limited settings. He conducts studies at the Center for Vaccine Development in Baltimore and at international sites in Burkina Faso, Mali, Malawi, and Uganda. The broad goal of Dr Laurens' research is to illuminate the mechanisms of vaccine-induced immunity, with the aim to inform development of vaccines and therapeutics. Dr Laurens directs the Typhoid Vaccine Acceleration Consortium (TyVAC), a partnership between the Center for Vaccine Development and Global Health at the University of Maryland School of Medicine, the Oxford Vaccine Group at the University of Oxford, and PATH.

Matthew Laurens MD, Professor, University of Maryland School of Medicine, Maryland, MD, USA

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