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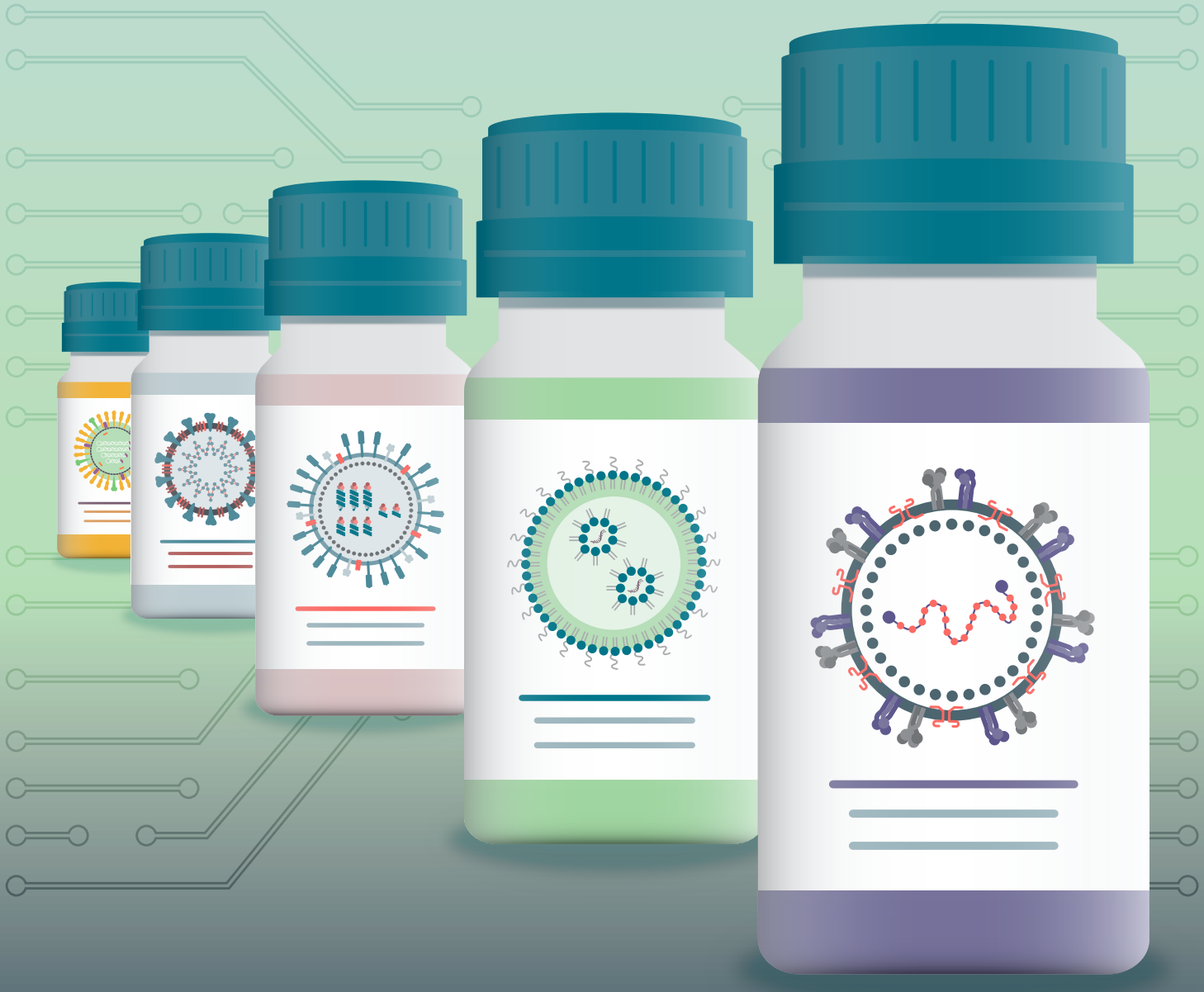
VACCINE INSIGHTS

SPOTLIGHT ON

Manufacturing: downstream, fill/finish, and delivery



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EXPERT INSIGHT

Advanced technologies enabling accessible and sustainable vaccine development and manufacturing for future pandemics

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The unprecedented speed and scale in vaccine development and manufacturing were key factors in ending the acute phase of the COVID-19 pandemic and lowering the rates of illness and death from SARS-CoV-2. Efforts to further accelerate vaccine development are still ongoing, with an increased focus on ensuring equitable access to vaccines and enhancing manufacturing sustainability with and without pandemic-scale demands. Significant advances have been made recently in the knowledge-based rational design of immunogens, the development of novel vaccine modalities, and the expansion of manufacturing capabilities integrated with process analytical technology and digitization. This review highlights innovative technologies and the need for continuous investment toward realizing innovations for the vaccine industry. In addition, alignment with global regulators is discussed to improve the practical implementation of the innovations and better prepare for future pandemics.

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INTRODUCTION

The emergence of novel infectious diseases has been a constant threat in recent years. Pandemics caused by SARS-CoV-1 (2002–2004), Middle East respiratory syndrome (MERS) (2012–current), Western African Ebola virus (2013–2016), and

SARS-CoV-2 (2019–2023) are only a few examples in the last two decades [1–3]. At the time of writing, the outbreaks of monkeypox (mpox) in the Democratic Republic of the Congo are spreading to neighboring countries [4], and the highly pathogenic avian influenza virus is impacting dairy cows in multiple states in the US and raising concerns about the potential transmission to humans [5]. On August 14, 2024, WHO eventually declared the mpox outbreak a public health emergency of international concern [6].

As demonstrated during the COVID-19 pandemic, obtaining marketing authorization for effective vaccines within 1 year of the pandemic being declared was crucial in controlling rapid viral transmission [7,8]. The rapid development of COVID-19 vaccines was possible only because there were decades of research and development efforts for novel technologies, such as mRNA and viral vectors [9–11], and prior experience with vaccine development against SARS-CoV-1, MERS, and respiratory syncytial virus (RSV) [1–3,7,12]. It reminds us again how critical it is to continue investment in vaccine technologies.

In addition to the record speed of vaccine approval, an unprecedented number of COVID-19 vaccine doses were produced and administered in 2021. 12 billion doses of vaccines were forecasted to be manufactured in 2021 by numerous developers [13]. BioNTech-Pfizer reported that 3 billion doses of Comirnaty were manufactured in 2021, within 12 months of the vaccine's initial emergency authorization in December 2020 [14]. AstraZeneca delivered 2 billion doses by November 2021, also within 12 months since its emergency use authorization was received in the UK [15]. About 4 billion doses of COVID-19 vaccines were administered globally by June 2021 [16].

Much investment and many resources were dedicated to COVID-19 vaccine development and manufacturing [14,16]. However, there were numerous challenges and constraints in supply chains, scaling-up and scaling-out of the manufacturing processes worldwide. This led to substantial delays in vaccine manufacturing and the loss of millions of doses due to quality issues [16]. It suggests that there is still a significant need to improve manufacturing processes and control strategies to deliver robust performance, consistent quality of vaccines at different scales and across different manufacturing sites, and better access.

Sustainability has become important an important principle for the vaccine industry, emphasizing the need to produce more vaccine doses while using fewer resources. This includes optimizing the use of raw materials, shrinking the manufacturing footprint, conserving human resources, minimizing energy consumption, and reducing the use of time, water, and other natural resources. It is important to advance vaccine manufacturing capacity globally and enhance vaccine accessibility. It aligns with the framework of Pharma 4.0, which includes the concept of digitization, automation, and artificial intelligence in manufacturing, and still complies with the pharmaceutical regulatory guidance [17–19].

This review article highlights noticeable technical advancements made in recent years, from vaccine design to manufacturing. There are numerous disruptive technologies under development to better prepare vaccine developers and manufacturers for future emergency. It could also be contemplated that the purposeful integration of key innovations could help to realize CEPI's 100 Days Mission [20]. A few examples in this article are taken from therapeutic areas rather than vaccines because those technologies present great potential for the vaccine industry.

IMMUNOGEN DESIGN

The increased understanding of protective immunity and vaccinology has laid a solid foundation for the rationale design of potent immunogens [21]. SARS-CoV-1 could be a good example for vaccinology. The understanding of the viral life-cycle of SARS-CoV-1 and

the role of spike protein (S protein), with its structural evolution on the viral infectivity, led to the finding that the ectodomain of S protein (known as S1) of SARS-CoV-1 was responsible for viral binding and fusion to host cells [22,23]. Especially, the prefusion state of the S protein exposes the receptor binding domain (RBD) and interacts with the cellular receptor (ACE-2) to trigger viral infection [24,25]. Immunization with prefusion S protein or the RBD induces strong protection by eliciting neutralizing antibodies [24,26]. Similarly, the fusion protein (F protein) of RSV causes the viral membrane fusion with target cells and the prefusion F is dominantly subjected to neutralizing antibodies induced by infection [27–29]. As prefusion F is metastable, the market-approved RSV vaccines by GSK (Arexvy®), Pfizer (Abrysvo®), and Moderna (mRESVIA®) all utilize the stabilized prefusion F protein by engineering the sequence [30–32].

The lessons from SARS-CoV-1 S protein and RSV F protein were successfully applied to SARS-CoV-2 vaccine development. SARS-CoV-2 is closely related to SARS-CoV-1 in the Coronaviridae family. The S protein of SARS-CoV-2 is a class 1 fusion protein, the same as the S protein of SARS-CoV-1 and F protein of RSV [25,29]. The vaccine and prophylaxis development against SARS-CoV-2 were initiated with the stabilized prefusion of S protein as immunogens. Some market-approved SARS-CoV-2 vaccines are developed either with their entire prefusion S protein or the RBDs, highly immunogenic in eliciting neutralizing antibodies [33].

More powerful immunogens than the first-generation SARS-CoV-2 vaccines have been developed through structural and sequence-based rational designs. The first-generation mRNA vaccines by BioNTech-Pfizer and Moderna include the full-length S protein with two stabilizing mutations (K986P and V987P) [33]. It was further discovered that six proline substitutions exhibit increased yields and stability of S-protein (HexaPro) [34]. In addition to better manufacturability, the HexaPro induces more S protein-specific serum antibodies, broadly neutralizing antibodies against other SARS-CoV-2 variants and higher cellular immune response in animal models [35].

A similar structure-based engineering strategy was explored with the RBD. Sequence variants of ancestral SARS-CoV-2 RBD presented improved manufacturability, in terms of yields and stability, and also exhibited high-affinity binding to ACE-2 and broad protection against other SARS-CoV-2 variants [36]. There have been continuous efforts to develop broadly protective vaccines to overcome viral escape [27,37–39]. More evidence suggests that heterologous antigen display or chimeric antigen confers a competitive avidity advantage to cross-reactive B cells and, therefore, elicits broad protective immune responses [27,37,40,41].

The accumulated knowledge in engineered immunogen and structural/functional characterization contributes to empowering computational and AI-driven immunogen design for various novel pathogens and facilitates rational design of vaccines rather than empirical approaches [42–46]. AI-generated, lab-tested, and verified immunogen designs are being advanced to develop a vaccine library against 'disease X.' It could be the foundation to respond quickly to an outbreak with any related pathogens from the library [47–49].

WHO defines disease X as "the knowledge that a serious international epidemic could be caused by a pathogen currently unknown to cause human disease." Disease X is a priority pathogen for WHO and CEPI [50,51]. Significant investment has been made in researching various viral families beyond known virulence, including the vaccine library development. It could enable rapid response to a health emergency, by achieving emergency use authorization of vaccines in 100 days from recognition of a pandemic pathogen (CEPI's 100 Days mission) [7,52].

NOVEL MODALITIES

By August 2023, more than 58 COVID-19 vaccines were approved by WHO. These

vaccines are produced in various forms, such as RNAs, viral vectors, protein subunits, or inactivated viruses [12]. mRNA is a non-infectious and non-integrating platform and has proven safe and efficacious during the COVID-19 pandemic. It can also be manufactured rapidly [10].

There are also alternative RNA technologies that could further improve RNA-based vaccines. Self-amplifying RNA (saRNA), trans-amplifying RNA (transRNA) and circular RNA (circRNA) are a few examples garnering interest, which could compensate for some weaknesses of RNA vaccines.

SaRNA contains alphavirus RNA replication machinery, and promotes amplification of RNA containing a gene-of-interest [53]. It is reported that saRNA is up to 100-fold more potent than mRNA; therefore, the saRNA dosage could be lower by two orders of magnitude. It could lower the cost per dose significantly compared to mRNA and potentially relieve the mRNA manufacturing burden [54–56]. A couple of saRNA vaccines already achieved licensure approvals for COVID-19, one in India and one in Japan [57,58].

As saRNA utilizes alphavirus replicon, safety concerns derive from the potential recombination with circulating alphaviruses. The risks appear to be minimal in laboratory experiments but the long-term safety impact needs to be studied carefully from real-world evidence [59].

TransRNA is a novel bipartite RNA system consisting of a vector encoding a gene-of-interest and a second vector delivering the alphavirus RNA replication machinery [60]. It could alleviate manufacturing challenges of saRNA due to its size being larger than mRNA (~10,000 nucleotides (nt) for saRNA and ~1,000 nt for mRNA) [56]. The second vector delivering the replicon could be manufactured separately and used for various RNA vaccines carrying different genes of interest. Cellular translation of a gene-of-interest from transRNA is comparable to saRNA [60].

CircRNA is a covalently closed RNA molecule that lacks 5′, 3′ ends and polyA tails [61]. It exhibits high stability and RNase resistance and, therefore, can be stored at room temperature. It also presents longevity *in vivo* and, thus, prolongs antigen expression [61]. Optimized constructs of circRNA could increase translation *in vivo* by several hundred folds compared to mRNA [62]. The circRNA encoding SARS-CoV-2 RBDs showed superior humoral and cellular protection to mRNA encoding the same antigens in mice [63]. A major challenge in adopting circRNA for vaccines is its low production efficiency and difficulty in purification [64]. Significant manufacturing innovation is still required to promote circRNA application.

Adenoviral vectors, such as type 5 (Ad5) and type 26 (Ad26), have been exploited as vaccines for decades. These adenoviral vector-based vaccines induced robust humoral and cell-mediated immune responses in clinics but also presented mixed clinical outcomes with pre-existing immunity or vaccine-induced thrombosis in a small population who received the vaccines [65,66]. Chimpanzee adenoviral vectors were used to immunize against SARS-CoV-2 infection. Heterologous immunizations with chimpanzee adenovirus type 6 (AdC6) and type 68 (AdC68) vectors presented robust immune responses in mice [67]. A type of chimpanzee adenovirus-vectored SARS-CoV-2 (ChAdOx1 nCoV-19, Vaxzevria) was approved for use and distributed globally with billions of doses [68]. Vaccine-induced thrombosis was also observed in a small population that received ChAdOx1 vaccine [65]. A novel adenoviral vector such as gorilla adenovirus is explored as a vaccine against SARS-CoV-2 in animal models [69].

Virus-like particles (VLP) are noninfectious nanoscale particles composed of self-assembling recombinant proteins. VLPs are safe, highly immunogenic, and versatile vaccine technologies in which various antigens could be incorporated [70–72]. The human papillomavirus vaccine (Gardasil®) and the Hepatitis B virus vaccine (Engerix-B®) are nonenveloped VLPs,

which do not contain lipid membranes [71]. There are enveloped VLP-based vaccines against SARS-CoV-2 currently under development [73]. SpyTag/SpyCatcher-based VLP is used to elicit broad protection against coronaviruses. The affinity tags (SpyTag and Spycatcher) allow conjugation of 60 antigens on the nanoparticle to form either homotypic particles with one antigen or mosaic particles with multiple antigens [40,70].

Novel immunogens can be further developed to vaccines only if they can be manufactured and satisfy quality specifications for the intended use. There have been substantial advancements in technologies leading to better productivity, quality, and control strategies for manufacturing across different modalities. Some of the key technologies are highlighted below.

CODON OPTIMIZATION

Codon optimization has been a critical technology for the recombination of genetic engineering for decades. It promotes the expression of a recombinant gene in a non-native host by reassigning the genetic codes favored by the host but does not alter the amino acid sequence [74–76]. Codon optimality is calculated using algorithms developed with various rationales of codon usage bias in the host cells, which results in increased protein production [74,77].

With increased computational capability, often empowered by machine learning, advanced algorithms for codon optimization have been developed. There are well-established codon optimization algorithms for Chinese hamster ovary (CHO) hosts, highlighting a significant increase in recombinant protein production post codon optimization [78,79]. It is possible that this technology could improve the productivity of difficult-to-express immunogens for vaccine development.

It was reported that codon optimization could influence the secondary structure of mRNA [46,80]. It has been discussed that the secondary structure of mRNA modulates mRNA translation efficiency by adjusting its half-life *in vivo*. The higher-order structure of mRNA is also known to modulate mRNA stability and immunogenicity, which has a significant impact on its application for vaccine development [46,80–85]. Baidu LinearDesign, empowered by artificial intelligence, generated mRNA without modified nucleosides but still yielded comparable or better immunogenicity and stability than the non-codon optimized mRNA with modified nucleosides [46].

DEVELOPMENT OF PROTEIN EXPRESSION SYSTEM

Process development of protein-based vaccines could take many months due to stable cell line development and upstream cell cultivation process, especially when mammalian cell lines are used as expression hosts. Traditionally, random integration of genes-of-interest has been used to develop stable cell lines. It requires a series of painstaking screening rounds of millions of cells to choose the best clone, exhibiting desirable attributes in productivity, product quality, and stability to ensure consistent manufacturing of the protein-of-interest [86,87].

Instead of random integration, targeted integration of genes-of-interest could accelerate cell line development and improve predictability regarding the attributes of stable cell lines [88]. CRISPR/Cas9 is a gene editing tool that can deliver a heterologous gene to selected genome regions in a host cell. However, it can only insert a small gene, is error-prone, and, therefore, appears inadequate for cell line development [89–92]. Alternative gene editing technologies have recently emerged. Cas-CLOVER, a high-fidelity site-specific nuclease, can insert a large gene with high fidelity [89]. Large serine recombinases have presented precise integration of large genes (as big as 10 kb DNA) in human cells [93,94]. These gene editing tools are primarily developed for potential

therapeutic purposes, but the technology could be democratized for vaccine development if proof-of-concept of these technologies can be demonstrated.

Yeast cells, such as *Pichia pastoris* or *Saccharomyces cerevisiae*, are considered attractive hosts to express immunogens because they are safe, cost-effective, and contain post-translational machinery. Therefore, these hosts have been utilized to express various recombinant proteins including vaccines [95]. It was reported that the RBDs of SARS-CoV-2 and multivalent RBDs of various SARS viruses, produced by *P. pastoris*, presented durable immunity against the homologous viruses as well as heterologous viruses in the selected animal models [96–98].

Cell-free protein synthesis is another potential technology for vaccine development. An advanced *Escherichia coli*-based cell-free system has demonstrated protein yields up to several mg/mL, scalability, and versatility in application, as the function of *E. coli* cell-free system can be enhanced by easy redesigning of the cellular machinery [99,100]. Engineered *E. coli* cell-free system, which incorporates non-native amino acids to the target protein, have successfully progressed antibody-drug conjugate candidates to clinical phases [101].

Although eukaryotic cells have the advantage over *E. coli* of having cellular machinery generating relevant post-translational modifications, the application of their cell-free systems has been limited mainly due to the poor yields and scalability. A significant improvement has been reported lately with the system derived from *Nicotiana tabacum* BY-2, named ALiCE®. ALiCE® presented a linear scale-up from a few microliters to 1 L and ≥1 mg/mL productivity of functional VLP for vaccine application [102]. It is also reported that difficult-to-express proteins could be produced better when the lysates of transiently transfected CHO cell lines with T7 RNA polymerase and the targeted integration into the CHO genome were utilized for the expression [103].

CONTINUOUS MANUFACTURING AND PROCESS ANALYTICAL TECHNOLOGIES

A major lesson learned during and after the COVID-19 pandemic is that the capacity of vaccine manufacturing needs to be flexible and resilient to meet the rapidly changing vaccine demands from a few million doses to a few billion doses or vice versa. A potential technology enabling flexible vaccine manufacturing is a continuous manufacturing process.

There is a growing number of implementations of continuous processing in the pharmaceutical industry and it allows end-to-end process integration. It was simulated with a therapeutic monoclonal antibody manufacturing process for the operational and economic impact of integrated continuous bioprocessing. This example indicated that the production capacity could be adjusted between 1 and 8-fold with a single-use bioreactor and an end-to-end integrated continuous process. It suggested that a single plant could be utilized flexibly for the rapidly changing market demands as needed [104].

In addition to flexibility, the improved sustainability of continuous processing has been evaluated by calculating process mass intensity (PMI). PMI is a ratio of total input mass to product mass and, therefore,lower PMI indicates better production efficiency per raw materials [105,106]. In general, continuous processing presented a multiple-fold better PMI than batch processing at the same scale. In general, the electricity usage and operation time of the continuous process were more efficient than those of batch processing [104–107]. The improved environmental sustainability suggests that continuous processing could fit better in regions with limited natural resources and infrastructure for vaccine manufacturing.

It was reported that integrated continuous manufacturing for a recombinant vesicular stomatitis virus (rVSV)-based COVID-19 vaccine, using perfusion cultivation and three chromatography runs in a counter-current mode, increased the vaccine productivity by

several folds [108]. It was also demonstrated that the continuous process could be further optimized to manufacture viral vaccines with better qualities [109].

Continuous manufacturing often comes with challenges in keeping consistent process controls, which can be overcome by the integration of process analytical technology (PAT) [110]. FDA guidance for industry summarizes PAT as a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, to ensure final product quality [111].

For successful implementation, appropriate PAT sensors need to be defined, according to the intended target attributes to monitor and the process steps to be integrated [112–116]. PAT acquires the measurements in real time or almost real time either in-line (directly in the process), on-line (in a built-in loop where samples are automatically fed), or at-line (samples are collected manually and analyzed next to the bioprocess site) modes [112,117,118]. The large amount of data acquired through PAT must be processed and analyzed via suitable data analysis models, qualitatively or quantitatively [112,114,116]. Eventually, the sensors and data analysis models should be validated to ensure the accuracy of generated data and its application for robust process control [119].

In practice, advanced PAT tools were applied to better control the manufacturing of live VSV with enhanced predictability of the quality of viral particles [120]. The integration of continuous processing and inline and online PAT tools was demonstrated successfully for the pilot-scale production of a monoclonal antibody [121].

mRNA MANUFACTURING TECHNOLOGIES

mRNA is produced in a cell-free system with well-defined platform processes [122]. It requires specialty reagents, such as T7 polymerase, 5′ cap analogue, modified nucleotides (such as pseudo-uridine or N1-methyl-pseudouridine), and ionized or ionizable lipids for formulation. These proprietary raw materials are expensive and, therefore, contribute significantly to the total cost of mRNA manufacturing. It is often considered that mRNA cost-of-goods is too high to ensure equitable access to life-saving mRNA-based vaccines [123].

There are several strategies to improve the cost-effectiveness of mRNA production. A fed-batch IVT reaction led to better utilization of T7 RNA polymerase, an increase in overall mRNA productivity, and, in turn, significant cost reduction [124]. Recirculation and re-use of raw materials such as enzymes, cap analogues, or modified nucleosides, was evaluated in continuous bioprocessing of mRNA [123]. It was also reported that integrated continuous processing of mRNA IVT and subsequent purification steps reduced overall operation costs [125]. QbD-based modeling helped to identify the optimal ratios of mRNA productivity to key raw material usage and, therefore, reduced waste of raw material usage [123].

As the economies of scale does not apply to mRNA manufacturing, small-scale and modular decentralized manufacturing could be rather economical for capital expense (CAPEX) and operation expense (OPEX). Decentralized manufacturing might also facilitate regional raw material supply and mRNA vaccine distribution [54,55]. The deployment of BioNTech's modular manufacturing units (BioNTainer) to Rwanda is a noticeable example, enabling clinical and commercial manufacturing of mRNA vaccines in Africa [126]. Biofoundry, empowered by microfluidic systems and continuous manufacturing with advanced PAT, is an emerging technology enabling the manufacture of mRNA in small scales at the point of care. Biofoundry development has been energized by funding from Wellcome Leap R3 program [127].

The size of saRNA (~10,000 nt for saRNA and ~1,000 nt for mRNA) makes it challenging

to formulate with lipid nanoparticles (LNP). An optimized lipid formulation yielded 80% encapsulation efficiency of saRNA, and it could result in a negative impact on stability [56]. Instead of LNPs, cationic polymer or nanostructured lipid carriers were reported beneficial to saRNA formulation [128,129]. There are also emerging and unconventional mixing technologies that could potentially improve saRNA-LNP encapsulation, such as Micropore and FDmiX technologies.

DIGITAL TWINS

QbD is an important principle for developing manufacturing processes and control strategies. It allows developers to gain a better understanding of processes by identifying critical quality attributes (CQAs), critical process parameters (CPPs), and multivariate design space in systematic and resource-efficient ways. It is also the foundation for robust manufacturing with batch-to-batch consistency [130].

Combined accumulated knowledge and experience in QbD principles, with empirical and mechanistic models describing each unit operation, could further enhance process analyses, controls, and decision-making [131]. The model-based approach also enables *in silico* simulation to accelerate development and ease uncertainties related to scale-up and technology transfer [109,131,132]. The process model of each process step could be integrated to depict the end-to-end operation and the integrated process model could assist FMEA-based risk ranking to determine CPPs and their proven acceptable range for all CQAs in a holistic way [115,133,134].

Moving forward, process models are pivotal tools for digital twin development. A digital twin is a digital replica of physical processes or systems connected with the physical systems through automatic data flow [135,136]. The models, presenting the digital replica, are calibrated and validated against the process data. Afterward, those could be integrated into manufacturing processes and PAT. The real-time process data collected by PAT are fed into the models so that they can prompt the advanced process controls to maintain the process performance within the design space and the quality of the product within the specifications. Ultimately, it can lead to automation of the entire manufacturing process and real-time release [130,137–140].

The proofs-of-concept of digital twins have been demonstrated in various applications, such as mRNA-based COVID-19 vaccine manufacturing [125], VLP production [130,138], pDNA clarification process [141], lyophilization [142], and quality control laboratories [143]. As the successes of digital twins are mostly achieved at the lab scale, the validation of digital twins at the manufacturing scale is further required to realize their potential for vaccine manufacturing.

CONCLUSION

As presented in this article, there are significant technical advances happening in vaccine development and manufacturing. These innovations provide vaccine developers and manufacturers with the capabilities to develop vaccines rationally rather than empirically, fine-tune the manufacturing processes for high quality and productivity, and better control manufacturing for robustness at various scales and sites. These can significantly increase the probability of success for the rapid development of novel vaccines, and equitable access, to better respond to the next pandemic. Furthermore, integrating innovative vaccine technologies and the Pharma 4.0 framework could generate powerful synergies toward the 100 Day mission, utilizing the digital transformation to increase manufacturing sustainability [19,144,145].

Harmonization with pharmaceutical regulators is critical in adopting innovation toward Pharma 4.0. In the past decades, regulators have published important guidance for industry, which could facilitate the adoption of Pharma 4.0. It includes ICH Q8(R2), Q9, and Q10 for QbD-based development,

risk assessment, and continual improvement in quality systems, respectively. ICH Q13 for continuous manufacturing and Q14 for advanced analytical development based on QbD could contribute to the adaptation of Pharma 4.0. In addition, the FDA recently issued a guidance for advanced manufacturing technologies designation program, which paves the regulatory path to implement innovative technologies in manufacturing processes. FDA also published discussion papers regarding AI and machine learning in drug development and manufacturing and sought public feedback [146,147]. In parallel, the EMA organized the Quality Innovation Group to learn about innovative technologies from the public [148]. The synergy between innovators and regulators will further accelerate the advancement of Pharma 4.0.

As Pharma 4.0 is in the early stages, significant investment is still required to generate more evidence to build stronger business cases and realize its benefits for accessible and sustainable vaccine development and manufacturing.

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VACCINE INSIGHTS

MANUFACTURING: DOWNSTREAM, FILL/FINISH, AND DELIVERY

SPOTLIGHT

EXPERT INSIGHT

Advanced drying technologies for vaccine products

Sue Behrens

Vaccines continue to be the frontline protection against infectious disease, preventing epidemics and taming outbreaks. Distribution of vaccine products is challenging; product quality must be protected during shipment and storage to ensure stability throughout shelf-life. Drying is a critical process for removing water and oxygen to prevent product degradation. Recent advances in technologies for aseptic drying may provide significant benefits in product quality, supply reliability and productivity, with reduced cost.

This review discusses factors that must be considered during product development across the range of currently available vaccine formats. Current operations are predominantly based on lyophilization processes using heating fluids in the shelves to sublimate vapor from product in vials. Over the last decade, equipment design has progressed to allow new options for final product manufacturing, including potential for continuous processing. Microwave energy is being developed as a new heating source with more homogeneous penetration leading to faster drying cycles. Spray-drying and spray-freeze drying are now available for aseptic production of a flowable bulk powder for dosing in a wide variety of container closure systems. These novel systems are driving reductions in cost and improved cycle time, while maintaining product sterility and stability.

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INTRODUCTION

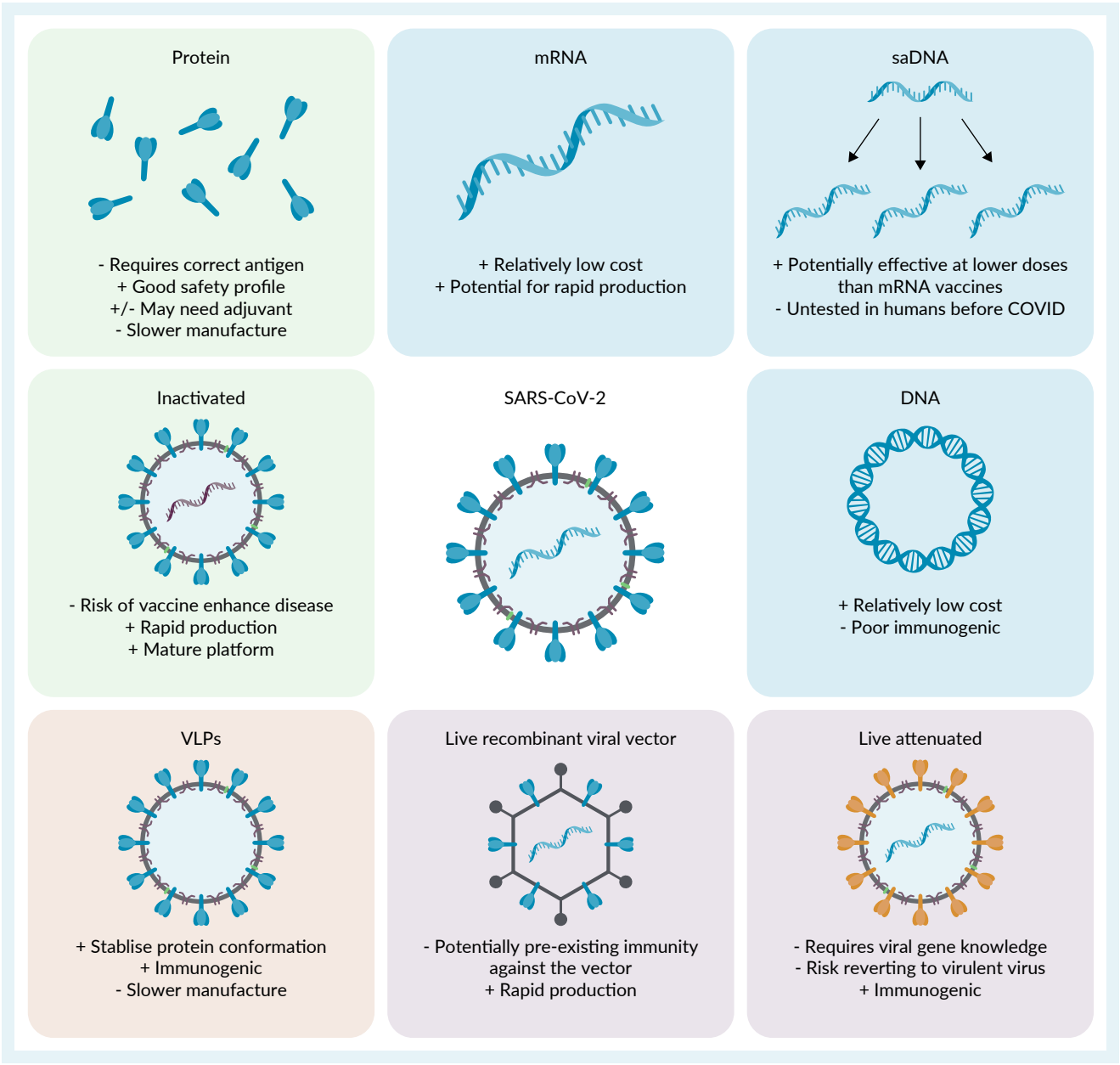
A broad set of manufacturing operations are used in making vaccines. Production of the Drug Substance requires a number of different product steps, which are often combine in dedicated processes for each vaccine. The final Drug Product steps transform large volumes of bulk drug substance into patient doses. Patient doses can be provided in multiple images or formats—liquid or dried in vials, syringes or other novel dosage forms. Aseptic manufacturing facilities can generate many different types of products as long as

they share the same final image. This review will describe key considerations for final product design for vaccines, including composition and alternatives for drying process steps needed to ensure stability and quality throughout shelf life.

Distribution of thermally unstable vaccines leads to several challenges. These products are biologic in nature and subject to thermal degradation [1]. A controlled environment is required to ensure quality is maintained until patient administration. Very large warehouses are needed for cold storage early in the supply chain: refrigeration at 2–8°C, frozen storage at 20°C or even 80°C may be needed. Smaller scale refrigeration and freezers are needed for intermediate storage along the distribution chain and in clinics [2,3]. Validated shipping

FIGURE 1

Multiple vaccine platforms evaluated for COVID-19 protection [14].



containers are essential, all the way to delivery in 'the last mile', where transportation may be a healthcare worker on a camel, a bike, hand-carrying a cooler or other local transport [4,5]. Recently, remote operated drones have been used to deliver vaccines to areas that are difficult to reach [6,7]. The vaccines must be protected against both heat and freezing, which means that temperature should be monitored, continuously if possible. Data loggers and vial vaccine monitors minimize waste by allowing identification of each container or vial that may exposed to temperatures outside the acceptable range [8,9]. Over the last decade, remote temperature sensors using internet-of-things technology have been developed and efforts focused on blockchain technology for tracking and security were initiated [10–13].

The COVID pandemic prompted development of new types of products, with limited time for process optimization (Figure 1). The earliest products were based on novel platforms using mRNA and viral vector DNA, which allowed rapid development and licensure due to prior work on gene therapy products. The mRNA (Comirnaty®, SpikeVAX®) and DNA products (Jcovden®, Vaxzevria®, Covishield, Convidecia) were quickly manufactured and used to mitigate the rapidly spreading pandemic in the US and Europe. In addition, inactivated virus products (Covaxin™, Covilo, CoronaVac) were used in many countries around the world. Later, recombinant protein subunit vaccines (Covovax, Nuvaxovid®) contributed to developing protection for COVID-19. For many years, other platforms, including virus-like particles, live attenuated virus, bacterial membrane polysaccharides and toxoids have been important for control of infectious disease.

Each type of vaccine has unique stability characteristics, depending on product-specific degradation pathways and kinetics [15,16]. To ensure product quality, the manufacturing process must safeguard both sterility and potency throughout the shelf life. The wide diversity of vaccine products requires different product formulation (composition) and processing (unit operations) for each different platform. The earliest COVID vaccines were developed without time for development of optimal formulation & process for stability, which required complex frozen cold chains for delivery to and use in clinics [17,18]. Efforts are in progress to improve stability for these novel vaccine formats, allowing broader use in the future [19–23].

Drying is a critical process for removing water and oxygen to prevent product degradation. These unit operations add both manufacturing cost and complexity for process control. However, the drying step will extend stability up to 3 years and enable distribution at ambient temperatures during shipment. The improvement in product stability enables a supply chain without requiring significant increase in shipment volumes [24]. Recent advances in technologies for vaccine drying may provide significant benefits in product quality, supply reliability and productivity with reduced cost.

VACCINE PRODUCT DESIGN

There are several potential components in a final product in addition to the antigen or antigen-generating nucleic acid. Often, an adjuvant is added to increase activation of the immune system and improve effectiveness. It is necessary to add buffers, product stabilizers, preservatives, and/or surfactants to control microbial contamination risk and ensure clinically effective products [25]. Lipid nanoparticles (LNPs) are used as a delivery mechanism and contribute as stabilizers for mRNA products *in vivo* [18]. Depending on the product image, diluent for reconstitution will be incorporated into the product or provided in a companion vial.

During product formulation and process development, degradation pathways are identified to assess the stability impact of changes in the manufacturing process and product composition [1,26]. Two of the

most important concerns for biologics are hydrolysis, which often results in opening peptide bonds, and oxidative degradation of amino acids, which may change molecular structures. Other pathways include deamidation and reduction reactions. Both chemical degradation and mechanical stress can cause physical changes, including aggregation and denaturation [27].

These molecular modifications result in product quality changes that can lead to a loss of function, off-target immunogenicity, or other adverse effects. A single change in the molecule can render it completely ineffective, depending on where the structure is affected. On the other hand, there are changes that have no impact because they are in an area unrelated to product activity. Due to the complexity of the antigen and adjuvants used in vaccine products, it is difficult to understand all the possible changes and their impact [28]. In general, product and process development scientists endeavor to minimize any impact during manufacture.

Therefore, all factors that drive degradation must be controlled and monitored in the drug product. These include pH, oxygen levels, and water activity in the system. All of these are important in both hydrolysis and oxidative pathways. Certain buffer species, ionic strength, and presence of trace metals can impact the stability of a product. Finally, exposure to light, multiple freeze-thaw cycles, and exposure to shear can cause product damage.

Product stability can be ensured by adding different types of stabilizers, which are identified during formulation studies depending on the type of product, degradation risks, and route of administration. Stabilizers include cryoprotectants, oxygen scavengers, proteins, gelatin, polyethylene glycol, and specific buffers to control pH [27,1].

Increases in temperature accelerate degradation across all pathways. Once the product composition has been defined by formulation scientists, environmental controls are necessary throughout manufacture and distribution [29]. Reduced temperatures, whether refrigerated or freezing, will protect product quality. Many degradation pathways can be limited by reducing the presence of water and oxygen and products are dried through lyophilization or spray drying and introducing an inert gas into the headspace [30].

Each type of vaccine is vulnerable to degradation pathways in various degrees and must be maintained under different conditions to ensure quality throughout shelf life. Table 1 summarizes platforms for vaccine products

TABLE 1

Vaccine product images and storage conditions.

	Product image			Long-term storage conditions			
	Liquid	Frozen liquid	Dry	Ambient	2–8° C	-20° C	-80° C
Live attenuated virus			●		●	●	
Inactivated virus/bacteria	●		●		●		
Virus-like particle	●				●		
Subunit—protein, polysaccharide	●				●		
Viral vector		●	○		○	●	
DNA		●	○	●	○		
mRNA/LNP		●	○		○	●	●

●: applicable; ○: potential.

and the types of final product used for each, along with long-term storage conditions. Frozen conditions can protect products in solution, but results in complex manufacturing and supply chain challenges [31,32]. Some liquid products cannot be frozen without impacting product quality, which is particularly important for those containing aluminum adjuvants. However, dried products can be stored at higher temperatures, often with less impact of temperature variation in shipping, minimizing quality risks.

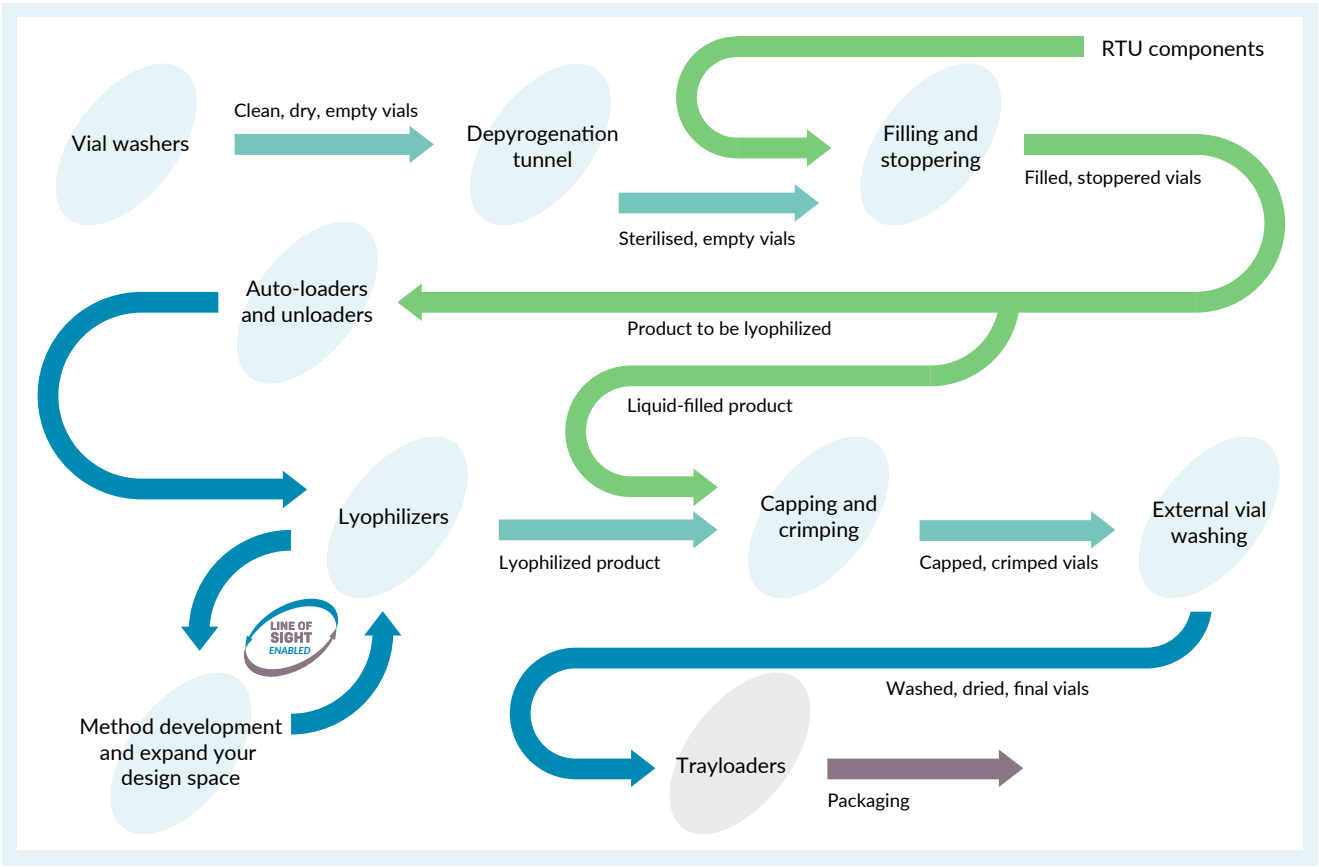
For each new vaccine, a process must be defined to manage the specific risks associated with storage, shipping, and distribution. Enhanced product stability will minimize supply chain complexity. Thus, drying steps in vaccine manufacturing are critically important [33,34].

Dried vaccine product is packaged in a variety of containers, which drives the operation selected for final dry product processing. The majority of vaccine products have been commercialized in vials, resulted in high global capacity for these images. Vial filling machines are very fast, flexible for different sizes of vials, and available in manufacturing sites around the world. In addition, vial filling capacity can be shared with liquid products, increasing utilization and the financial return for the high capital investment in aseptic filling facilities.

Lyophilization equipment has been designed to optimize production of single or multidose units in glass vials [33]. For lyophilized products, a custom diluent may be needed, which doubles the demand for filling capacity. The need for additional components and second manufacturing operations increases cost of

FIGURE 2

Process operations for liquid and lyophilized products [38].



goods sold [35]. At patient administration, two sets of materials introduce extra steps and increase the risk of error in the clinic. Hence, dual-chamber options have been developed for convenience of reconstitution [36].

Vaccines are typically administered parenterally, and the final stages of manufacturing must be done under aseptic conditions [37]. Containers must be washed and sterilized in line or purchased ready-to-use at increased cost. Filling into the container is the first product step. Products to be lyophilized are only partially stoppered prior to transport to the lyophilization cabinets. Systems have been designed to allow direct transfer from filling lines to the cabinets to assure continuity of aseptic conditions for the product and containers. Traditional lyophilization *in situ* processing removes water vapor from final product containers, using heat under deep vacuum conditions. The steps (Figure 2) in a traditional lyophilization process include [38]:

1. **Filling:** product is dispensed into vials to ensure consistent dosing
2. **Stoppering:** vials are partially stoppered to allow water vapor to escape
3. **Loading:** vials are transferred into the lyophilization cabinet
4. **Freezing:** energy is removed to rapidly freeze the product
5. **Primary drying:** vacuum is applied, sublimation occurs at low temperatures
6. **Secondary drying:** vacuum is maintained with heating to remove bound water to meet low moisture content
7. **Vacuum release:** sterile gas is introduced to return cabinet to atmospheric pressure; dry, inert gases are often used to protect product
8. **Stoppering:** stoppers are completely inserted to close the vials prior to exposure to ambient conditions

For dual-chamber syringes, differences in the process require specific equipment to allow lyophilization in the syringe, followed by filling of diluent and application of liquid stopper and plunger [36]. Since the equipment is dedicated to this type of product, ensuring proper utilization can be difficult. The cost for dual-chamber products is increased by the number of components, number of steps, and the reduced capacity of the equipment. Although these products simplify administration, additional manufacturing steps are required to lyophilize in one chamber and fill diluent into the second chamber.

Another recent product format for dried products is based on microneedles, which deliver product across the outermost later of the skin into dermal tissue below. These systems are convenient for administration and minimally invasive with slight to no pain reported by patients. In addition, product deposited on to the microneedles is dried and very stable. Vaccine microarray patches (VMAPs) are an important option for increasing access to vaccines and improved global health [39,40].

VMAPs can be generated using one of several different methods (Figure 3) [41,42]. The microneedles can be used to generate cavities for introduction of the vaccine or coated with the active product. The active product can be formed in the microneedles, which would dissolve into the tissue. The microneedles may be hollow with the antigen solution introduced through the needles in more traditional manner, or the microneedles may form hydrogels which release the product. Due to the minimal product depth on each needle, the drying step may use vacuum or direct exposure to dry air at ambient pressure. System design for each configuration may require different process operations.

Full-scale manufacturing methods and capabilities for VMAP products are being established globally [43]. Identification and resolution of technical and regulatory challenges is underway to allow commercialization of these products [44,45].

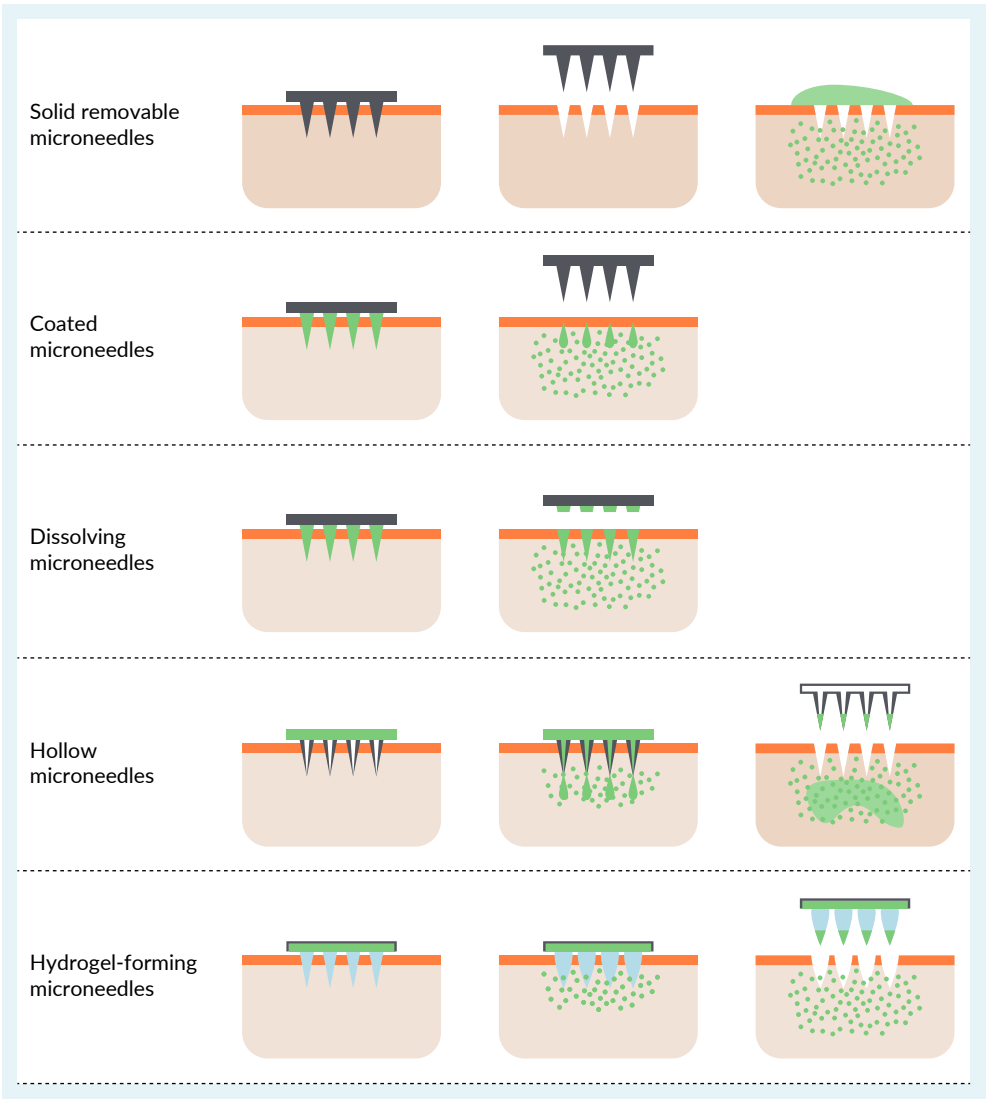
VACCINE DRYING TECHNOLOGIES

Advances in manufacturing technologies for dry vaccine product provide capabilities for new product images, with increased productivity and improved quality assurance. Decreasing the duration of the drying operation provides a significant opportunity for increased productivity. The major limitation for cycle completion is the rate of moisture removal, which is often constrained to maintain low product temperature and protect product stability. Both heat and mass transfer must be properly controlled throughout the process to deliver an optimal cycle [46, 47].

Hence, improvements in heat and mass transfer will significantly shorten cycle times. This principle can be applied to drying for all product types, including vials and dual chamber devices. Some of these technologies allow drying *in situ*, while others generate a dried product that must be distributed into containers at the proper dosage. Production of a bulk dried product provides opportunities for development of novel formats beyond the typical vial, with improved stability.

FIGURE 3

Options for development of microarray patches [41].



For the last century, the most common drying process used for vaccines has been lyophilization. Aseptic lyophilization cabinets use heating fluid flowing inside each shelf to freeze the product and to drive vaporization and remove water from the product. For both freezing and heating, energy must be transferred across the shelf through the bottom of the glass vial and the full volume of the product, which can be inefficient. Vapor removal occurs through a condenser operating under vacuum, which often results in inhomogeneities throughout the cabinet due to mass transfer differences at the walls, between the shelves, and various vial locations on the shelves.

Any process improvements must meet aseptic requirements to provide appropriate product quality. The equipment must be sterilized and operated in a highly controlled environment to maintain the highest levels of sterility assurance; current trends for new installations include implementation of closed systems wherever possible. All inputs must be sterilized at the point of use through sterile filtration or aseptically introduced as pre-sterilized materials. The room classification is determined based on the equipment design and interventions required.

IN SITU DRYING PROCESS ALTERNATIVES

Currently, *in situ* drying in final containers such as vials and dual-chamber syringes is the most common method for commercial products. Lyophilization cabinets are the most common process equipment. Improvements in traditional lyophilization have been investigated for all steps in the cycle. The freezing step is critical to allowing a proper porous macroscopic structure and enhanced mass transfer for vapor removal. Developments have focused on improved control of nucleation and increasing surface area for drying steps (Arsiccio 2020; Assegehegn 2019) [48,49]. Primary and secondary drying steps can be improved by focusing on the delivery of energy to drive vaporization inside the container. Heat transfer required from the shelf fluid through shelves to the glass is often inefficient due to space. Continuous processing could lead to improvements in productivity as well as consistency.

VACUUM FOAM DRYING

Foam drying does not require freezing and is conducted at ambient temperature under vacuum for evaporation [50,51,52]. The bubbles that form provide membrane-like areas with high surface area for drying [53]; process development focuses on controlling the size of the bubbles and the thickness of the membrane to ensure a rapid and efficient drying process [54]. The high surface area generated during this process will lead to reduced reconstitution times. Due to the increased volume of dried foam product, larger containers are required for the same dosage.

MICROWAVE VACUUM DRYING

Microwave energy can be used to replace heating fluid in the shelves, with application in traditional lyophilization cabinets that could allow continued use of current equipment. Rapidly cycling water molecules in a microwave field results in heating at or near the molecular level and evaporation of water without significant heating of sensitive products [55]. In addition, the energy penetrates immediately across the entire system without generating localized heat and mass transfer effects observed in traditional lyophilization. Frequency control of the microwave field provides a tool for optimization of the system during process development for individual products [56].

Microwave vacuum drying (MVD) technology has been demonstrated for vaccines and other biopharmaceuticals [57,58]. The energy necessary for sublimation is provided by magnetrons, which generate microwaves in the drying chamber. A dry ice condenser is used to condense the water vapor, and

product vials are stoppered at completion of the process.

Merck [59] investigated MVD using EnWave pilot scale systems, where the product is frozen offline in vials and transferred to a FreezeREV® dryer. Cycle times for MVD were 87% shorter than for lyophilization. Results from the early pilot-scale study showed equivalent moisture content and activity when compared to traditional lyophilization.

CONTINUOUS PROCESSING

Continuous production systems improve productivity by increasing equipment utilization. Continuous lyophilization methods for vial products that leverage existing principles and vial filling capacity would be the simplest to implement [60].

A continuous process for *in situ* drying was proposed by Pisano and colleagues [61]. Vials are moved through modules, using specially designed transfer connections to maintain the appropriate pressure, temperature, and gas composition for each. This system was designed as an opportunity to produce dry product in a rapid and continuous manner using traditional and familiar process operations.

BULK DRYING PROCESS ALTERNATIVES

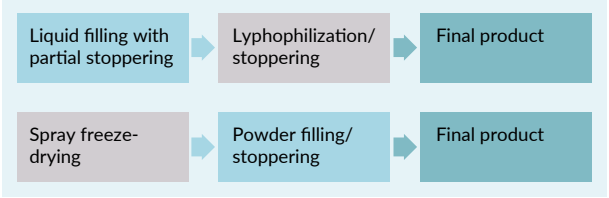
Improvements in heat and mass transfer can be achieved through more direct contact of the vaporizing energy and moisture removal medium with the product. The presence of containers and stoppers during *in situ* drying processes is the main source of resistance leading to increased cycle times. Efforts to improve efficiency of the drying operation have focused on producing dried bulk product, followed by dispensing in unit dose containers.

Overall, the number of process steps remains the same as for *in situ* drying, although the order of filling and drying steps is reversed (Figure 4). Manufacture starting with dry product in bulk requires aseptic powder filling capability, with flexibility for novel reconstitution-injection systems [62]. Aseptic powder fillers have been in use to fill sterile antibiotics and other parenteral products since the mid-1900s. The process can be challenging for amorphous materials due to inconsistency in powder flow and segregation of product and excipients during material handling. While blending and milling are often required to achieve particle size targets for materials produced by spray-drying, these extra steps are not common for injectables as they are reconstituted and administered after dissolution. Larger pellets can also be filled using variations of existing powder filling equipment. The risks of generating fine particulates and contamination of external vial surfaces are also reduced when filling material is composed of structured particles versus amorphous powder.

Implementation of bulk drying followed by powder filling provides new opportunities to support a broader range of images. Maintaining homogeneous composition with improved flowability of bulk materials provides the opportunity for consistent product quality at reduced costs.

FIGURE 4

Traditional lyophilization compared to bulk drying processes.



SPRAY DRYING

Spray drying, which results in a dried powder comprised of spherical particles, is the focus of many recent advances for production of stable vaccine products [63]. In spray drying, a liquid with excipients flows through a nozzle that disperses the product into fine particles. In the traditional mode of spray drying,

hot gas flows through the system to drive evaporation. Aseptic processing requires sterile gas for product drying; an inert gas such as Nitrogen or Argon may be used to limit oxidative degradation.

For many applications, the gas temperature can be up to 120°C, which could cause degradation of sensitive biologic products, although short-term exposure and evaporative cooling will limit product temperature. Advantages of spray drying include a homogeneous feed containing the final formulated product, the ability to control particle size by tuning the spray nozzle, and an opportunity for continuous operation.

Aseptic designs that allow for continuous drying and powder production using spray drying are available. One example is the Aseptic SD™ system from GEA Niro (Soeborg, Denmark). The system uses a standard spray-dryer design, which has been upgraded to allow sterilization and aseptic operation [64]. Another system developed by Ziccum AB (Lund, Sweden) uses a mesh nebulizer to create spherical droplets in a dry nitrogen laminar flow. In addition, dry nitrogen is provided in counter-current flow along a membrane to remove moisture from the gas. The system runs at ambient temperatures to limit heat stress on sensitive products. Proof-of-concept of the technology has been demonstrated for mRNA–LNP products with excellent results for maintaining encapsulation efficiency, particle size, and distribution, and in vitro and *in vivo* mRNA activity [65,23]. Other examples of alternative technologies are discussed below.

SPRAY FREEZE-DRYING

Spray freeze-drying is an alternative developed to provide advantages of spray drying to the established lyophilization process [66, 63]. The steps include droplet freezing, followed by vacuum sublimation in a drying chamber. Historically, freezing prior to sublimation has provided quality product with strong stability profiles. Incorporating spray-freezing eliminates the requirement to fill product into containers prior to drying. In addition, there is a strong regulatory understanding of quality parameters for lyophilization which minimizes regulatory risks for transition to a new process. Two commercial equipment vendors currently provide spray freeze-drying options.

IMA Life, Tonawanda, NY, USA, has designed a continuous spray freeze dryer [67]. Droplets are generated from the spray nozzle and frozen as they drop through a liquid nitrogen-cooled column, which leads to the formation of small diameter spherical particles. The frozen spheres transit multiple spaces on shelves through different levels of pressure and temperature to achieve a low-moisture product. The final product is filled directly into an appropriate container, which is sealed in an aseptic manner to be used as a feed for a powder filling system.

Meridion, Müllheim, Germany, has built systems based on droplets freezing in a tower followed by vacuum drying of the frozen pellets in a unique rotating drum, with demonstrated product yields of up to 97% [68].

CONCLUSION

Vaccines continue to provide significant benefits for global public health. Improvements in manufacturing for these products will enable broader access for products that are more stable and convenient to administer at lower cost. Alternative systems are in development to provide opportunities for more efficient manufacturing of dried vaccines, to achieve highest possible quality and support additional product formats.

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VACCINE INSIGHTS

MANUFACTURING: DOWNSTREAM, FILL/FINISH, AND DELIVERY

SPOTLIGHT

Strengthening cold chain maintenance systems in resource-constrained environments: insights from Nigeria

Tahir Buhari
eHealth Africa, Nigeria

"Proper resourcing includes dedicated budgets for spare parts, fuel, maintenance supplies, and specialized training programs, as well as a reliable supply chain to ensure timely availability of necessary components."

VIEWPOINT

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Maintaining vaccines at optimal temperatures in resource-constrained environments, like Nigeria, presents significant challenges [1]. Strengthening cold chain systems is vital for ensuring reliable vaccine delivery to all communities, thus achieving universal health coverage, and reducing zero-dose children [2]. This article presents findings from a recent assessment across 12 Nigerian states and insights from the Cold Chain Data Hackathon Workshop held in Kenya [3], to offer advice on addressing the challenges of cold chain systems in low-resource settings.

Immunization is one of the most cost-effective public health interventions [4], yet vaccine-preventable diseases account for over 22% of childhood deaths in Nigeria and other low- and middle-income countries [5,6]. Inefficient cold chain systems lead to vaccine spoilage and wastage, undermining immunization efforts.

CHALLENGES IN COLD CHAIN MAINTENANCE: INSIGHTS FROM NIGERIA

Cold chain maintenance in Nigeria faces structural issues common to resource-limited settings. Aging equipment frequently fails to maintain optimal temperatures, with only 55.2% of state and 63.2% of local facilities currently functional [7]. Shortages of skilled technicians impede timely repairs, and lack of regular training can cause suboptimal use of tools and spare parts. Limited funding further restricts routine maintenance, reducing the availability of functional equipment. Additionally, poor data integration hampers diagnostics, and the absence of a decommissioning plan for unserviceable equipment further decreases efficiency. A phased approach prioritizing foundational maintenance, capacity building, and data integration is critical to reinforcing vaccine delivery and supporting continuous immunization across Nigeria.

PHASED APPROACH TO STRENGTHENING COLD CHAIN SYSTEMS

Countries must first invest in procuring high-quality cold chain equipment and replacing aging, obsolete models to establish a foundation of reliable, current-generation equipment. With this base in place, the next priority is to maintain the proper function of equipment through consistent maintenance, training, and reliable funding. Programs like the Gavi Cold Chain Equipment Optimization Platform [8] demonstrate how targeted investments in both equipment renewal and ongoing upkeep can reinforce long-term cold chain performance.

Next, the focus should shift to building a dependable maintenance capacity. This involves increasing the number of trained technicians, bolstering their skills through ongoing capacity-building initiatives, and maintaining an adequate inventory of spare parts.

Once equipment is in place and a regular maintenance schedule established, data integration becomes essential for optimizing maintenance efforts. Real-time data and system-wide analytics can enable predictive maintenance, enhance vaccine logistics and inventory management, and increase the overall efficiency and value of maintenance activities. This phased approach ensures cold chain systems operate at peak performance to safeguard immunization programs.

LEVERAGING DATA AND INNOVATIVE APPROACHES FOR MAINTENANCE

The Cold Chain Data Hackathon Workshop held in Nairobi, Kenya in 2024 illustrated the importance of combining foundational maintenance with data solutions to strengthen system resilience. Experiences from other countries highlight that digital tools are effective only when integrated with a solid maintenance foundation, for example:

- In Tanzania, real-time temperature monitoring succeeded because a maintenance infrastructure was in place, allowing swift responses to data-identified issues.
- In Uganda, the inventory system, based on ODK-X (free, open-source software), improved data collection and coordination in low-resource settings but relied on trained technicians and established maintenance protocols.

These examples reinforce that while data and predictive maintenance tools enhance efficiency, they cannot replace essential maintenance capabilities.

KANO STATE'S FLOATING ASSEMBLY MODEL: AN INNOVATIVE SOLUTION

Kano State in Nigeria has pioneered the Floating Assembly Maintenance Model to address cold chain maintenance challenges, with impressive results. In December 2020, the equipment functionality rate stood at 71%. After the Floating Assembly team began maintenance in three zones, this rate improved to 76% by December 2021, and between January and December 2022, equipment uptime reached a high of 89%. Although functionality dipped to 69% in 2023, likely due to resource constraints and system strain, it rebounded to 85% by October 2024.

The Floating Assembly team, consisting of 10 mobile engineers equipped with vehicles stocked with diagnostic tools and spare parts, enables efficient on-site repairs for over 1,400 facilities. By establishing a foundation of reliable equipment and a dedicated maintenance team, Kano's Floating Assembly exemplifies the phased approach required to build resilient cold chain systems. This initial investment in maintenance capacity has driven sustainable improvements in functionality, even without advanced data tools. Now, with a strong foundation in place, Kano is well-positioned to integrate predictive analytics and real-time data tools as the next phases in cold chain optimization.

SCALING THE FLOATING ASSEMBLY MODEL

The Floating Assembly Maintenance Model could help optimize the cold chain maintenance landscape in Nigeria and beyond. Nigeria's cold chain infrastructure follows a hierarchical structure, with the National Strategic Cold Store at the top, supported by six Zonal Cold Stores, State Cold Stores, and over 19,000 health facilities. This extensive network, staffed by technicians across multiple levels, ensures operational continuity but requires robust maintenance strategies to remain effective.

Implementing the Floating Assembly Model at a national scale could strengthen each level of this structure, enhancing operational efficiency and reducing equipment downtime across all facility types.

To achieve this, it is essential to advocate for a well-resourced effort involving government agencies, donor organizations, and local communities. By demonstrating the model's success in Kano State and its potential to improve cold chain performance nationwide, Nigeria can take a leadership role in securing the necessary funding, equipment, and personnel to scale this model effectively.

Proper resourcing includes dedicated budgets for spare parts, fuel, maintenance supplies, and specialized training programs, as well as a reliable supply chain to ensure timely availability of necessary components. By investing in resources to support the Floating Assembly Model across Nigeria's hierarchical infrastructure, the country can establish a robust, sustainable maintenance system for its cold chain network.

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TAHIR BUHARI is a global health program manager with over a decade of experience in leading high-impact health projects across Sub-Saharan Africa. Specializing in health systems strengthening, cold chain optimization, and immunization infrastructure for low-resource settings, he applies a phased approach, data-driven strategies, and sustainable maintenance models to enhance vaccine security and distribution systems. His recent work provides actionable insights into improving cold chain reliability and advancing safe vaccine delivery in challenging environments.

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VACCINE INSIGHTS

INTERVIEW

From Ebola to Mpox: developing International Standards to accelerate vaccine development

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, speaks to **Giada Mattiuzzo**, Head of Viral Vaccines (R&D), MHRA, about the importance of developing International Standards for vaccines, especially during public health emergencies.

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Q How did you get involved in the vaccine field?

GM: I did my PhD in Molecular Virology at University College London, and soon after joined the National Institute for Biological Standards and Control (NIBSC), which is now part of the UK's Medicines and Healthcare products Regulatory Agency (MHRA). My career in vaccines really kicked off with the Ebola outbreak in 2013–16, when I joined the response team involved in the production of standards. Afterward, I worked on the Zika virus, and

in 2019, we started collaborating with the Coalition for Epidemic Preparedness Innovations (CEPI) to develop physical standards for measuring antibody responses, ultimately supporting vaccine development.

Q What are you working on right now?

GM: I lead the Viral Vaccine group in the Vaccines R&D Team at the Science Campus of MHRA. My group works on a range of projects, with a primary focus on assay development and standardization. We also support the MHRA's statutory functions, such as control testing, and we are part of CEPI's Centralized Laboratory Network.

Additionally, I am working on developing an International Standard for antibodies against monkeypox virus, which has become a high priority since the WHO declared Mpox a public health emergency of international concern in August 2024.

Q What is the importance of reference materials in vaccine development?

GM: When developing a vaccine, it is critical to employ methods that can quantify and measure responses to the vaccine consistently within a group and ideally across globally distributed laboratories. A reference reagent serves as a standard that allows results from these assays to be expressed in the same units. Much of my work is done on behalf of the WHO to produce International Standards, which are the highest order of reference materials.

The International Standard serves as the primary calibrant and establishes a 'common language' so everyone can express results in the same way, allowing for greater comparability. If such a standard is not available, comparing results becomes challenging. For instance, if one laboratory expresses the results in arbitrary units while another uses μg/ml, it is nearly impossible to assess if they are obtaining the same results.

Without standardization, vaccine development progress slows down. Laboratories may still develop their own assays and produce results but comparing them becomes incredibly challenging. The critical role of an international standard is to enable comparability across different laboratories.

The use of reference materials helps to make informed decisions and enables an early understanding of vaccine efficiency in preclinical phases. For example, if a vaccine is not performing as expected, it can be removed from the pipeline, saving time and effort. Later, in Phase 3 or after the vaccine has been licensed, standardized immune response data can be gathered across studies. The data can be collected from other studies of different vaccines and, if sufficient, can help establish a correlate/surrogate of protection. This 'magic number' indicates a level of immune response that protects against infection and/or disease, which is useful to make

"...it would be ideal to establish an international infrastructure before epidemic or pandemic situations."

go/no-go decisions during vaccine development, reducing time and costs of the clinical phases, and can support licensing application.

Q What are the key challenges in developing reference materials for vaccines and how can these be overcome?

GM: The value of an International Standard lies in the rigorous process employed to develop it, closely following the WHO guidelines. The entire process usually takes 2–3 years, and the primary bottleneck is sourcing the material that will serve as the candidate international standard. For antibody standards, specifically, the ideal material should closely resemble clinical samples used in laboratories—usually serum from recovered or vaccinated people.

When dealing with emerging viruses and pandemics or epidemics, sourcing this material becomes challenging, especially in regions directly affected by the outbreak, where local resources are understandably focused on public health needs rather than providing materials for standards development. Obtaining materials from where the outbreak is happening, and having reagents available is critical in being able to develop and validate assays.

To overcome these hurdles, it would be ideal to establish an international infrastructure before epidemic or pandemic situations. Such a framework would include globally distributed organizations with pre-established legal agreements, which would dramatically expedite the sharing of these critical reagents.

Q What are the key challenges during a public health emergency?

GM: The main challenge during a public health emergency is speed. If the world is to achieve the 100 Days Mission set out by CEPI, everything needs to be done to tight timeframes. However, sourcing the materials and creating an international standard both take time. To address this challenge in an emergency, we have developed research reagents that resemble International Standards, although they lack the full characterization typical of such standards. We conduct in-house characterization, thereby offering a product in which we have confidence. Once these research reagents are produced, we can include them in the multi-laboratory collaborative studies for evaluation of the candidate International Standard, which allows us to back-calibrate these reagents to the units of the International Standard. Consequently, once developers have acquired these reagents and run their assays, they can simply convert their

"There is a growing awareness that the next outbreak may not come from a known virus but rather an unknown but related one."

units to the international units, automatically translating their values to align with the standard. This process helps bridge the period prior to an International Standard being available.

Regarding diagnostics, to detect the viral RNA of Ebola virus, instead of using the virus itself, we created virus-like particles based on an HIV structure, into which we inserted the Ebola virus genes. This construct was ideal for controlling the entire testing procedure, from RNA extraction to gene amplification. Crucially, this was a reference material that was safe to use in typical diagnostic laboratories as it does not have the properties of either Ebola virus or HIV. We later adapted this method for other pathogens, such as Lassa fever virus and SARS-CoV-2.

For antibody standards, we collect serum from convalescent individuals, treat it to minimise the risk of presence of harmful pathogens, aliquot it, test it in-house to ensure it contains sufficient antibodies to generate a dose-response curve, and make it available as a research reagent. For Mpox, we developed such a research reagent in 2022, with CEPI's support, which is now progressing toward becoming an international standard.

We are also proactive—we follow priority lists for pathogens of interest, and aim to prepare materials for potential use in case one of these pathogens, or a closely related one, emerges to be of public health concern.

Q How is the vaccine field developing, and what advances do you hope to see in the future, especially regarding assay development and standardization?

GM: The biggest shift has been in the approach of moving from response to preparedness. Another change relates to the priority pathogen lists. The latest WHO R&D Blueprint priority list emphasizes virus families rather than focusing on specific pathogens, although prototypes for each family have been listed. There is a growing awareness that the next outbreak may not come from a known virus but rather an unknown but related one. Therefore, preparing in terms of virus families enables us to have enough information to respond promptly to a new, related pathogen.

Regarding novel vaccine platforms, we are all exploring ways to act quickly, with mRNA emerging as a vaccine platform which can be developed at pace. Although promising, it may not be the perfect platform for every specific pathogen. Therefore, extensive studies must be conducted in advance of an outbreak to determine which platform is best suited to elicit the best immune response for a given pathogen, ensuring we have this information ahead of time.

From a biological standardization perspective, we aim to strengthen the collaborations we currently have. We are fortunate to work on behalf of the WHO, which facilitates excellent collaborations with organizations around the globe. When these organizations participate in our studies, we do not provide any compensation—they invest their time and resources to be part of projects that are recognised as important for public health.

CEPI has introduced us to many potential donors of materials, usually large organizations. We must not forget the individuals who donate their blood to assist us, however. There is a growing understanding that what we do is important because it expedites research, accelerates assay development, and ultimately increases the speed at which vaccines become available to patients.

Q What projects will you work on in the next few years?

GM: Collaborating with CEPI's Centralized Laboratory Network to develop standards and achieve harmonization in the industry has been incredible, and we will continue to work together. The aim is to develop assays quickly and ensure that no matter which laboratory receives the clinical sample, they all achieve the same results. Currently, we are working on one of the top priority pathogens, the Lassa fever virus, but we aim to continue to collaborate on other pathogens in the future as well, to continue our support for vaccine and diagnostic development and use.

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GIADA MATTIUZZO is the Head of the Viral Vaccines (R&D) group at the Medicines and Healthcare products Regulatory Agency (MHRA), South Mimms, Hertfordshire, UK. Dr Mattiuzzo joined the National Institute for Biological Standards and Control (NIBSC), now part of the MHRA, in 2010 after obtaining her PhD in Molecular Virology at University College London, London, UK. The research interests of her group focus on assay development and standardization to evaluate the immunogenicity and potency of viral vaccines. She has led several projects for the development World Health Organization (WHO) International Standards on emerging viruses, including SARS-CoV-2, MERS-CoV, Lassa and Rift Valley fever virus, and currently Mpox.

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BIOINSIGHTS

Thank you to all our peer reviewers in 2024

At BioInsights, we use double-blinded peer review to minimize bias and focus the evaluation solely on the quality of the research rather than personal identities. However, despite their invaluable expertise and thoughtful feedback, peer reviewers often go unrecognized and are not formally acknowledged in publications. Their contributions are crucial to our editorial process, helping us assess articles and advance fields such as cell and gene therapy, nucleic acids, vaccines, and immuno-oncology, and we deeply appreciate the time and effort our reviewers dedicate to this process, enabling us to publish content of the highest quality. To honor their contributions, the editors of BioInsights are proud to publicly acknowledge our top reviewers of 2024 and look forward to collaborating with them in the future.

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