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SPOTLIGHT ON:
CMC and analytics

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Stability assessment for vaccines: recent trends & learnings from accelerated scenarios

Cristiana Campa

In recent times, outbreaks and pandemics have prompted the need to consider new paradigms for the stability assessment of vaccines. This article provides an overview of key CMC strategies to support the advancement of stability assessment approaches, including the relevance of product understanding and strong analytical packages, risk-based approaches based on advanced modeling, and increased reliance on prior knowledge. The considerations reported here are based on the current dialogue between Industry and Regulators and need to be assessed on a case-by-case basis; nevertheless, they enable the establishment of a structured path to secure global vaccine access in accelerated scenarios.

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BACKGROUND

It is widely known how important is to identify the right antigen(s) and an appropriate vaccine platform to prevent diseases. It is equally imperative that patients receive safe and efficacious vaccines, considering the time from manufacturing and release, product storage conditions, product transportation to vaccination sites, as well as product handling by healthcare professionals before administration. In addition, understanding degradation pattern is critical information used to verify the impact of a manufacturing change on product quality and to support product comparability evaluation. In other words, assessment of the stability is a key deliverable for the development and lifecycle of vaccines.

International guidelines have been issued to provide guidance on stability for vaccines and (bio)pharmaceutical products in general. As an example, WHO [1] issued a specific guideline on stability considerations for vaccines, covering aspects like stability evaluation at different stages of production and use, regulatory considerations to support clinical trials, licensing, and post-licensure, as well as stability studies design and data analysis. These considerations complement ICH Q5C [2], providing guidance on biotechnological/ biological products; shelf-life acceptance criteria discussion is also mentioned in ICH Q6B [3].

In addition, excellent publications are available on the topic; for instance, a collection of reflections resulting from workshops on vaccine stability evaluations (co-sponsored with the Korean FDA in April 2008 and IABS in October 2008) was published in 2009, along with individual contributions from regulatory and industry experts [4].

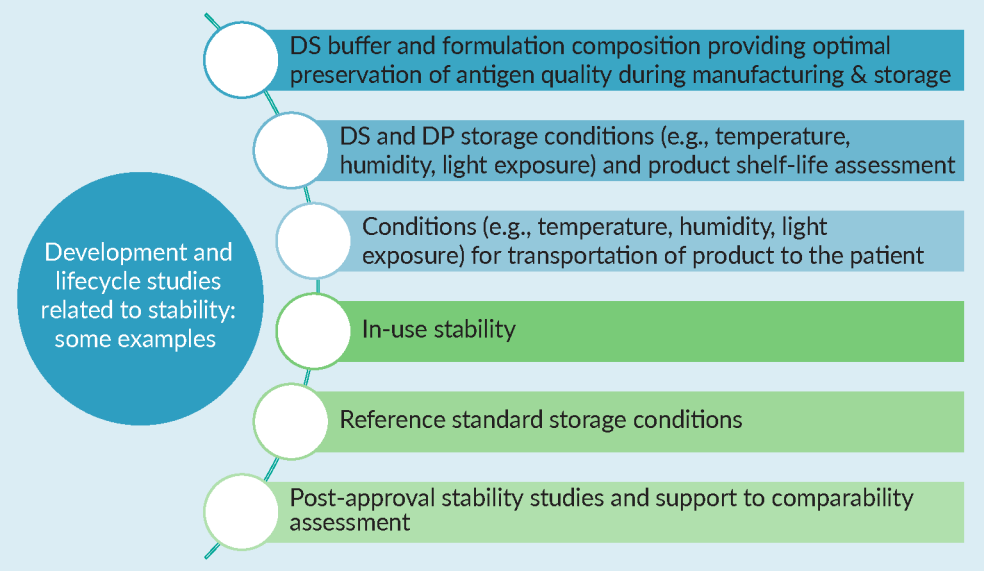
Figure 1 reports some illustrative examples of studies related to stability assessment of vaccines.

Over the last few years, industry and regulators are facing new challenges, including the necessity to accelerate development to address unmet medical needs (e.g., oncology, pandemics), the introduction of new modalities and platforms (e.g., mRNA vaccines), and the opportunity to introduce advanced analytical technologies. In addition, Regulatory Agencies may provide diverse views on CMC acceleration enablers, depending on the region and on the pharmaceutical modality. These challenges have triggered the establishment of a dialogue between Industry and Regulators on CMC expectations in accelerated scenarios [5], which also included reflections on shelf-life and storage conditions; in fact, during the development of drug substance and drug product and in medicine supply, stability is frequently on the critical path. For instance, as evaluated by the Vaccines Europe/ IFPMA CMC COVID task force, to address the global need for COVID vaccines, the rigid application of ICH Q5C indications, like the core stability data package requirements for real-time data, is not compatible with the accelerated pandemic vaccine development and industrial plans [6–8].

Coherently with these reflections, the ICH Quality Discussion Group has proposed the revision of ICH guidelines on stability and specifications. One of the main

FIGURE 1

Some examples of development and lifecycle studies related to the stability of vaccines.



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triggers for these updates is the clarification and modernization of technical and regulatory expectations. Key points are the integration of contemporary science/ risk-based approaches (e.g., Quality by Design, use of prior and platform knowledge, modeling strategies), which are expected to facilitate accelerated product development and lifecycle management [9].

This article will provide an overview of approaches to vaccine stability assessment in an accelerated/pandemic context, considering the ongoing dialogue of regulators with industry [5], recent guidelines and positions from regulators [10], as well as COVID learnings [11, 12].

INSIGHTS & FUTURE PERSPECTIVES

The main challenges and opportunities for stability assessment of vaccines in accelerated scenarios are related to three main areas:

- Justification of stability- indicating critical quality attributes and their acceptance criteria
- Stability data packages, including modeling strategies
- Appropriate analytical strategy

Key points of attention for each of the three areas are summarized in Figure 2 and described in this manuscript.

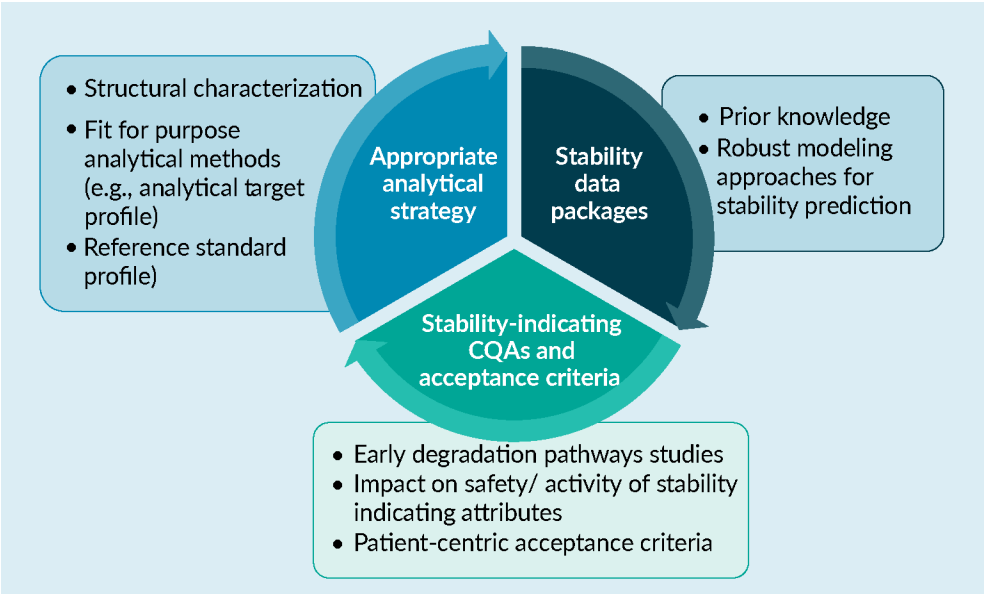
Justification of stability-indicating critical quality attributes & their acceptance criteria

Initial assessment of degradation pathways

Stability assessment for vaccine efficacy is traditionally driven by potency loss verification. Despite the firm relevance of this attribute, there are some points of attention, especially related to accelerated scenarios. First of all, potency tests have higher variability compared to other analytical methods, with consequent challenges in rapidly assessing stability trends, which may partially be addressed upon optimizing the placement of stability points, and upon

FIGURE 2

Main challenges and opportunities for stability assessment of vaccines in accelerated scenarios, and related points of attention.



increasing the number of tested sampling points and representative batches. In addition, the simple observation of a potency loss does not necessarily provide clarity of the structural root cause(s) for stability behavior; such understanding is based on an integrated assessment of physico-chemical tests and potency and it is key to proactively defining optimal formulation and storage/ transportation conditions, as well as early confirmation of trends (real-time or predicted). So, a critical step for the assessment of stability is the identification of the structural features of the antigens and of formulation components (excipients, adjuvants, delivery systems), which have an impact on safety or efficacy and may be altered in certain storage conditions. When feasible (e.g., for subunit or mRNA-LNP vaccines), this biophysical understanding is a key enabler of stability assessment and should be established as soon as possible during development, along with accurate degradation studies. The main drivers for such activities are reported below:

- Stress and acceleration studies help identify the actual liabilities of the antigens [13]. This knowledge helps tailor analytical testing in an accelerated context and secures focused studies for verification of antigen degradation and degradation products that impact safety and efficacy.
- Fit-for-purpose analytical methods can be selected for stability assessment, upon exploring the technologies that detect relevant changes upon stress/ accelerated conditions.
- Study of different formulation components compositions and pH values is important in stress/ accelerated settings, to select the conditions ensuring the most preserving conditions for the combined antigens and adjuvants/ delivery systems, as appropriate.
- These initial accelerated studies can help identify temperatures to be considered in designing stability studies to support shelf-life prediction through stability modeling
- The material generated by the accelerated/ stress studies can be used for experimental studies to support criticality confirmation of quality attributes (see next section)
- Studies under stress conditions may be useful in determining whether accidental exposures to conditions other than those proposed (e.g., during shipping and distribution of the product) are deleterious to the product. This is very important for rapid and effective global supply

Identification of the impacted CQAs

Once the actual liabilities of the antigens/ formulation components are verified, it is important to understand which degradation products are having an impact on safety and/ or efficacy (i.e., the stability-related critical quality attributes, CQAs). Indeed, some of the potential degradation products could be product-related substances of the antigen (as per ICH Q6B definition [3]) or they could have an impact on safety/ efficacy, i.e., being CQAs.

Early identification of stability-indicating CQAs is important, to focus analytical strategy and specifications on attributes that are relevant for the product quality, hence streamlining activities, especially in case of accelerated scenarios. Such identification may be based on prior knowledge. For those attributes for which such knowledge is not yet available (e.g., efficacy- related product-specific attributes for new vaccines/ platforms), experimental studies may be performed, comparing the biological activity of the degradation products with respect to the target product [14].

In general, the use of prior knowledge may be limited by the diversity and complexity of vaccines. For instance, a given structural motif may have different criticality for similar antigens. An example is the O- Acetylation of meningococcal polysaccharides [15]. It is worth mentioning that the correlation between *in vivo*/ *in vitro* and clinical results

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is typically not available in early development, hence criticality confirmation studies at this stage of development have a comparative nature only (e.g., structural variant vs target antigen) and are not typically used for defining acceptance criteria for CQAs.

Despite these challenges, there are some very good examples of prior/ platform knowledge in the vaccines field, like the recent COVID mRNA vaccines for variants [16] and the Flu seasonal vaccine. In both cases, there is also evidence of the correlation between antibody titers and efficacy, which facilitates rapid assessment of new vaccines against different strains; it is important to note, however, that the protection is likely associated with multiple factors beyond antibody titers [17,18].

Definition of acceptance criteria for (stability-indicating) CQAs

As reported in ICH Q6A/B, acceptance criteria for specifications should also consider results from stability studies, as appropriate to ensure the specification is suitable for the product's shelf life. Such limits or ranges will also define the boundaries for other stability evaluations, e.g., transportation, in-use stability, etc. So how to define suitable acceptance criteria for release and shelf life?

In view of quality by design principles, specifications acceptance criteria should be set considering clinical relevance. According to a recent cross-industry paper on strategies for setting specifications for biotherapeutic products, clinically relevant specifications are "a set of tests and acceptance ranges to which product quality attributes should conform for the product to be safe and effective when used as labeled. Justifications for acceptance ranges focus on risk-based assessment of the impact to patients". "Commercial acceptance criteria based solely on statistical ranges may result in the rejection of an acceptable product or, if the process was historically highly variable, the release of batches that may be of unacceptable quality" [19]. This does not necessarily mean that every CQA range needs to be verified in a clinical study, but that a justification linked to safety and efficacy expectations is appropriate (e.g., based on prior knowledge, nonclinical models as relevant, dose-finding studies, quality characteristics of clinical lots...), in alignment with the principles of quality by design [20].

The same principles apply to vaccines. Clinically relevant specifications are particularly important in accelerated scenarios, where there is typically a limited number of lots to support statistically driven acceptance criteria, especially when limited platform/ prior knowledge is available. Also, since process optimization activities may be deferred, manufacturing flexibility can be supported by acceptance criteria set with a link with patients. In addition, this approach supports comparability assessment and lifecycle plans, when process improvements are planned after launch. Finally, patient-centric approaches avoid wasting good lots due to over-restrictive specification limits set with a limited number of lots and not fully representative of potential stability excursions/ manufacturing variability. For instance, COVAX Regulatory Advisory Group (RAG) reflections on COVID vaccines highlighted the following [11]:

> "since Phase 3 trials are generally used to demonstrate clinical consistency, there is a tendency to use lots that are relatively consistent in terms of quality attributes narrow specification ranges. The tighter the quality specifications are, the more likely batch rejections will be for potentially useful clinical lots. Hence, it is recommended that during early clinical development, sponsors should aim at established clinically meaningful ranges for specific CQAs. This would typically occur during dose-finding studies to support CQAs such as potency. When correlates of protection are not defined, perform a broader set of immunological assays, in coordination with regulatory authorities."

Dose-ranging studies may be designed to support evolving product knowledge and future changes during development and lifecycle, especially in case of accelerated scenarios. During development, the target antigen amount in the final product should be ideally higher than the minimum active dose demonstrated in the clinical trials of the antigen under study (if there are no safety concerns). This can support the justification of vaccine stability. As an example, during storage of a subunit vaccine, degradation could generate a structural variant impacting efficacy. If the actual antigen amount is lower than the target but still higher than the minimum active dose, the product will still be effective. Of course, control over the structural variants to appropriate levels (including stability considerations, as applicable) should be ensured [5, 14]. This dose selection strategy can also be of help to define clinically relevant specifications (lower limit) for potency testing, including shelf-life expectations. Although dose-ranging studies may often be helpful in setting product specifications to encompass product changes that may occur over the course of the product's shelf life, other changes may happen that require additional clinical ad hoc studies (e.g., structural changes not described by dose reduction). In such cases, to support the evaluation of acceptable CQA variations, it may be useful to study lots with different time of life/storage conditions, including them in clinical trials [21] or in nonclinical studies, where correlation with the clinical response is expected. In some instances (e.g., when there is limited prior knowledge and a short time to support full early product understanding), it may be useful to conduct clinical studies at the end of the product's intended shelf life, to demonstrate that there are no unaccounted or hidden variables that are changing that may affect efficacy.

Stability data packages

Appropriate information must be generated to justify the preservation of quality during manufacturing, shelf life, transportation, or in-use conditions.

For vaccines, the World Health Organization (WHO) estimated that cold chain breaks (i.e., excessive temperature excursions outside of the recommended storage conditions) are responsible for around 50% of vaccine wastage ([6] & references therein), pointing out how the temperature is one of the key parameters impacting the stability of vaccines [13]. For this reason, the considerations in this section will mostly focus to study the impact of temperature.

According to WHO and ICH guidelines, primary data to support a requested storage period (expiry) for either a drug substance or drug product should be based on long-term, real-time, real-condition stability studies, in support of INDs/IMPDs or marketing applications.

During accelerated development, it may be challenging to generate full real-time stability packages to support shelf-life assignment; therefore, stability evaluation exclusively based on real-time data may represent a bottleneck for rapid and global vaccine access.

In the context of cross-company discussions and dialogue with regulators, the use of prior and platform knowledge, along with the use of predictive stability modeling are considered key enablers of stability assessment in accelerated scenarios. Stability modeling is a well-established approach for small molecules [22], while for biologics specific reflection is needed, given the more complex kinetics involved, the necessity of an appropriate analytical characterization to understand the relevant attributes to monitor, and the demanding elucidation of the degradation pathways. The recent EMA toolbox guidance [10], as well as the 2018 EMA/FDA workshop on early access [5], describe opportunities for stability predictions for monoclonal antibodies, relying on platform knowledge, which is also reported in industry publications [23]. For a given vaccine platform (e.g., mRNA, viral vectors), prior knowledge elements could be considered for

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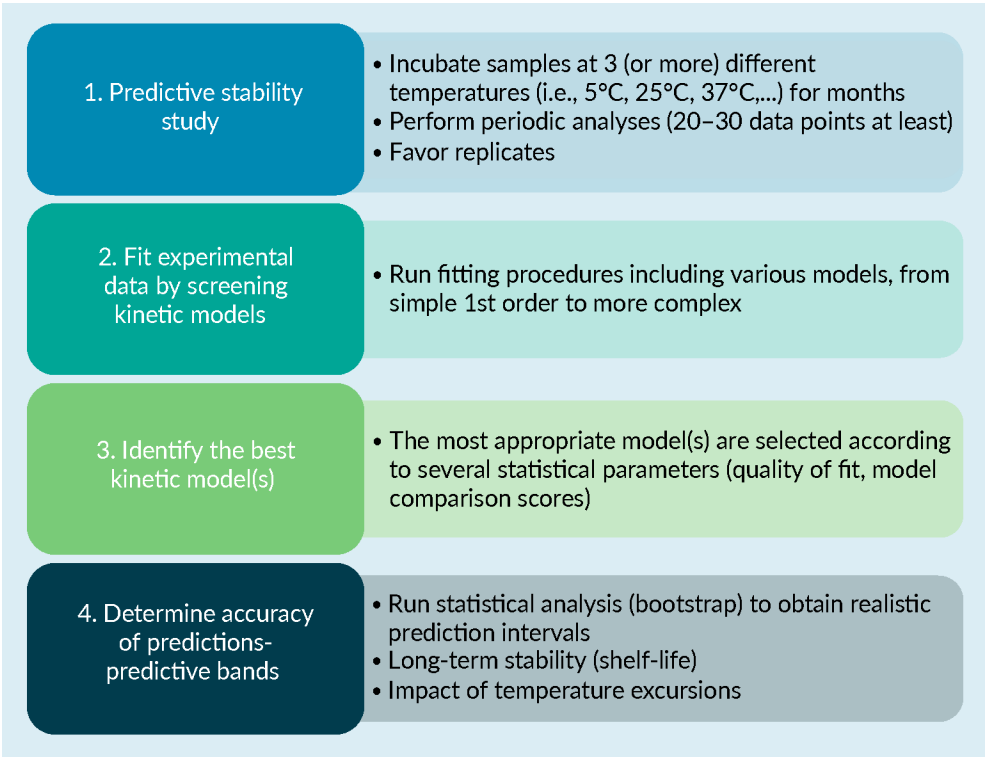
stability predictions. For instance, it can allow study design using the best time points and temperatures of interest or reduction of the required stability data; this has also been done for Flu vaccines [24] and proposed for viral vectors [25]. As demonstrated in a recent cross-company publication, tailored-modeling approaches are appropriate, depending on the monitored attributes and degradation pathways of the vaccine, ranging from first-order kinetics with simple-step activation to more advanced two-step models [6]. Figure 3 shows an illustration of this concept, as part of the best practices for modeling for vaccines, presented at the COVAX workshop on best practices for determining and updating storage temperatures and shelf-life [26].

Such advanced-kinetic modeling makes it possible to go beyond the current ICH and WHO recommendations for stability predictions of products. Furthermore, such models can also be used to adequately predict the degradation of products in real-time under standard storage conditions (i.e., 2 – 8°C) and under fluctuating temperature conditions (cold-chain breaks) [27–29]. Beyond the vaccine vial monitor (VVM) and extended controlled temperature chain (ECTC) initiative of the WHO, the integration of kinetic modeling in supply chain product management could dramatically improve the monitoring of the quality of vaccines during their shipping and use, averting or significantly reducing product wastage, even after experiencing minor excursions [6].

The emergency posed by COVID-19 has fostered further discussions on stability predictions for vaccines. For instance, the above-mentioned COVAX workshop dedicated to stability strategies highlighted the importance of using stability models for COVID-19 vaccine development and supply. In a WHO document on considerations for the evaluation of COVID-19 Vaccines, it is mentioned that [31]:

FIGURE 3

Best practices for stability modeling of vaccines.



Adapted from [6,26,30].

"with appropriate justification and discussion with the WHO, a scientific risk-based approach to determine the proposed vaccine shelf life in the absence of time stability data on the commercial batches may be considered. For example, data generated from smaller lots, such as clinical or engineering lots, and/or data generated on a different vaccine using a similar process and/or manufacturing platform, may be appropriate for submission in support of the initial recommended shelf-life for the vaccine. Consideration of platform stability data, prior knowledge from early clinical batches or statistical modeling may also be applied to forecast expiry of product"

Stability modeling may also help with the rapid introduction of vaccines for COVID-19 variants. For instance, in the EMA reflection paper on vaccines for COVID-19 variants [16], it is mentioned that

"confirmation of the suitability of the active substance and finished product registered shelf life needs to be demonstrated (e.g., by available real-time stability data, predictive stability models, early stability data under accelerated storage conditions). Confirmatory real-time stability data need to be provided post-approval."

Appropriate analytical strategy

As previously mentioned, extensive structural characterization warrants early understanding of degradation pathways and sets the basis for a fit-for-purpose analytical strategy for stability assessment, grounded on identification of (stability-indicating) CQAs and on the ability of analytical methods to detect changes during product storage, transportation, or distribution. In this context, the knowledge of analytical method performances is important to inform the interpretation of stability trends and is a key input for effective stability modeling strategies. During accelerated development, an appropriate analytical strategy is critical to fulfilling phase-appropriate product quality expectations, while product and process understanding evolve. This objective can be achieved upon pre-defining the performance expectations for attributes testing in the Analytical Target Profile (ATP), now described in the ICH Q14 draft, USP <1220> [32,33], and some recent literature [34,35]. The ATP can be constructed to include the total error (combination of accuracy and precision) considering product/process expectations and is technology-agnostic. For this reason, it sets the basis for technology-independent analytical procedure validation acceptance criteria and provides suitability criteria for the introduction of new analytical technology during development and across the lifecycle. For stability and specifications setting in general, reliance on ATP (and not on the specific analytical procedure) minimizes the risk of changes in specification ranges or stability trends due to analytical procedure/ technology changes.

Another crucial element for a robust analytical strategy during stability evaluation is the use of appropriate reference standards, which are critical especially for biological assays. The stability plan should therefore include verification of the best storage conditions not only for the product but also for reference standards (at DS and DP level), ideally more protective than those considered for the vaccine product commercial distribution. Lots used in the clinical trials that established safety and efficacy serve as ideal reference standards for (potency) testing and comparability studies of PPQ and early commercial lots. In this context, these clinical reference lots should be stored for as long as possible, to help in understanding potential issues with manufacturing or potency testing at early commercial stages. Reference standard characterization and storage strategy are therefore key, in accelerated scenarios, for risk mitigation in case of potentially incomplete product & process development studies, and for the execution of comparability studies [13, 36].

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CONCLUSIONS

This article has provided an overview of recent developments related to the stability assessment of vaccines, focusing on accelerated scenarios, considering the current dialogue between Industry, Regulators, and Institutions like WHO and CEPI. Challenges and opportunities have been discussed, focusing on early product understanding, use of prior knowledge, robust modeling approaches for stability prediction, and smart analytical and reference standard strategies. The implementation of these tactics, facilitated by early dialogue with Regulatory Agencies, will be an enabler of rapid access to new vaccines without compromising safety and efficacy.

TRANSLATION INSIGHTS

Product understanding, prior knowledge, and advanced modeling approaches allow reliable assessment of vaccine stability behavior and shelf life. Multiple examples mentioned in the text and references evidence that such risk-based approaches are successful for different vaccine platforms, and indispensable to enabling rapid and global access to vaccines. It is of fundamental importance to divulge examples of novel approaches for vaccine stability evaluation, as this will foster trust and discussion with Regulatory Agencies and WHO. Such dialogue can be facilitated by individual vaccine developers through early engagement of the relevant Regulators. The most powerful approach, however, is the discussion of cross-company experience and approaches, to enable awareness of the industry and regulatory agencies' needs, foster technical competencies building proactively, and discuss the level of risk/ benefit associated with the acceleration options. As illustrated in this manuscript, some progress is being observed in this context, although much effort is still required to gain broad acceptance and harmonization of expectations in different world regions.

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AUTHORSHIP & CONFLICT OF INTEREST

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SPOTLIGHT

COMMENTARY

A fresh look at analytical methods for vaccines

Timothy Schofield

Recent guidelines have promoted a lifecycle approach to analytical methods. Mimicking a similar paradigm in vaccine product development, this draws attention to a science and risk-based pathway and tools which help ensure successful analytical method development and lifecycle management. However, adoption and implementation of this approach faces serious hurdles, some cultural and others regulatory. This article will describe some key elements and give notification of challenges which must be considered as the vaccines industry and regulators embark on implementing a lifecycle approach to vaccine analytical methods.

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INTRODUCTION

Recent guidelines have invited industry and regulators to adopt a lifecycle approach to analytical methods [1–4]. This follows nearly two decades of change in product development and lifecycle management [5–8]. Similarities between analytical and product approaches may be driven by the viewpoint that an analytical method produces a product for a customer [9,10]. In this case the product is a reportable value from a procedure using a method (i.e., the final result from using the method such as a release value or a stability slope) while the customer is a decision maker who uses this to address the goal of a study. A parallel to product quality thus relates to the quality of the reportable value which impacts on the statistical risk (i.e., probability) of making the wrong decision.

In fact, a lifecycle approach to analytical methods directly parodies the lifecycle approach for a product. Both follow a development paradigm called quality by design

(QbD) which emphasizes science and risk-based methods to "build quality into a product" [11]. It is this perspective that uncovers strategies and tools that are common to vaccine product and analytical development and lifecycle management.

While guidelines don't make a distinction this article will differentiate the analytical method from an analytical procedure [12]. Briefly a method is the wet chemistry, design, calculations, and controls which yield a measurement. A procedure is a 'study' using a method which results in a reportable value or result. Following this an analytical method is fit for use if it can be developed into a procedure that leads to quality to the decision maker (and ultimately to patients). This may be a process characterization procedure that is used to identify and define process controls, or a release procedure that is used to clear vaccine lots for use in the clinic or into the market. While seemingly unrelated these and other procedures are employed throughout development and lifecycle management to help ensure quality of vaccines.

This dichotomy is useful for distinguishing method versus procedure design. Method design includes the framework of the method (e.g., the technology, sample and standard preparations, calculations, and system controls), as well as optimization of method parameters. A procedure is designed with the aim of minimizing uncertainty and the statistical risks associated with making a decision. Thus, a release procedure can be designed using replication to minimize uncertainty of the average [13], while a stability procedure can be designed with adequate replication and strategic time points to minimize the uncertainty of the regression slope. The two come together to design a procedure which ensures that a lot is within specifications at release and throughout the vaccine shelf life [14].

Other terms which will be used throughout this article are method parameter (a condition which can be controlled or monitored such as pH), performance characteristic (a performance descriptor such as accuracy, precision, or total error which combines bias and variability), study (synonymous with procedure), decision maker (the customer of analytical results), uncertainty (a measure of the quality of analytical results, which is associated with statistical risk), and statistical risk (the probability of drawing the wrong conclusion from the a procedure using a method). The term specification will be taken to mean the acceptance criterion for a reportable value.

This article will begin with an examination of specifications and their role in the vaccine analytical method lifecycle. Elements of the lifecycle approach will be discussed, including knowledge management, the analytical target profile, lifecycle stages, and the analytical control strategy. This will be followed by a summary of some statistical opportunities and will end with some viewpoints on clearing the way for a lifecycle approach to vaccine analytical method development and validation.

SPECIFICATIONS & THEIR ROLE IN THE VACCINE ANALYTICAL METHOD LIFECYCLE

Specifications are an expression of vaccine quality. This requires an unambiguous definition of quality. At its root quality is associated with value to the patient; i.e., safety and efficacy of a vaccine. This association and its relationship to other development concepts is illustrated in Figure 1.

In this illustration models [f(x), g(y), etc.] are experimentally derived (and using prior knowledge where appropriate) across areas of development and trace to a common goal – satisfactory patient outcomes. Each model is jointly developed between corresponding functional areas. Working backwards through each model yields limits which predict satisfactory patient outcomes:

1. A definition of satisfactory patient outcome (e.g., equal to 95% efficacy) is translated to a limit on a vaccine biomarker (correlate of protection);

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2. The correlate of protection is used in vaccine clinical studies to define a limit on a critical quality attribute (a specification limit);

3. The specification limit is used to define limits on critical process parameters (a design space).

It is noteworthy that method, process, and formulation development, as well as 'lifecycle management' (a ranged dedicated to addressing product and method changes; see Change Management) share the specification limit range and their impacts must sum up to fit into that range. This can be characterized as a form of development planning whereby each function develops towards their allocated budget (see Figure 2) [15,16].

Thus, analytical development must ensure acceptable accuracy and precision at release (blue vertical arrows), formulation development must ensure adequate stability throughout the vaccine shelf life (blue sloped line), process development must ensure on-target performance and acceptable variability (normal process distribution), and lifecycle management must ensure manufacturing stability within the release limits (areas between control and release limits). Thus, limits in Figure 1 (e.g., or Design Space) are derived from the appropriately budgeted portions of the specification range.

Several of the models in Figure 1 (and their resulting limits) represent current practice in vaccines development. The identification of correlates of protection is carried out in Translational Medicine while a design space is the outcome of Process Characterization. Typically overlooked, however, is the model linking critical quality attributes and clinical biomarkers. In order to make that link CMC and clinical development must make common cause to bridge their areas of development.

Accurate coupling across CMC development is often compromised by the use of different methods (or the same method without adequate bridging). To realize the vision in Figure 1 CMC methods should be standardized to the same measurement scale (i.e., similar units; ideally defined by the units of the specification) across functions.

A significant issue, however, is lack of agreement about the basis of specifications. Specifications practices should be examined and harmonized to facilitate implementation of the vaccine method lifecycle (and development as a whole). A common practice is for the company to wait until the end of development to calculate specifications from product variability [17]. This is reinforced by regulatory expectations. It is difficult in this case to define the requirements needed to guide analytical (or process and formulation) development. Said otherwise, without an early vision for product specifications in the quality target product profile or QTPP [5] there is limited basis for product and analytical development.

These issues not withstanding the development of the method control strategy, seen linked to the specification in Figure 1, will be illustrated later in the article.

ELEMENTS OF A LIFECYCLE APPROACH

The lifecycle approach has been described in 3 stages:

1. Method design and development;

2. Method qualification (i.e., validation);

3. Continued performance verification [1].

This construct masks, however, the view that the lifecycle approach is part of a continuous process, beginning with identification of a critical quality attribute which needs to be controlled, and addressing this through selection, design, control, and maintenance of an appropriate method.

While these stages have their counterparts in classical method (or procedure) development, validation, and maintenance, in a lifecycle approach they are seamed together with line of sight towards uses of a method.

The gains realized through this approach lead to a more robust method coupled with knowledge that is gained, preserved, and utilized throughout its lifecycle.

This section will describe important elements of this approach and explore their applications in a broader context.

Knowledge management

Knowledge management is the foundation for a lifecycle approach, either as a reservoir of prior knowledge used for method development or as a basis for continued learning that brings additional knowledge and supports lifecycle management. Distinguished from a classical approach of documenting method performance at points in time (e.g., pre-validation, validation, and revalidation), information obtained throughout the lifecycle can be used to evaluate method performance in real time, and to improve an established method.

Knowledge management is also key to development of 'platform methods'. Platform methods might be viewed as 'plug and play' where a method is utilized across vaccine programs. Thus, the accumulated knowledge gained from previous applications of a method can be used as prior knowledge, to expedite method development in subsequent programs. Performance characteristics such as method precision may be agnostic to the vaccine being tested. Thus, previous optimization related to precision need not be re-performed, while conformance to the ATP can be verified using routine analytical control.

A vision for knowledge management should anticipate the kinds of data that will be useful throughout the method (or platform) lifecycle. This includes identification and capture of method parameters (pH, incubation time, temperature) and metadata (analysts, reagents, equipment) that link parameters and components to method performance.

The knowledge gathered throughout the method lifecycle can be used to evaluate performance characteristics (e.g., accuracy and precision) against expectations. Those expectations are captured as part of the analytical target profile.

The analytical target profile

The vaccine method lifecycle is guided by an analytical target profile (ATP) [1,3,10,11]. Without this there is no basis for judging success of method and procedure development, nor for guiding method and procedure quality throughout its lifecycle. The ATP serves several purposes:

1. It expresses requirements on performance characteristics of a method when it is used in a procedure to make a decision; and/or
2. It acknowledges business requirements such as throughput, timeliness, and capabilities in a production laboratory. While some organizations consider business requirements to be out of scope for the ATP, putting these together with performance characteristic requirements allows the laboratories to balance the costs and benefits associated with technology selection versus development effort.

Due to its role in specifying requirements on performance characteristics when a method is used to make a decision, the ATP applies to a procedure, a use of the method. An informal definition of the requirements in an ATP might be 'the reportable value from a procedure with a 'combined bias and variability' should not fall outside a given acceptance criterion with more than a low pre-specified 'probability'. Taking this apart, 'combined bias and variability' represents the requirement and can be taken to be on bias and variability separately, or together as total error; the 'acceptance criterion' can be viewed generically as a decision rule associated with a procedure; and the 'probability' is the maximum statistical risk of making the wrong decision when using a procedure.

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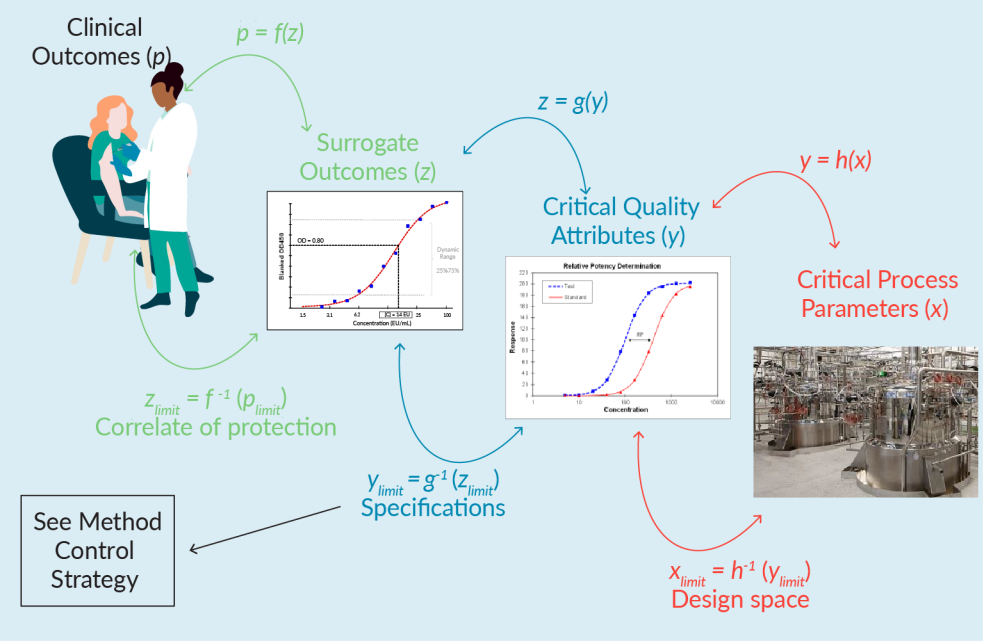
FIGURE 1

Bridging quality across vaccines functions.



An ATP can be formulated to support vaccine development. Thus, for example, requirements may be placed on a procedure supporting process characterization, where DOE studies are performed to identify critical process parameters (CPP's). The process characterization procedure can be designed to detect a difference in response to changes in process parameters, which is the basis for CPP determination. In this case 'difference in response' is the ATP of the process characterization procedure. This and similar procedures (which will be discussed in the section on Change Control) can be designed based on method variability, an acceptance criterion, and a limit on the level of statistical risk [18].

Developing the ATP for a commercial release procedure is less straightforward. This is especially the case when the specification limits are calculated late in development from manufacturing variability, where the release assay variability is a component of the overall variability seen of the manufactured lots. This is further complicated since the process and the assay have limited long term history, thus restricting the scope and experience of the calculated limits to process and method variability (or when combined, manufacturing variability) to a short and early period of time. Finally, accepting the view that the release procedure ATP is a guiding principle in method (or procedure) design and development, the entry of specifications late in the process complicates the use of the ATP for its intended purpose (i.e., guiding method development). An early ATP might be built on initial assumptions, then adapted or improved as product and process knowledge become available.

Given a lack of an ATP an alternative approach might be to base method development on the expected "capability of the art" of the technology (e.g., HPLC or binding) and method design, or based on prior knowledge (e.g., from a well-established platform). The method is then optimized to meet this expectation. Once optimized the method can be "qualified," using multiple ruggedness factors to forecast long term variability [19,20]. In this case the qualification is a precision study, yielding information which is useful for procedure development. In the spirit of "building quality into

the process" the ATP for the commercial release procedure can be satisfied through procedure design (i.e., a replication format) once a commercial specification has been established [19]. Once established release procedure performance can be monitored and addressed through continued verification.

The method control strategy

Like a product control strategy, the method control strategy (MCS) is based on accumulated knowledge related to method performance and control, and line of sight to the method lifecycle. Defined simply, the MCS is the combination of method parameter and attribute controls, as well as studies supporting routine method changes (e.g., transfers, standard qualifications, and technology bridging). As important, it should be viewed as the source for continual method (or platform) knowledge.

The MCS will be broken down into two parts, method control and change management. These will be described from the point of view of a method ATP. While it is acknowledged above that the ATP refers to requirements on a procedure (here, a release procedure), for this discussion it is assumed that the release procedure is fixed and that requirements on the method can be derived from requirements on the release procedure.

Method control

Similar to the view of overall product development (Figure 1), control elements of the MCS can be derived from models between critical method and suitability parameters, and performance characteristics (see Figure 3).

In this depiction the specification limit (or the analytical budget,) is used to define the ATP , where v and w represent performance parameters such as accuracy and precision. The relationship between a critical method parameter (u) and performance parameters can be used to derive a method parameter limit (, in red), while the relationship between a suitability parameter (s) and the performance parameters can be used to derive a system suitability limit (, in green).

The model relating critical method parameters and performance characteristics [$(v,w)=k(u)$] can be established utilizing multifactor design of experiments (DOE) [21]. Applying the ATP (or the analytical budget, accounting for method replication) to the estimated model yields the method operable design region (MODR).

System suitability parameters provide additional control. Like critical parameter parameters, these can be established through a model between performance characteristics and similarity parameters, also driven by the ATP (or the analytical budget, and accounting for method replication). It is noteworthy that the limits on suitability parameters can be used as proxies for performance characteristics in the determination of the MODR. Thus, for example, rather than replicates at points in the method optimization design, a limit on the slope of the calibration or dose response relationship might be used as a proxy for precision and to define limits on critical method parameters.

Change management

Parameter and attribute controls act to ensure satisfactory method performance on a run to run basis. However, vaccine analytical methods are subject to routine changes over their lifecycle. Those changes should be supported by studies which demonstrate lack of impact on the decisions made using a method. While holistically change management is conducted using prior knowledge about a method as well as studies driven by the statistical risks associated with the change, this section will discuss only the latter. It is noteworthy that both can be combined to implement both an efficient and effective change management exercise.

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The studies supporting a method change might be classified either a 'qualification procedure' or a 'calibration procedure'. Qualification procedures seek to demonstrate the lack of a 'meaningful difference' in method performance due to the change, while calibration studies are used to derive a 'calibration factor' which can be used to adjust results or design of the method.

The qualification procedure can be formally designed and analyzed using an equivalence test together with, or instead with a noninferiority test [22,23]. The basis of such a test is an equivalence (or noninferiority) margin which must be statistically satisfied to conclude that the change has "no impact." When specifications have been formulated scientifically rather than calculated from manufacturing variability a basis for the equivalence margin is taken from Figure 2 (see Figure 4).

In this depiction the manufacturing distribution can move by an amount labeled delta (Δ, the equivalence margin) before there is unwanted excess statistical risk of failing the release specification limit (shown as a small red area below the lower release limit). Here, the equivalence margin represents the requirement in the qualification procedure ATP. The qualification procedure ATP might include a noninferiority margin on variability alongside of the equivalence margin on the target, or a requirement on total error [22,24].

The equivalence procedure is designed and carried out as a 'two one-sided test' (TOST) by showing that the 90% confidence interval on the difference in results between the comparison groups (e.g., two laboratories in a transfer) falls within the equivalence margin (Figure 5).

While an equivalence approach is useful in the case where the laboratory wishes to conclude no impact due to a change (e.g., method transfer and technician qualification), a calibration procedure can be used to 'adjust' the method to assure continuity. This is appropriate for a change in a material component, when the source is subject to high or unknown variability. The calibration procedure proceeds with a design to estimate a difference between materials (e.g., new and current standards), or of the level of a method component which regularizes the method (e.g., a dilution of a reagent that generates overlapping standard curves). The calibration procedure ATP specifies the maximum uncertainty allowed in the determination of the calibration factor, and resultant estimated endpoints. This is identical in effect to the qualification procedure, with the calibration procedure ATP requirement equal to the equivalence margin.

While these change management practices are not new to the vaccine analytical laboratories, adherence to the principle of

FIGURE 2

Analytical limits and functional budgets.

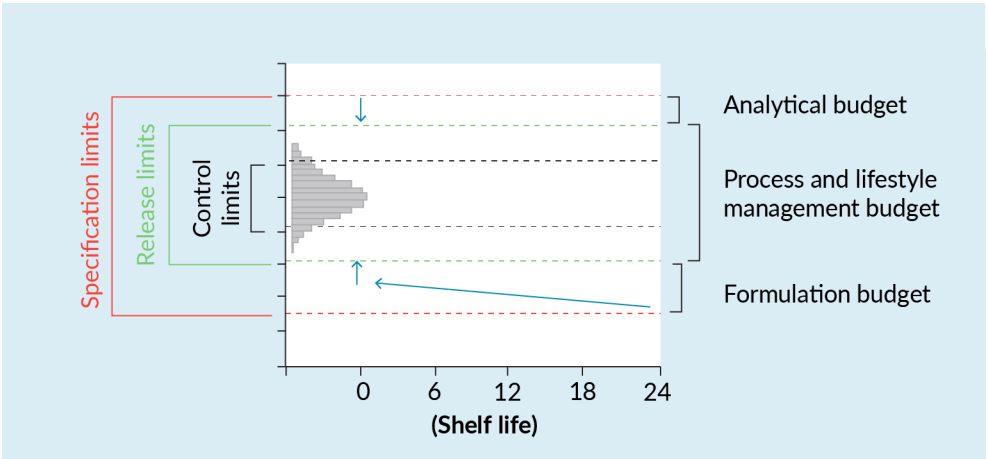
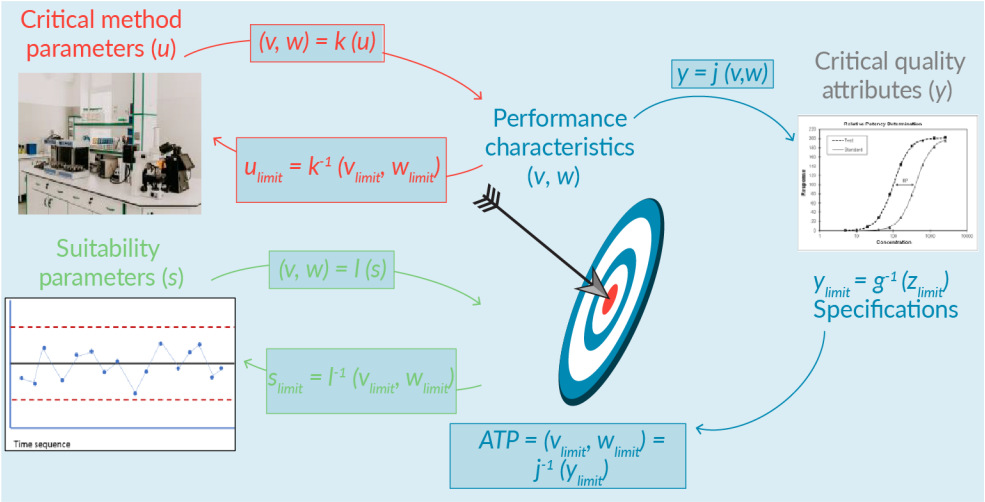


FIGURE 3

Control of a method through method (*u*) and suitability (*s*) parameters, which are driven by the ATP (y_{limit},z_{limit}).



risk management invites the laboratory to consider appropriately defined acceptance criteria (formulated as a change management procedure ATP), sound experimental design, and appropriate analysis in order to control the statistical risk associated with putting the change into practice.

Some statistical opportunities

The term statistical risk has been used throughout this article to mean a statistical probability of an unwanted outcome. This can be the risk of an undesirable patient outcome, the risk of a reportable value being outside of specifications (OOS), or the risk of a negative study outcome.

Statistical risk is directly proportional to uncertainty in a reportable value (e.g., a release value or a difference between laboratories). Thus, risk can be minimized by controlling uncertainty. The statistical basis for controlling uncertainty is procedure design. In its simplest form the uncertainty (U) in the reportable value (RV) from a release procedure which generates an average from n-independent measurements, when the underlying method variability is equal to sigma (σ) is: $U\_RV=t_{\alpha,\ n-1}\ (\sigma/\sqrt{n})$. The factor is a statistical constant associated with a probability equal to α and with n-1 degrees of freedom. The statistical constant decreases with an increase in n; so also, the ratio decreases with larger n. Thus, overall uncertainty decreases with larger n. This is discussed in greater detail for a release procedure in [11] while a broader understanding can be obtained in most textbooks on design of experiments. Uncertainty is a powerful tool for assessing the risks associated with use of a procedure, or more appropriately for designing a procedure to minimize statistical risk.

While statistical design principles are useful for implementation of some elements of the vaccine method lifecycle, of equal value

FIGURE 4

Lifecycle management budget and equivalence margin delta (Δ).

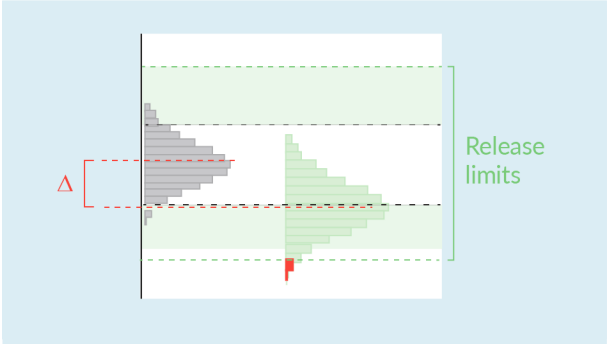


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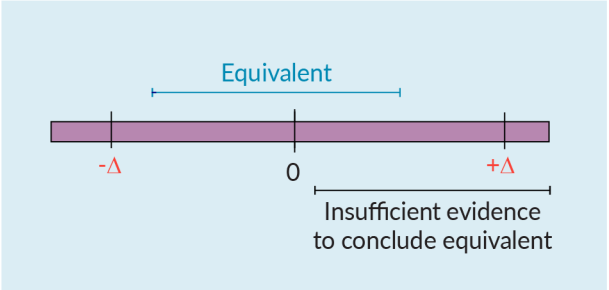
FIGURE 5

Illustration of TOST showing a confidence interval fully inside of the equivalence margin (±D) and partially outside, indicating equivalence and not.



is a statistical basis for incorporating prior knowledge into decision making. This falls into the area of Bayesian statistical methods. Put simply, Bayesian methods combine prior knowledge with information from a study (procedure) to generate probabilistic predictions about a question of interest. Practically speaking Bayesian methods can reduce the need for prohibitively large studies by 'borrowing' information from previous experience. This also produces answers in the language of statistical risk, or probability, which can be used to make internal decisions or to communicate the basis of regulatory decisions to authorities.

CLEARING THE WAY FOR A LIFECYCLE APPROACH TO VACCINE ANALYTICAL METHODS

In this article a framework has been described along with some elements of a lifecycle approach to vaccine methods. Various weaknesses have been alluded to (uses of different methods across laboratories) and contingencies (the basis of specifications) that will challenge the vaccine analytical laboratory as it transitions from traditional method development and validation to a lifecycle paradigm. These and other challenges need attention as a basis for clearing the way for implementation.

Before going further with challenges, however, it is worth pointing out some advantages of a lifecycle approach. At one level the lifecycle approach is a template for method development, validation, and maintenance. It encourages a vaccines organization to focus on the goal together with the principles and tools that can be replicated across methods and vaccines programs. This together with an emphasis on prior knowledge and platform methods provide the opportunity to deliver robust methods and procedures more efficiently for accelerated as well as standard vaccine programs.

Much of the savings occur after a method has been developed and put into commercial use. A procedure using a robust analytical method and supported by risk-based change management is less likely to generate false OOS results (and be more sensitive to true OOSs), while a strategically crafted method control strategy can help preserve method performance and supply information useful for improvements and platform development. This positively impacts on costs of discarded materials, OOS investigations, and unanticipated regulatory interactions. Further efficiency can be had through the filing of established conditions [7], whereby an ATP coupled with a change management plan replaces method details which when altered are subject to lengthy prior approval.

Challenges to implementation

Advantages not withstanding, some challenges (real or perceived) require attention prior to implementation of the lifecycle approach.

A significant challenge within industry relates to development and commercial siloes. As previously illustrated (Figure 1) it is necessary for development functions to coordinate on the principle of quality. Modeling CMC and clinical outcomes leads to meaningful targets for analytical as well as product development. 'Throwing the process and methods over the wall' should be replaced with technology transfer accompanied by 'feed-back and feed-forward'. This approach includes anticipation of commercial

challenges during development and facilitates commercial product and analytical investigations and improvements. These and other commercial experiences serve also to build on method knowledge which should be co-owned between development and commercial laboratories.

Barriers exist also due to lack of harmonization in guidelines and expectations. Some rules are ambiguous, leading to development driven by risk aversion over science. This is no less evidenced than in the FDA Guidance on Investigation of OOS Results [25]. While scientific principles support the use of replication to reduce uncertainty and the inherent statistical risk in managing quality against specifications [13], the OOS guidance states: "In cases where a series of assay results (intended to produce a single reportable result) are required by the test procedure and some of the individual results are OOS, some are within specification, and all are within the known variability of the method, the passing results are no more likely to represent the true value for the sample than the OOS results. For this reason, a firm should err on the side of caution and treat the average of these values as an OOS result, even if that average is within specification".

As described in this article planned replication and averaging should be used to good cause, to reduce uncertainty and statistical risks associated with making the wrong decision. This is particularly true of vaccines methods such as bioassay, which are best managed through standardization (relative potency) and replication [19]. The FDA guidance engenders a disincentive for use of sound design principles [26]. For this reason, the pathway to adoption of a lifecycle approach, including appropriate use of averaging, can be facilitated with further refinement of this guidance.

A less obvious ambiguity is related to validation, i.e., should a company 'validate the method' or 'validate the release procedure'. Some vaccine release procedures are considerably burdensome due to the type of method or the use of replication to manage decision risks. This poses the choice of whether to validate the procedure (with replicates of the method) and reduce the number of study factors or validate the method with a design inclusive of relevant long-term factors. The former is a 'test of the release procedure' while the latter treats validation as an opportunity to predict long term performance of a vaccine method, and to use the results of the study to design procedures using the method.

Finally, practices and expectations related to introduction of advanced technologies should be examined. Current practice is to submit a prior approval change request for review by authorities. The effort and amount of time for global agreement creates a disincentive to innovation. A focus on method performance (the ATP with requirements on specificity as well as accuracy and precision) rather than the technology and design will open the door to improvements inherent in the adoption of advanced technologies.

Overall, adoption of a lifecycle approach to vaccine methods requires more focused attention on scientific and risk-based principles. The practice of 'building quality into the process', during design and development, and maintaining quality through sensitive markers of method performance and strategic change control should replace 'testing in quality' through validation. Cultural barriers within a company, and between vaccines companies and regulatory authorities must be identified and resolved, while ancillary practices such as specifications must be examined to smooth the pathway to implementation. The motivation for industry and regulators should be enhanced knowledge and improved control of vaccines, leading to improved vaccines safety, efficacy, and supply.

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VACCINEINSIGHTS

CMC & ANALYTICS

SPOTLIGHT

EXPERT INSIGHT

Considerations for the stability of vaccines as liquid-, frozen-, or lyophilized presentations

Ramin Sabet Azad & Raafat Fahim

An important aspect of vaccine process development is that the final product maintains its stability specifications throughout its intended shelf life. Storage of vaccine products at temperatures of -20°C and lower may be readily available in High- and Upper Middle-Income Countries (HIC) but such storage- and distribution conditions may not be widely available in Low- and Middle-Income Countries (LMICs), which may hinder their usability for addressing the needs of such regions. Thus, enabling equitable access to vaccines in LMICs may be best achieved by ensuring long-term stability of vaccine drug products at more readily available cold chain temperatures of 2–8°C or above. This in turn necessitates an assessment early in vaccine development of the appropriate steps to ensure that the desired stability is achieved. This document is intended as a potential guide to vaccine developers when considering storage conditions appropriate for their intended use.

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The COVID-19 pandemic has brought to light the challenges of equitable access to vaccines that organizations such as the Coalition of Epidemic Preparedness Innovations (CEPI) are addressing. While financial-, vaccine nationalistic- and geopolitical challenges are among the main causes of such disparity, vaccine process development initiatives can play a role in facilitating equitable access by providing vaccine drug products with stability characteristics suitable for countries with limited cold chain capabilities

and/or distribution infrastructure. Thus, it is important that vaccine developers of all platforms (protein, nucleic acid and viral vectors) integrate formulations with favorable stability characteristics at readily available temperatures to facilitate access of vaccines to LMICs. Commonly for all vaccine platforms, developers must demonstrate that a vaccine candidate's stability attributes including potency and quantity are within specification over the product's intended shelf-life as well as confirming its identity [1,2]. Stability studies of the vaccine product must also include measurements of potency and quantity under accelerated conditions to determine the highest temperature at which the product is sufficiently stable for a suitable period of time and to potentially allow modelling of real-time stability.

There are numerous strategies that can be implemented for understanding vaccine storage and transportation limitations and thus potentially improving the stability of vaccines during vaccine development. Kinetic modeling based on data from accelerated stability studies can be performed to understand optimal storage conditions, temperature excursions (cold chain breaks) and product degradation in various formulations [3]. Optimisation of formulations can be performed by the addition of excipients such as sugars, salts and peptides to the final product [4].

A widely used and readily available strategy to increase the stability of vaccines and in particular viral vaccines at higher temperatures, is lyophilisation. Lyophilisation, also known as freeze-drying, is a process where sublimination (transition of a solid to gas without passing the liquid state) is achieved at very low pressures, thus limiting degradation/denaturation and hence loss of potency of the vaccine candidate as no heating is required [5]. The lyophilised powder can then be reconstituted with a diluent at point of use.

Lyophilisation as a strategy to increase stability of vaccines should be considered carefully as it may not be appropriate for all vaccines. As such, it is recommended to assess the need for lyophilisation early during drug development and revisit it continuously as data is collected for the vaccine candidate and the associated manufacturing process.

Figure 1 is intended as a general guide for such an assessment and will be referred to throughout this text. It should be noted that deviation from the proposed guide is expected in some cases since products and their processes differ due to their specific manufacturing requirements. Moreover, the criticality of vaccine stability under certain circumstances should be considered. For example, during a health emergency outbreak, a pandemic or other emergency response situation, regulatory requirements might differ and therefore a shorter shelf life for a liquid or frozen vaccine could be more acceptable and may obviate the need for a more stable lyophilised products.

In general, whenever possible, liquid vaccines are always preferable for numerous reasons; lyophilisation of vaccines require extensive additional development work, acquisition and validation of lyophilisation equipment (or external lyophilisation capacity), could lead to high losses in yields, require lyo- and cryoprotectants, and introduce additional complexities related to reconstitution of the vaccine product at the clinic. In many situations , a liquid vaccine presentation stable at 2–8°C for a minimum of 6 months after release might not need any further stability optimisation and can be accommodated in existing cold chain managements (Q1, in Figure 1). On the other hand, during outbreak emergencies, a shorter shelf life at 2–8°C may be acceptable from a regulatory perspective [6], as long as vaccine safety and efficacy can be demonstrated through appropriate studies. However, as evident by the COVID-19 pandemic this short shelf-life may not be practical as it could lead to challenges during distribution of vaccines in some LMICs where the infrastructure is not equipped to handle the required roll-out for a vaccine with a short shelf life [7]. It is therefore advisable that small scale lyophilisation

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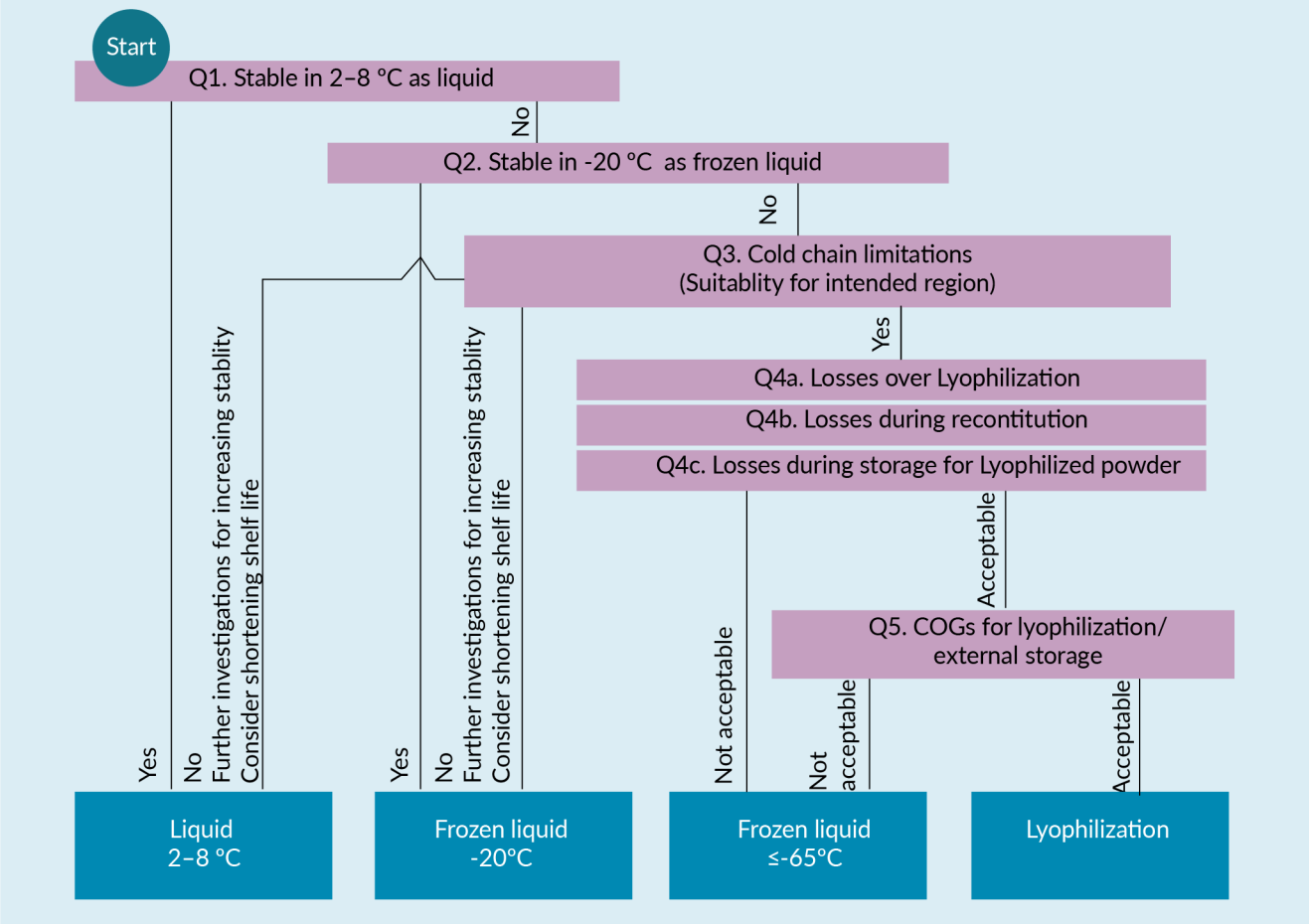
FIGURE 1

Guidance tree for vaccine developers to assess optimal presentation of vaccines as either liquid-, frozen or lyophilised product to maintain stability specifications throughout the vaccines intended shelf life.



COGs: Cost of goods.

development studies be initiated if the liquid vaccine is shown to lose stability during short term storage of 3 months or less at 2-8 °C. In parallel other stability optimisation development such as evaluating different liquid formulations should be investigated.

If stability of the liquid vaccine product cannot be demonstrated at 2–8°C for 6 months, a lower temperature of -20°C can be considered for long term storage (Q2, in Figure 1) followed by short stability at 2–8 °C for 3 months. Hence, a shelf life of 12 months or longer as frozen liquid at -20 °C in facilities equipped to accommodate such a temperature which is then followed by 3 months or longer at 2–8°C in LMICs may be acceptable. Obviously, the stability studies would require the inclusion of formal studies to demonstrate stability at 2–8 °C following freezing at -20°C and may include repeat freeze thaw cycles.

In situations where stability can not be demonstrated for liquid vaccine products at 2–8°C for a reasonable duration or as frozen liquid at -20°C for long term storage followed by 2–8°C for a short duration, then cold chain distribution limitations in the intended region(s) should be considered (Q3, in Figure 1). As such, if the target disease is endemic to regions where the distribution infrastructure is advanced (such as in High Income Countries), developers should consider filing for licensure of the vaccine with a shorter shelf life while simultaneously

optimising the vaccine formulation so as to increase the shelf life of the drug product to acceptable levels as discussed above.

In healthcare emergency situations in LMICs where storage- and distribution at low temperatures are limited and further stability optimization of the liquid formulation have not been successful, development of a lyophilised product is warranted. This may be appropriate, despite the limitations of lyophilisation regarding product losses, during the lyophilisation process itself (Q4a, in Figure 1), after reconstitution of the lyophilised product at time of use (Q4b, in Fig 1) as well as during storage of the lyophilized product at 2–8°C (Q4c, in Figure 1). Collectively, these losses need to be assessed in relation to overall process yields of the vaccine. This is important since higher losses result in lower number of doses per batch, which in turn leads to higher number of batches to meet the demand and ultimately decreased capacity and increased Cost Of Goods (COGs). In addition, as seen during the COVID-19 pandemic, potential supply chain challenges could also be expected related to shortage of manufacturing capacity and availability of raw materials [8]. Moreover, developers must consider the additional complexity introduced at point of use of reconstituting lyophilised vaccines using appropriate diluents. Studies have demonstrated that the risks of immunisation errors are higher when practitioners are required to reconstitute lyophilised vaccines, compared to handling liquid vaccines [9,10]. It is of importance that validation studies are performed demonstrating the stability of a reconstituted vaccine product for the duration of its utilization period after reconstitution, as outlined by WHO Guidelines [11].

If recovery of vaccine activities is acceptable over the process of lyophilisation and over the intended shelf life, cost of goods sold (COGS) and capacity limitations should be finally evaluated (Q5, in Figure 1). If COGS of lyophilisation is relatively high and/or lyophilisation capacity is limited then lyophilisation may still not be appropriate. . In this case, stability and suitability of a liquid frozen presentation should be compared to lyophilization considering the availability and costs associated with storage at freezing temperatures (transport, energy requirements and space) [12] relative to the capacity and costs associated with lyophilization.

Like all process steps in a manufacturing process, the regulatory impact of lyophilisation should be taken into consideration. It is essential that the developer follows the correct regulatory guidelines for process, equipment, and cleaning validation [13], which clearly adds another level of complexity. Furthermore, the addition of lyo- and cryoprotectants lead to a different excipients profile of the drug product.

Ensuring equitable access of vaccines require products that are sufficiently stable for use in the intended countries (HICs, UMICs as well and LMICs). Stability and storage requirement in the Target Product Profile (TPP) of the vaccines can differ depending on the use of the vaccines for routine immunisation versus outbreak health emergencies. Other process related aspects and financial considerations may influence the choice of introducing lyophilisation as a means of increasing the shelf life of a vaccine.

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VACCINEINSIGHTS

CMC & ANALYTICS

SPOTLIGHT

INTERVIEW

Advancements in assay & analytic development in the vaccines field

Charlotte Barker, Editor, BioInsights, speaks to **Jessica White,** Vaccine Formulations Team, PATH, about her career in immunology and infectious disease, and her recent work on *in vitro* inhibition ELISA assays.

JESSICA WHITE is a virologist who has been working at PATH for the past 10 years on the Vaccine and Pharmaceutical formulation technologies and the Chemistry and Manufacturing Controls teams. She has a PhD in Comparative Pathology from University of California, Davis and completed two postdoctoral positions at University of Washington as a research fellow in Infectious Diseases and Immunology and one at PATH in vaccine formulation. Jessica supports the development of vaccine candidates for Shigella, E. coli, Rotavirus, Measles and Rubella, Novel Oral Polio Virus, Non-Salmonella Typhoid, and SARS CoV-2. To improve global access to these critical immunizations, her team specializes in producing novel thermostable and needle-free vaccine formats, such as fast dissolving tablets. Currently, Jessica is leading the work to produce and characterize monoclonal antibodies specific against the SARS CoV-2 variants for use by vaccine manufacturers and researchers.

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Q What initially sparked your interest in immunology and infectious disease, and how did you specifically end up working in vaccination?

JW: I have always been interested in the details of how things work, specifically in adaptations to increase survival. The push and pull relationship between host and pathogen is interesting to me, including how different species or pathogens adapt to their environment.

I got my bachelor's degree in biotechnology at the University of California, Davis. After that, I worked at the California National Primate Research Center in a pathogen detection lab. Our goal was in assessing the primate colony's health. I learned about many different pathogens and infectious diseases through maintenance of a specific pathogen free colony. This began my interest in infectious diseases specifically.

From there, I went to graduate school, where my fascination was expanded. I focused on the epidemiology of a herpes virus. Through that work, I was able to ask a lot of questions about why the virus was taking on certain characteristics and how those increased its survival. I know you are not supposed to personify a virus but looking at why a virus would choose to an adaptation was always my interest.

In my post-doc at the University of Washington, I decided to look at that same host-pathogen interaction but from the other side, focusing on the host. I studied the host immune response to a herpes virus infection. My main interest was to understand that relationship from both sides. This is where, I first learned about PATH, and I was drawn to its mission of accelerating health equity through innovation and the ability to be involved in early research, clinical research, and licensing. It was attractive to me to be able to have that impact at several different stages of research.

I felt like what I was doing in academia was focused on specific questions and it was hard to step back and see the overall impact. At PATH, I was exposed to projects with a wide team, including people looking at the science in addition to people looking at the business, regulatory strategy, commercial partnering, and licensing. I find it interesting to have that whole picture upfront to help you think about how to solve a problem. This enables you to make sure the solution not only solves the scientific problem but also can be used and that there is a market for it.

Q What is your role at PATH, and what main projects are you working on?

JW: I am part of the Vaccine Formulations Team. Much of my work is to support vaccines in development, through our Center for Vaccine Innovation and Access (CVIA). Most of my direct work is on assay development, largely potency assays which measure the quantity of vaccine antigen administered per dose. This can include generating or identifying reagents to be used for a specific vaccine in development, designing the potency method, and taking that method through qualification.

We do a lot of tech transfers to partners. This can include harmonization testing between our lab and a partner's lab. Frequently, we will design a method, qualify it, transfer it to a partner,

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and then support our partners in method validation and application. This includes troubleshooting if issues arise down the road.

What I love most about this is that I get to work on a wide variety of things. I have several projects that are COVID vaccine-focused, as well as several different shigella vaccine candidates, enterotoxigenic *E. coli* (ETEC) vaccine candidates, rotavirus candidates, and polio candidates. I also work with a mucosal adjuvant and with non-typhoid salmonella. I work on many enteric pathogens, mainly because diarrhea is such a large burden of mortality in children under five, in low- and middle-income country (LMIC) settings. It can be overwhelming, but it also is interesting and keeps me engaged. There are many commonalities or lessons that I learn from one project that I can apply to another.

"We do a lot of work with partners for specific vaccine candidates to help screen reagents for use in method development. We can either generate reagents *de novo* or screen from available commercial reagents."

Q What are then some of the key challenges in developing potency or stability assays for vaccines?

JW: One of the largest challenges we run into is that programs usually start too late to think about a potency assay. It can be challenging to prioritize that work – finding specific reagents for your pathogen, ensuring your method has the right sensitivity for your dose, and measuring changes or damage to your vaccine candidate, can take up a lot of time. You do not want to rush setting up a potency method and validating it. You need to take time and think about whether it is telling you everything you need, and if it is sensitive and reliable enough. I frequently get called into projects already in Phase 2 without a reliable potency assay. Trying to then establish one and establish the stability of your product is challenging. So, start early.

Related to that, is selecting a reference lot. Many potency methods that we set up are relative potency, so you compare your test vaccine to a standard lot. Often those are a clinical lot so that we can bridge to clinical data. Early in development, there is usually limited material, so people may switch between different lots for reference. That can make the data choppy, or hard to interpret and understand.

Q How is your group at PATH working to address some of these challenges?

JW: I get the privilege of working with many talented scientists all over the world. We do a lot of work with partners for specific vaccine candidates to help screen reagents for use in method development. We can either generate reagents *de novo* or screen from

available commercial reagents. We are trying to de-risk some of that decision-making process for partners as well as enable open access to reagents.

For example, right now, I am leading a project that is working to generate monoclonal antibodies that are specific for COVID variants of concern. Once these are identified, we are scaling them up for use by researchers and LMIC vaccine development partners. The goal is to have a large pool of preselected antibodies that are well characterized for multiple partners to use. Part of our selection process is performing proof-of-concept potency assay development. We will share all that information with partners and then it is up to them to take it and use it. Our aim is to provide standard antibodies enabling development of COVID vaccines modified to protect against new pandemic virus variants, such as Omicron BA.5.

PATH has also worked on developing standards for other assays such as our ongoing work on Sabin Inactivated Polio Vaccine (sIPV) to generate international standards for use across manufacturers to have a standard unit that is relatable when you get the same vaccine from a different manufacturer.

In addition to identifying reagents, our goal is to share as much relevant information and data as possible.

Q Tell us more about the *in vitro* inhibition ELISA assays you've been working on – how are they being applied and what are the benefits of this approach?

JW: In a nutshell, with inhibition ELISAs, you incubate your detection reagent, usually an antibody, at a constant concentration across a dilution series of your vaccine. That allows the antibody to bind to your vaccine. After that binding has occurred, you can remove the supernatant and transfer it to an ELISA plate. Then, any unbound antibody is measured on the ELISA. This gives an indirect measure of the specific antigen present in your vaccine candidate.

I see this method being used more because you only need to identify one specific reagent. You only need one specific antibody to set this up. Traditionally, you would need two antibodies for a sandwich method, or another way to capture your antigen. This method streamlines reagent identification. If you select a specific neutralizing antibody, it can also allow you to tie that data to a relevant clinical outcome. It can give you a lot of information in one ELISA.

Another benefit is that they can be conducted in the presence of an aluminum adjuvant or other complex matrices. This means that frequently, you can use the same method for drug substance and drug product. It does not necessarily require you to purify the drug substance before testing. In the past, developers may have developed one potency assay for a drug substance, and a separate potency assay for the drug product. Developing a good inhibition ELISA may eliminate that need.

Q How have you been applying those assays?

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JW: I have been supporting a trivalent rotavirus vaccine development program, and that vaccine does include an aluminum adjuvant. We developed three separate inhibition assays for each of the antigens present in the trivalent vaccine. We recently published the correlation of those ELISAs with the animal *in vivo* potency. We were able to demonstrate that the ELISA was potentially more sensitive to changes in the vaccine than the animal model. Being able to apply these *in vitro* methods to demonstrate more sensitive characterization and eliminate the need for *in vivo* testing is important and inhibition ELISAs are helping us make that transition.

I am also supporting some COVID vaccine trials, and we have generated some inhibition assays. There is a Newcastle disease virus (NDV) vaccine candidate, NDV-HXP-S, originally developed by Icahn Mount Sinai and the University of Texas, Austin. The candidate was then transferred to three vaccine manufacturing partners, Institute of Vaccines and Medical Biologicals (IVAC) in Vietnam, Government Pharmaceutical Organization (GPO) in Thailand, and the Butantan Institute in Brazil. For that work, we developed two different potency assays – a direct potency assay where you coat the antigen onto the ELISA, as well as an inhibition assay. Right now, the manufacturers are in the process of transitioning to the inhibition assay.

As with COVID, this NDV-HXP-S project encouraged a lot of open sharing and community among scientific researchers from multiple institutions around the world. We were able to harmonize between each of the three labs and the PATH lab to establish the assays for use for each of the manufacturers. They have been very open in sharing data, so it has moved quickly. One upside to COVID has been the increase in sharing in the scientific community.

Q What advice would you give to scientists who are engaged in assay development for vaccines?

JW: Start early. Think carefully about how you see yourself using the method over time, including how frequently you are going to test, and how you are going to set up your plate design. How many reagents are you going to need? How much standard should you freeze? How big should the aliquots be? The sooner you start thinking about those logistical questions, the better.

Also, have a strategy for how you will try to connect the *in vitro* method to a clinical outcome. Either using a clinical lot as your standard or using an antibody that recognizes a neutralizing epitope is key for the regulatory strategy and demonstrating the importance of that method.

Q What are the barriers to solving the issue of the need to start sooner in planning? Why do people frequently leave it too late?

JW: It could feel like a distraction. When trying to make a vaccine candidate, you are trying to characterize it, look at total protein, and get it in animals to see if it does anything. Taking some of that and making antibodies to it and seeing if you can measure it in a potency

assay is a big scope of work. Sometimes, it can be hard or distracting to think about doing that at the same time.

It is important because it can tell you a lot about what you are generating in those early development stages. It often gets delayed because people do not know if they have a vaccine candidate yet. That tends to draw away from trying to start a potency assay. You need to take that time to stop, take some of this precious material that you are using for animal studies, and use it to generate reagents.

"The next decade looks to be an exciting time for innovative vaccine development."

Q Are there any other advances you are particularly excited about in the vaccine field, in assay developments and analytics, or beyond?

JW: I am excited more generally about new vaccine development platforms. Obviously, mRNA has opened up a lot, but even self-amplifying RNA will get more attention now, as well as other ways to encapsulate RNA. It has been exciting to see those advances come so quickly.

I also think the use of multiple adjuvants is more accessible right now than it has been in the past. There could be a lot of benefits to those approaches.

The other area that our work focuses on is improving thermostability for vaccines, thus increasing access and reducing wastage. In addition, we are looking at alternate routes of delivery. As more vaccines are introduced, the immunization schedule is becoming very full. Looking at alternate routes, possibly with the potential for self-administration in the future, is exciting.

While still in the early stages *in vitro* approaches to observe or predict the immune responses elicited are interesting. As ELISAs have shown, the more *in vitro* characterization we have, the more we can move away from animal testing and get more detailed characterization and information on vaccine candidates. The next decade looks to be an exciting time for innovative vaccine development.

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VACCINEINSIGHTS

CMC & ANALYTICS

SPOTLIGHT

COMMENTARY

Process modeling in the CMC of vaccines: are we doing it right?

Harini Narayanan & J Christopher Love

With a wider variety of vaccine platforms becoming available, manufacturers need more efficient ways to perform CMC. Machine learning and artificial intelligence hold the potential to reduce the time and cost associated with process modeling and data analysis in CMC workflows. However, we believe that significant changes in data collection and experimental approaches are needed, as historical datasets are insufficient to realize the full potential of these models. This article discusses some key challenges and offers practical solutions to incorporate machine learning and artificial intelligence into vaccine CMC.

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INTRODUCTION

Optimizing processes required for Chemistry, Manufacturing, and Controls (CMC) when developing vaccines takes substantial time and resources. Some of the critical steps include:

- Optimization of individual operations
- Process monitoring and control
- Process scale-up

The existing approaches to these tasks in vaccine development can be inefficient since they often require a *de novo* understanding of each operation for each component of the vaccine, depending on the target product profile sought. There is significant interest, therefore, in how process models based on mechanistic modeling [1], machine learning (ML)

[2,3], and hybrid modeling [4–11] could augment different tasks in the CMC process development workflow.

The advantages of process models may include [1,3,12–14]:

- *In silico* process optimization, accelerating the development time and reducing resources
- Monitoring and control, by forecasting evolutions and taking corrective actions
- Scale-up, to account for scale-specific effects in decision-making early on

Many biopharmaceutical companies have undertaken efforts to adopt these technologies in areas of their businesses beyond basic research and discovery. Digitalization and Artificial Intelligence/Machine Learning (AI/ML) have fostered substantial interest across the biopharmaceutical industry with respect to how these tools could guide workflows in CMC for all biologics, not just vaccines. Most of these efforts, however, have focused on applying ML, AI, and process modeling to historical data collected on existing products (and their corresponding processes) to attempt to discern models capable of guiding future development work in bioprocesses.

While this approach is suitable for anomaly and outlier detection, to serve as reference standards (for instance to have an initial estimate of possible titers or expected recoveries), and to some extent to assess the capabilities within the historical dataset itself. However, in most instances, historical data is often unable to provide the breadth and depth needed to realize the most transformative benefits of these machine-guided approaches to new products and processes. This article focuses on highlighting the motivations and potentials of using process modeling tools for the goals of the biopharmaceutical industry and identifies some of the key challenges and limitations in the current practices that limit its application. Subsequently, we provide some guidance on strategies on how further structured approaches to data collection and testing could realize new capabilities for AI-enhanced workflows in bioprocess development for CMC.

BOX 1

Short description of CMC tasks for vaccines.

- The goal of Chemistry, Manufacturing, and Controls, abbreviated as CMC is to ensure that the therapeutic product commercially sold in the market is similar to the ones used for clinical trials in all respects. Additionally, it aims to assure that the drug meets the standard and is consistently manufactured. In this direction, developing vaccine formulation and establishing a suitable process for the therapeutic products are key activities under CMC.
- The final therapeutic vaccine product comprises not only the biomolecule (e.g., antigen, mRNA, or other vaccine formats) but also a suitable adjuvant, delivery system, and series of excipients (to ensure shelf life and long-term stability of the product). These auxiliary substances that form part of the final vaccine product are called its formulation.
- The process aspect of CMC involves the development of a process to ensure the large-scale manufacturability of the antigen with a desired quality profile and potency and the ability to control the process to produce the antigen consistently.
- Typically, this process starts with the unit-operation-specific screening of critical process parameters and subsequent process optimization at small-scale and lab-scale systems. Thereafter, pilot scale studies and data recorded there-in are used to demonstrate that the therapeutic product can be stably and consistently produced. During this phase, strategies must be set in place to ensure the consistency of the product (e.g., monitoring and control of the system). Finally, the process must be further scaled up, implemented, and validated at a commercial scale.

MOTIVATION TO USE *IN-SILICO* APPROACHES

Biologics are receiving increased attention as therapeutic solutions and global demands are increasing rapidly over the years as highlighted specifically for vaccines [15]. The industry is constantly looking for ways to produce these molecules consistently and in higher quantities, while at the same time aspiring to reduce the time and cost of process development. These challenges faced by the industry are further exaggerated for vaccine

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development and manufacturing especially during an ongoing pandemic, as realized by everyone during the recent COVID-19 pandemic.

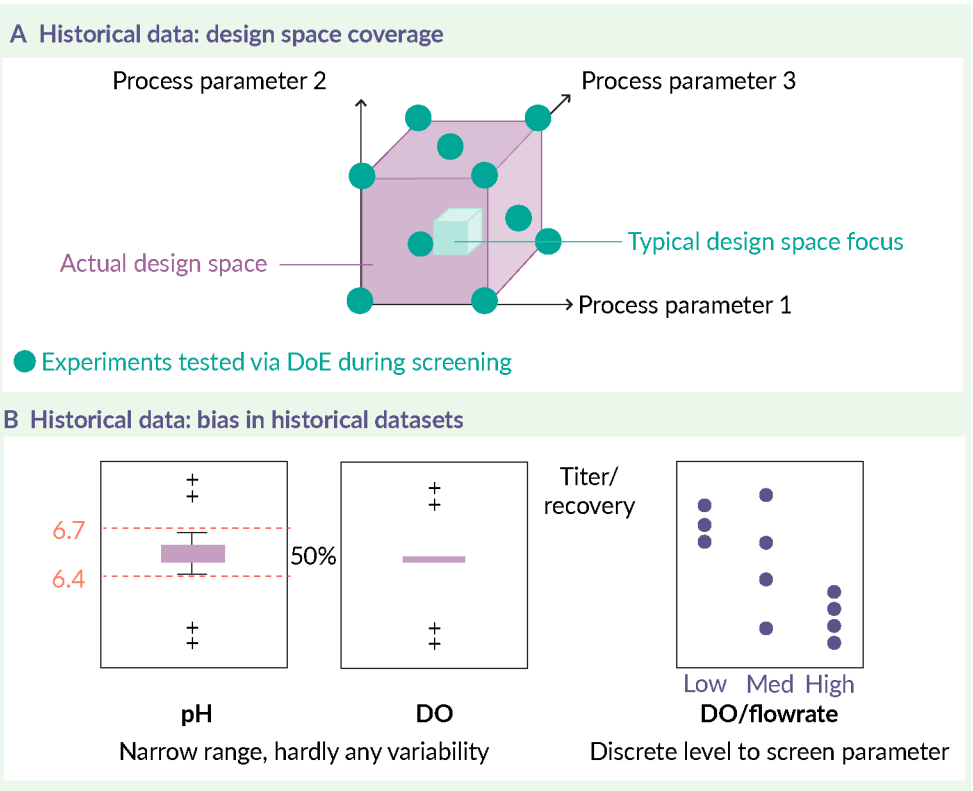
With the increasing variety of distinct and novel therapeutic modalities of vaccine components (and biologics in general) the current recipe-based processes and expert-based decision-making and manual control are rendered inefficient [16]. Sophisticated methods are required to systematically approach process development, scale-up, monitoring, control, and digitalization. The production of vaccine components and biologics is a complex process influenced by a plethora of process parameters that interact in a manner that is not completely understood. Process modeling approaches based on AI/ML (solely or supported by physical constraints) are promising to learn these complex patterns and interactions in higher dimension process parameter space.

The biopharmaceutical industry is realizing the potential of such approaches and looking to adopt them to answer questions such as: 'What are the best culture conditions (pH, Temperature, Dissolved oxygen) to use for the production of vaccine components?', 'What is the best resin to purify the produced mRNA or antigen?', 'How can I ensure in real-time that the process is going as expected and to make automated decisions to control it?' etc.

To fully exploit the benefits of these techniques to achieve the goals of the biopharmaceutical industry, however, there are a few challenges in the current practices of data collection, access and model application.

FIGURE 1

A) Schematic representation of the design space covered in most historical data pertaining to bioprocess parameters and objectives of interest (titer, quality). B) The distribution of the different process parameters tested in the historical data is represented as box plots and an illustration of level-based (low, medium, high) experimental testing through a scatter plot between the process parameter and target where the different dots indicate the process outcome quantified during different experiments.



IDENTIFYING THE CHALLENGES

Challenge 1: historical datasets harbor intrinsic bias

In the conventional setting, process development has been independent of *in silico* modeling and reliant on experience-based decision-making. Experiments planned and data collected historically perturbed the design space to facilitate the interpretation of the collected data by process scientists or engineers. Most experiments in bioprocess development were designed to generate specific insights about the influence of one or two process parameters on product quality attributes based on either (i) prior knowledge or (ii) expert opinion of the conditions that will likely produce the best results. This approach means historical data addresses only a very narrow region of the design space (Figure 1).

Even when screening analyses are performed, they are typically based on fundamentally sound scientific approaches that may allow for straightforward interpretation by a trained scientist or engineer, like one-factor-at-a-time (OFAT) testing (i.e., varying only one process parameter while fixing the others). This approach leaves a very large unexplored design space and limited insights into how multiple factors or variables may interact to alter the results (or output). Although there is widespread use of Design of Experiments (DoE) methods in the field [17,18], these methods are intrinsically constrained to inferring only linear and quadratic relationships among the inputs and outputs [19,20]. Furthermore, these methods are static designs that require a set number of experiments to be performed. Table 1 summarizes common DoE methods and the corresponding number of experiments required. The number of experiments, associated resources, and time required to execute the full DoEs increase significantly with the increasing number of process parameters, thus rendering it inefficient for handling very high dimensional spaces. As a result, the industry has adopted DoEs to test only selected parameters while constraining many other parameters, ignoring their potential influences in the process. This approaches necessarily have balanced the costs and time required for testing all possible states, and the interpretability of such results, to provide an understanding of certain relationships among interacting parameters.

Models built on such data, however, will have less utility for the application goals of the industry given that the model has learned the patterns based on a narrow and biased dataset. For instance, using a dataset studying the effect of different pH at fixed dissolved oxygen, temperature, and other conditions it would not be possible to answer what is the best culture condition to operate the bioreactor. Or to build a model-based controller that can forecast anomalous behavior due to a sudden drop in dissolved oxygen. Subsequently, the applicability of the models to making a generalized robust prediction is compromised since the dataset used did not present the relevant multivariate interactions (which existed in the real system but were not captured by the data).

Thus, testing different modeling approaches on the so-called historical data can often yield models with narrow predictive value or robustness restricted by the conditions observed while building the model. This limitation subsequently hampers its applicability to new products or other combinations of process conditions even for the same product.

Challenge 2: misinterpretation of the 'best' process model algorithm

Simple statistical models such as partial least square regression (PLSR) have been used extensively as the 'go-to' model in the biopharmaceutical industry [21–28]. This now serves as the benchmark modeling approach for comparing new advanced non-linear modeling techniques which the industry seeks to adopt. The transition to more sophisticated

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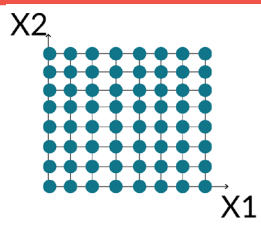
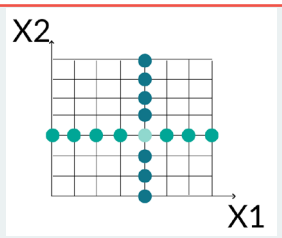
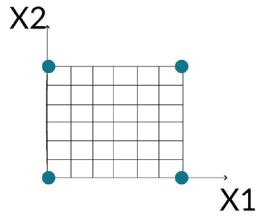
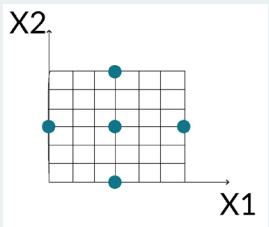
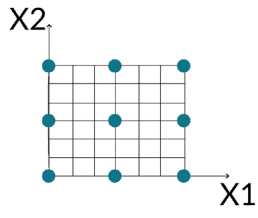
TABLE 1

Different types of experimental design approaches and the corresponding number of experiments required.

Design method	Response surface assumption	Design plot	Number of Experiments
Grid search/ full screen	Independent		$(levels)^k$
One factor at a time	Independent		k (levels)
Factorial design	Linear		$(levels)^k$ $(levels)^{k-r}$
Central composite design	Quadratic		$2^k + 2k + 1$
Box behnken design	Quadratic		$2k(k-1) + 1$

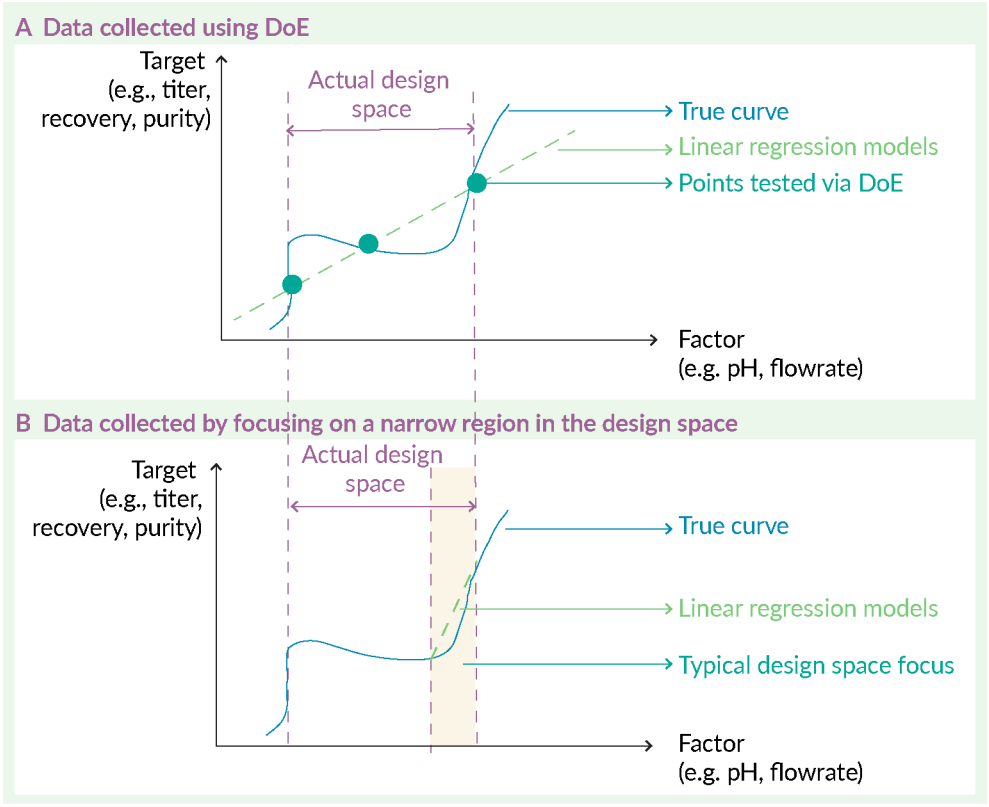
k: number of process parameters; level: number of discrete levels to be considered for each process parameter.

non-linear modeling approaches is justified by the fact that the production of vaccine components and biologics is a complex process influenced by manifold process parameters that interact in a manner that is not completely understood and which cannot be fully captured using linear statistical models such as the PLSR [6,7,29].

Nonlinear models, however, may often perform comparably to the PLSR-based

FIGURE 2

Data collected and its implication for model selection.



A) Data collected using DoE. **B)** Data collected by focusing on a narrow region in the design space. The x-axis represents an exemplary factor while the y-axis indicates the target quantity (e.g., titer, recovery, purity).

models when built on historical datasets. Does this outcome mean that the system tested has limited or no non-linearity? Is it fair to conclude that the system is linear from this approach that inherently seeks to impose such a relationship?

The interpretation of the models is naturally constrained by the data used to create the models. As noted above, data are often collected in a very narrow region of the design space and (or) using statistical DoE strategies which already intrinsically assume a linear (in parameters) response function. The former results in a linear approximation of a small region of the non-linear surface that may describe the multi-variate space of the process parameters and outcomes; the latter inherently imposes a linear-like relationship on the data collected (Figure 2).

As a result, even though non-linear models can be implemented for historical data, the broader benefits or potential added value of non-linear process models, typically based on ML (or hybrid modeling), are rarely realized from such biased historical datasets such that data is intrinsically collected to capture a linear trend (Figure 2).

Challenge 3: inconsistent data collection

In addition to the limited variability and range of data collected, historical datasets are also often inconsistent in their membership and structure. These inconsistencies result from the different aims of experimentation in the different phases of development, the

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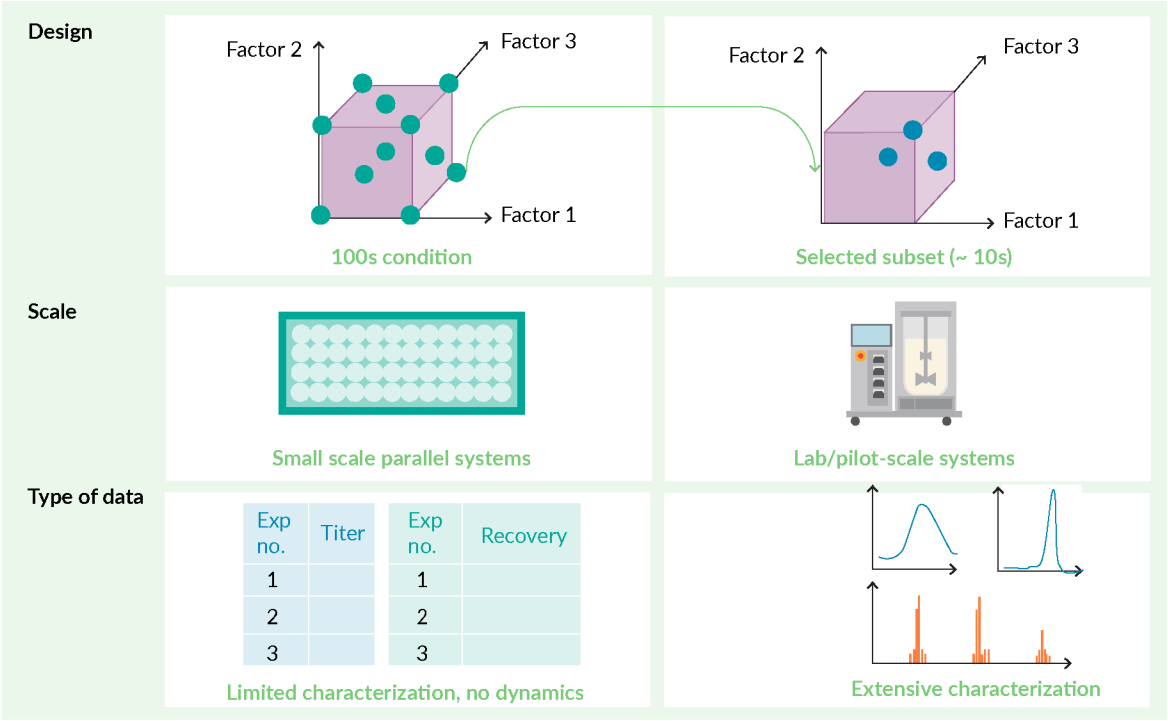
FIGURE 3

Resolution of data collected in the different phases of CMC mapped to the scale of experimentation and the coverage of design space.



equipment used, or the personnel performing the work, among other extrinsic factors. Subsequently, the target for the modeling approach is not uniformly set and can limit the development of generalized models and the transferability of such models across production scales (e.g., bench to pilot-scale to commercial).

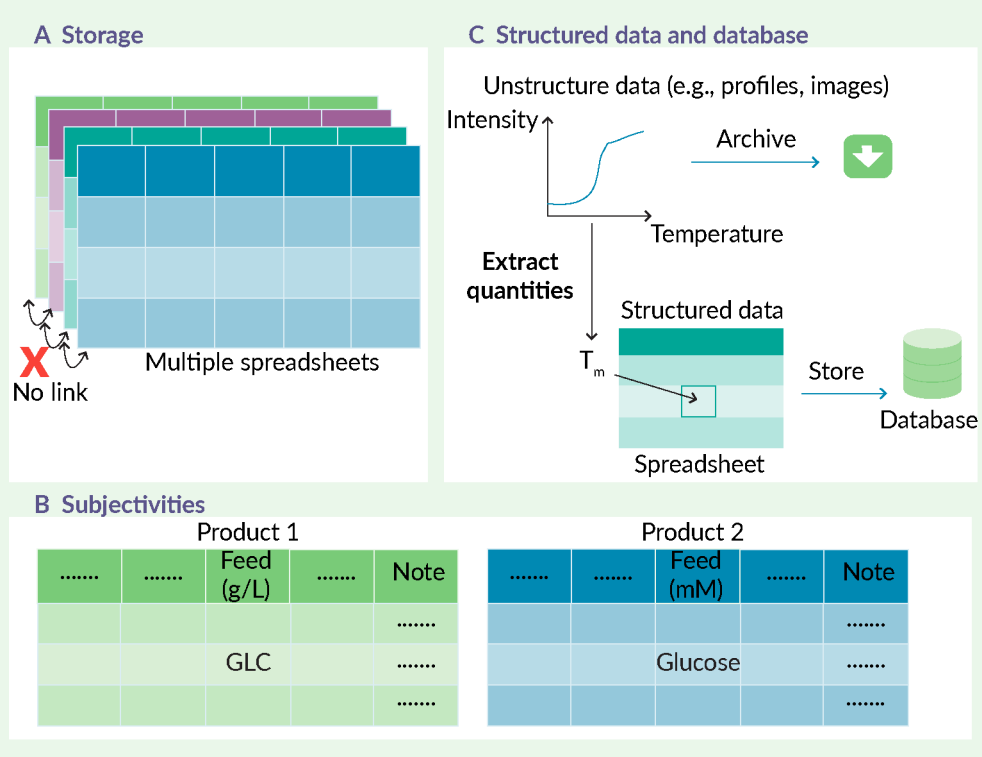
For instance, during screening studies of cell culture, dynamic process variables (e.g., viable cell density, metabolite concentrations) and titer may only be recorded on the final day of the culture when prepared in high-throughput plates (Figure 3). In lab-scale bioreactors and pilot-scale systems, however, the evolution of these parameters is typically recorded throughout the cultivation. Similarly, during screening studies, quality attributes are often recorded only for certain conditions: for instance, those known to generate acceptable titers. Such inconsistencies in the type and frequencies of measures impact data collected in protein recovery as well. For instance, high-throughput screening studies often record only the final fraction for each step which is used to select specific conditions for small-scale column-based studies in which stage dynamic chromatograms are recorded.

Such approaches limit the available information-rich data (e.g., dynamic evolution of the process) to only a certain subset of process condition/ design space. This constraint leads to lost knowledge about other regions of the design space that may be critical to understanding the connectivity of such data across physical scales of production or purification. These choices may be justified for traditional approaches of the rational design of processes in order to maximize human interpretation and minimize resource utilization, therefore, pursuing in-depth only those conditions identified to be promising for optimizing the outcome(s) of interest.

The adoption of advanced technologies like AI/ML, however, requires reconsideration of the workflow to generate the necessary data to realize the full potential of these methods. Thus, it becomes important to consider other

FIGURE 4

Inconsistencies in data storage and reporting.



A) Unlinked spreadsheets of information. **B)** Subjectivities and inconsistencies within the same type of information that recorded at multiple time points. **C)** Reporting aggregated quantities following a database approach to store structured data and lack of storage of unstructured data.

strategies to use resources effectively while ensuring information-rich datasets are collected. One strategy is the Active or Reinforcement Learning [18,30]. An advantage of this method is that it inherently incorporates the intention of creating predictive models during the initial planning and refinement of experiments to map relationships between process parameters and outputs of interest.

Challenge 4: inconsistency in data (& metadata) reporting & inefficient data storage

A fourth challenge presented by typical historical data is the reporting, recording, and storing of the data and associated metadata. It is worth noting that this challenge may not hinder the development and application of modeling approaches per se but often demands time and resources to gather and organize historical datasets from past projects. In most cases, no centralized, uniform format is used to record data across all products and processes. Even if there are established protocols [usually in the form of several spreadsheets (Figure 4A)], there is often extensive subjectivity in its organization (Figure 4B). Moreover, as operational teams change, formats are readapted and reapplied, resulting in inconsistencies across programs or products. This natural variance results in multiple formats of information and potentially even different variables, or data reduction methods used and recorded over time.

Furthermore, all the data associated with every experiment are often not stored or consistently mapped. As schematically shown in Figure 4C, often, only derived properties

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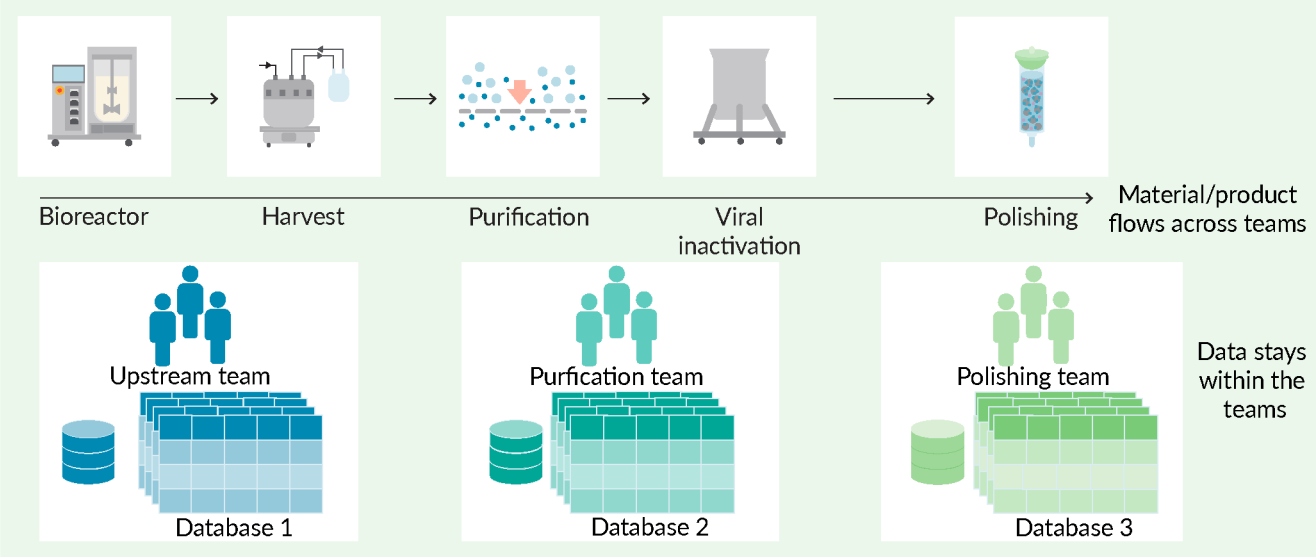
FIGURE 5

Schematic demonstration of the limited data transfer across teams and the resulting existence of several unmapped databases for the same molecule and process.



from the analytics are recorded in spreadsheets while the actual acquisition from the analytics is neither stored nor mapped. In other words, only structured data that can be summarized in a single cell (corresponding to one experiment and one property) may be recorded while the unstructured data (e.g., time-dependent profile information, images, etc.) are not stored or are archived separately from the reduced datapoint. In some sense, this practice already introduces bias in the analysis and modeling since specific features or attributes are extracted from the analytics performed based on a preconceived value of a certain calculation or metric that may be standard in the field.

This dichotomy is further exaggerated when looking at the holistic synchronization of data at a process level, beyond the data for individual unit-operations. In many organizations, each part of the data and optimization for steps in a process are generated and handled by different teams in the organization (e.g., fermentation/cell culture development team, separations development team, analytics team, etc), and the data generated at each stage usually resides within the domains of the specific team (Figure 5).

These aspects make the acquisition/parsing and utilization of such data in an automated manner challenging and time-consuming, since substantial manual work is required to re-adapt formats and interpret essentially what different things mean owing to the subjectivity in recording and reporting data.

Challenge 5: lack of transparency & subsequent reproducibility

Finally, compared to other fields such as computer vision, robotics, and even clinical applications of ML-based approaches [38], there are no common/universal benchmark datasets/databases available for training and testing the performances of the different competing algorithms that have been proposed. Due to concerns about Intellectual Property and Patent rights, most data in the biopharmaceutical industry are proprietary to the company and little is shared in public forums. Often these datasets are never published. Thus, most algorithms have been developed and demonstrated either on:

- Synthetic data collected *in silico*;

- Limited data collected in a research lab or;
- Anonymized industry data with minimalistic information about the process or variable ranges.

Subsequently, each proposed modeling approach is tested on a completely different dataset with no possibility to compare one to another as to which one performs better or to evaluate the relative strengths and weaknesses of different approaches. Thus, no generalized conclusion about the 'best' modeling approach can be drawn. In other words, the lack of a common dataset with which to benchmark has impeded the general development of algorithms (or modeling approaches) for the biopharmaceutical industry to apply in Chemistry, Manufacturing, and Controls (CMC)-related activities.

One potential approach to address this shortcoming might be for the various stakeholders in the area (biopharmaceutical companies, CDMOs, government agencies and NGOs, and academic institutes) to undertake a collective effort to create a common database or ecosystem to store certain data collected in the different phases of the CMC workflow. The best approach to generate the depth and breadth of data most useful for developing and testing algorithms would involve the deposition of data in a de-identified manner or controlled access as is common for genomic datasets like NCBI or Db-GAP (clinically relevant genomic data). In the pharmaceutical domain, the MELLODY consortium [40] is an example that serves as a common ecosystem for several pharmaceutical companies to store their drug molecule information. No such system exists, however, for vaccine components or biologics – neither for molecular information nor for CMC-related data. Alternative approaches could invoke public policies for data transparency or open access to data generated using federal funds or otherwise requiring federal support (such as regulatory licensure). Strategies that could balance the importance of proprietary knowledge for commercial competitiveness with the aggregation of sufficient information to benefit the manufacturing of biologics like vaccines generally should be considered.

HOW TO MOVE FORWARD?

One immediately accessible way to move forward would be to devise studies that consider the application of ML and process modeling in support of a new product in a commercial pipeline or an ongoing CMC activity, with an organizational commitment to apply such machine-guided approaches from the start through the end of the CMC workflow. We outline below three considerations for such strategies that could be adopted or refined for such purposes.

Consideration 1: unit operation level experimental design & optimization

As previously indicated, it is of interest to develop useful process models that can capture the interactions of different process parameters among themselves and the corresponding influence on process outcomes (e.g., titer or recovery) such that they can be used to perform process optimization, scale-up, monitoring, and control. It is, however, also important that this experimental coverage is achieved with minimal resources. Some works in the literature have suggested the application of intensified DoE approaches to reduce the number of experiments compared to classical DoEs [31]. However, techniques from specific domains of machine learning such as active learning [18] or reinforcement learning [2,30] might assist in further reduction of the number of experiments required by coupling the purpose of experimentation (e.g., maximization of titer, increasing recovery, etc) with the goal to cover or characterize the multivariate design space. Thereby, characterization of the multivariate interaction between input and output and achieving the optimal target can be handled simultaneously

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by performing sequentially or iterative experiments suggested by the algorithms. Such an approach to experimental design has been shown to significantly reduce the number of experiments required and has been shown to provide improved solutions, as illustrated, for instance, in [32] for reaction screening, and using Bayesian optimization in the case of formulation development for biopharmaceuticals [18].

Many of these strategies inherently rely on building models of the system that are used to guide experimentation. These models are in fact mathematical formalizations of the process that can be used to transfer knowledge from one product to another, or across scales. On the other hand, for those sequential experimental design methods that do not use models (e.g., model-free reinforcement learning approaches), process models can be built following the experimentation, thus providing a predictive framework that can be used as a basis for future vaccine components or biological molecules.

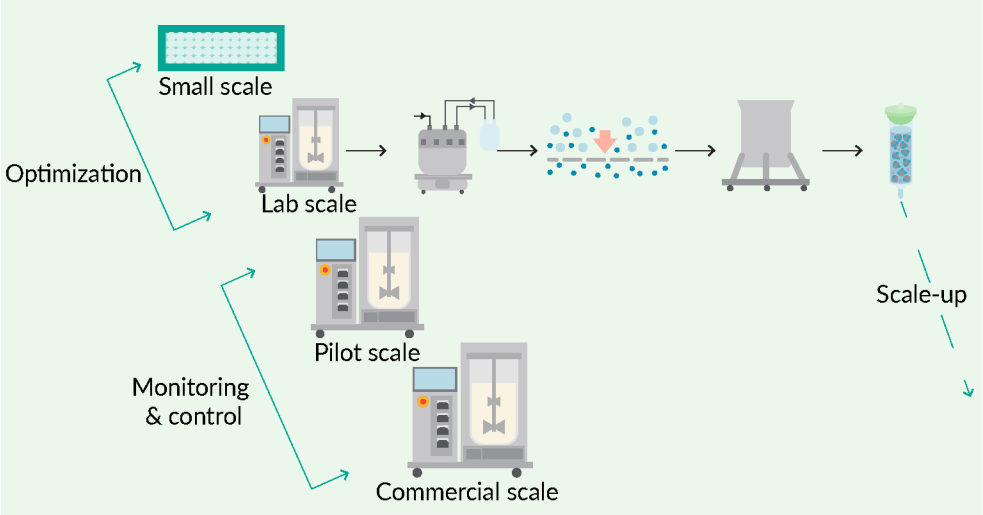
Such approaches can be of further relevance to vaccine development activities, for instance, when developing processes for vaccine products against different variants of the same virus or a related family of virus. Major underlying principles or patterns can be transferred from the past product to adapt to the nuances of the vaccine component against the new variant. In contrast to the traditional approach of applying heuristic-based conclusions from past experience (E.g., pH 6.6, DO less than 25% was the best), it allows transferring all the learnings in the form of patterns stored in the model. In addition, strategies will be required to record information-rich datasets, such as the dynamic evolution of different variables, profiles of different analytics, images, etc., for all or a calculated subset of these experiments.

Consideration 2: traversing the length, breadth & depth of the CMC activities

A second consideration relates to efficiently incorporating ML and process modeling approaches across all aspects and stages of CMC development to avoid incoherent or redundant implementations. Typically, CMC teams undertake activities across different dimensions of the process, including the sequence of unit operations, different

FIGURE 6

The length (different unit operations), breadth (different scales), and depth (different goals) of the CMC development process.



scales of experimentation, and different aims of experimentation. We might view all of these vectors as the 'length, breadth, and depth' of the CMC development process (Figure 6).

A vaccine component or other biologics produced in the bioreactor is harvested, purified, polished, transferred into the designed formulation, and packed. Thus, it might be beneficial to perform experimental design, modeling, and optimizations when considering the entire process holistically [33] or at least, across relevant related subsets of the process operations.

When considering specific unit operations, different scales are often used for different stages of experimentation. For instance, considering the production of biologics, small-scale parallel systems (e.g., plate reactors, Ambr systems) are used for screening conditions during process development, and subsequent optimization and characterizations are performed in lab-scale reactors (3–5 L). Subsequently, process validation is performed in pilot-scale reactors and a further larger scale is used for commercial manufacturing. Similar strategies can also apply to other unit operations involved in the purification and polishing of vaccine components and biologics. The effects or observations, however, do not often translate linearly across scales, since scale-specific effects enter into play (e.g., stirring, oxygen distribution, and concentration gradients in the case of bioreactors). The scaling 'laws' are a critical aspect to be considered to ensure that the material produced for clinical studies can be replicated in the commercially produced product, and while many empirical equations and models are often used, predictive AI/ML-based models could help relate specific scales and equipment types across a manufacturing network if considered holistically in development.

Finally, there are different goals associated with experimentation (e.g., optimizing the process, and ensuring stable and controllable production) and hence, modeling at different stages of CMC is commonplace. For instance, during process development, optimizing a process's total efficiency becomes a key objective primarily achieved in small-scale and lab-scale systems. At later stages, being able to control the process and have consistent production becomes important, typically considered in pilot scale and commercial units.

When considering the implementations of process models (either solely based on ML or in combination with physical laws), it might be beneficial to devise problem statements, and solutions considering all these three dimensions of the CMC workflow, such that the different teams handling these specific aspects of CMC come together and align on how and where to apply process modeling. This organized approach could potentially assist in choosing appropriate inputs, outputs, and algorithms aligned across unit operations, scales, and experimentation purposes such that the CMC's length, breadth, and depth can be traversed more efficiently.

In this regard, transfer learning approaches could be possible solutions to handle data collected at different scales wherein models developed at a small scale could be transferred to a subsequent large scale to retain the primary interactions between the input and output, while a reduced number of additional experiments could be performed to account for the scale-specific interactions [34,35]. Similarly, models developed during screening and process optimization could be applied for process monitoring and control by combining model predictions with real-time measurements (acquired through different probes and spectroscopic techniques) using filtering techniques such as extended Kalman filters or particle filters [8,36,37]. This holistic approach is likely to be more robust since data collected during screening and optimization studies are typically information-rich and capture the interrelationships between process parameters and relevant process outcomes than conditions tested at larger scales where monitoring and control applications become relevant.

Overall, if strategized and aligned appropriately across different fronts of the

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workflow of CMC, implementation of the modeling approaches could be made more efficient, non-redundant, and multi-purposed.

Consideration 3: non-technical yet critical

A third consideration that is crucial is appropriate documentation and storage of data. Since machines must read and use the data for ML analysis or developing models, it would be worthwhile to spend the time and resources to design machine-readable data files upfront. Some features to be ensured could include:

- Selection of appropriate file format for relevant tasks (e.g., spreadsheets to store value in matrix format, text document for protocols and notes, specialized image formats like .png or .jpeg for images from analytics);
- Usage of descriptive, precise variable names to reflect the quantity recorded along with consistent units (e.g., avoid using names like property1, property2 etc);
- Measures to reduce the level of subjectivities (for instance, avoiding typos in a spreadsheet by incorporating the use of predefined dropdowns)

Furthermore, it would be beneficial to store all the data acquired within a unit operation, across different scales, and across various unit operations such that it is accordingly linked and mapped in a centralized storage system shared across the different respective teams. As highlighted in section '*Challenge 4: inconsistency in data (& metadata) reporting & inefficient data storage*', storage of unstructured data (e.g., spectral acquisitions, profile from the analytics) should be considered and appropriately mapped to the corresponding derived quantity and the relevant experimental conditions. This requirement might invoke hierarchical ordering of data/information, which should all be accompanied by different levels of synchronized keys. Additionally, well-written documentation might prove extremely useful for future team members and across different teams in the organization to understand the type and content of stored data, navigate and access it, and subsequently interpret it.

To facilitate some of these hierarchical centralized storages of both structured and unstructured data, several firms in other sectors are transitioning from databases (purely based on several spreadsheets) to data lakehouse-based storage architectures [39]. Adoption of this approach across much of the biopharmaceutical industry remains in its early stages of adoption and implementation.

CONCLUSION

Vaccines are crucial biologic medicines for prophylactic protection against infectious diseases. The COVID-19 pandemic has recently re-emphasized the importance of vaccines for global health. CMC development for vaccines is necessary to ensure a safe and efficacious commercial product, and this work takes up a significant amount of time. With increasing types and formats of vaccines (proteins, viral vectors, mRNA, nanoparticles), and biologics in general (cell therapies, gene therapies), more efficient ways of performing CMC are required for organizations with inherently limited capacity for development (the available staff and experimental resources). The use of ML and AI for process modeling and data analysis in CMC workflow promises to reduce time and resource requirements, making CMC more efficient and ultimately, more predictable. We posit, however, that a substantial change in how data are collected and experimental approaches implemented is needed, and historical datasets, though pragmatic, are not ideal to realize the full potential of these models.

This article has highlighted some of the key challenges faced. First, it brought forth

the limitation of historical data that often lacks sufficient quality and resolution, hampering the depth of transferable insights gained from the best modeling approaches. Furthermore, inconsistencies in the types of analytics and data collected at different phases of CMCs, poor data organization, and lack of centralized data stores also make transitioning to new models more difficult. Finally, the absence of a benchmark dataset for comparing all the data analysis and modeling approaches was highlighted as an area of need for evaluating new approaches to modeling and predictions from them.

We outlined how ML and AI technologies could be incorporated functionally into the CMC workflow in ways to realize its potential completely. It is important to incorporate the design and testing of such models into the development of the CMC strategy for new products from the beginning of development. A priori planning of the desired applications and uses of ML/AI that traverse the length, breadth, and depth of CMC is important and its implementation requires alignment of stakeholders across the organization. Finally, the requirement to supplement efforts of generating and acquiring better and more efficient data with improved data organization, reporting, and storage protocols was discussed. Collective approaches among multiple organizations (academia, industry, government) and capital investment in such efforts could offer substantial strength in enhancing the applications of these predictive tools for vaccine development and other biopharmaceuticals. Similar to other shared resources like the internet and power/water distribution, basic investment in the infrastructure for accelerating therapies from the lab bench to commercial-scale production would benefit many, from companies delivering medicines to the patients receiving them to the healthcare systems that manage the distribution of them.

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VACCINE INSIGHTS

LATEST ARTICLES:

VACCINEINSIGHTS

COMMENTARY

Why vaccinate children against COVID-19?

Lancet Commission on COVID-19 Vaccines and Therapeutics Task Force
David C Kaslow, Carolina Batista, Maria Elena Bottazzi, Onder Ergonul, J Peter Figueroa, Mayda Gursel, Mazen Hassanain, Peter Hotez, Gagandeep Kang, Bhavna Lall, Heidi Larson, Denise Naniche, Timothy Sheahan, Samba O Sow, Nathalie Strub-Wourgaft, Prashant Yadav, Annelies Wilder-Smith

Any guidance on vaccine use prioritization, including booster dose policies, cannot ignore the current, ongoing profound inequities in global COVID-19 vaccine access and coverage. While higher-income countries expand their vaccination programs to children as young as 6 months old, and in some countries, multiple booster doses to a large proportion of their populations, many lower-income countries still struggle to get access and coverage of a primary vaccination series for their highest priority-use groups, including older adults and healthcare workers who comprise only a small proportion of their populations.According to the updated WHO Roadmap, averting severe disease and deaths and protecting health systems remain the primary objectives of vaccine use in the context of the global COVID-19 response [1]. The WHO Roadmap, however, also considers vaccine use for resuming socio-economic recovery, particularly the priority of maintaining uninterrupted education to keep children connected and learning. Here, we examine the rationale for vaccinating children based on consideration of those objectives, together with a potential surplus of currently available vaccines.

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The World Health Organization (WHO) Roadmap identifies priority-use groups to optimize the public health impact through recommendations that seek to ensure equitable distribution and urgent vaccine access to those most at-risk, no matter where they live.

To achieve the primary objectives of averting deaths by preventing severe disease and protecting health system impact by reducing hospital admissions and preventing intensive care from becoming overwhelmed, older persons and adults with comorbidities were allocated to the highest priority-use category. Children and adolescents with underlying health conditions that put them at higher risk of severe COVID-19 outcomes are in the medium priority-use groups. Healthy children were allocated to the lowest priority-use group based on their lower risk of COVID-19-related severe disease and death. Age-disaggregated cases reported to WHO from December 30, 2019, to July 4, 2022, showed that children less than 5 years of age represent 2% (6,607,392) of reported global cases and 0.1% (2,627) of reported global deaths; older children and younger adolescents (5–14 years) account for 11% (28,256,515) of cases and 0.1% (1,935) of deaths, while older adolescents and young adults (15–24 years) represent 14% (37,438,185) of cases and 0.4% (9,019) of deaths [2]. Patients less than 25 years represented less than 0.6% of reported global deaths.

The global burden of pediatric COVID-19 is not insignificant. According to United Nations Children's Fund (UNICEF), nearly 20,000 children (under age 20) have died from COVID-19 globally [3], and even this number is considered an underestimate. More than 1,000 pediatric deaths have occurred in the USA alone [4], such that COVID-19 outranks many other causes of vaccine-preventable deaths in the USA. But case counts and death rates are not the only outcomes relevant to the health and well-being of children. Despite a lower risk of severe disease, the COVID-19 pandemic and its control measures disproportionately affected children and adolescents. The most damaging and long-term effects relate to school closures, which disrupt the provision of educational (and in some cases health and nutritional) services and increase emotional distress and mental health problems [5]. Consistent and continuous school attendance is critical to the well-being and life prospects of children and parental participation in the economy.

Beyond educational setbacks, school closures and stay-at-home orders have been associated with increased domestic violence [5], including sexual assault, adolescent pregnancy, and child marriage. These traumas are further exacerbated by the increased probability of missing further education and of poor pregnancy outcomes. School closures also lead to loss of access to a wide range of school-provided services, including school meals, monitoring of health and welfare, social skills training, and services targeted to children with special needs. As schools moved online, impoverished children experienced dramatic educational setbacks [5], contributing to inequalities and long-term hardship.

While school closures during the peak of a pandemic may contribute to rapidly flattening the curve, greater overall health and well-being benefits come from keeping schools open by implementing comprehensive, multi-layered measures to prevent the introduction and spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in educational settings. That is why, very early on in the pandemic, WHO and UNICEF advised against school closures and developed guidance on how to minimize transmission in schools and keep schools open [5].

Vaccinating school-aged children has been recommended in some countries to help minimize school disruptions by reducing the number of infections at school and the number of children required to miss school because of quarantine requirements. Vaccinating children and adolescents has also been advocated to reduce intergenerational transmission, an important additional public health objective. Prior to the emergence of the Delta variant, the risk of symptomatic cases in household contacts of vaccinated cases was reported to be about 50% lower than that among household contacts of unvaccinated cases [6]. However, the impact of

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vaccination on reducing transmission in the context of the more transmissible Delta and Omicron variants appears to be significantly lower and less durable [7]. As such, the use of current COVID-19 vaccines to directly protect teachers, family members, and other adult contacts of children and adolescents is likely to have a greater impact on reducing severe COVID-19 and deaths in the contacts of children than vaccinating children to indirectly protect their contacts.

The emergence and spread of the Omicron variant showed that hospitalizations in younger children (all generally unvaccinated) became more frequent, reflecting increasingly widespread community transmission. Although children and adolescents can experience prolonged clinical symptoms (known as 'long-COVID', or post-acute sequelae of SARS-CoV-2 infection), the frequency and characteristics of these conditions remain under investigation. One large study from London found that approximately 14% of COVID-19-infected children suffer symptoms lasting more than 15 months [8]. Additionally, a hyperinflammatory syndrome, referred to as pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 in Europe and multisystem inflammatory syndrome in children (MIS-C) in the USA, may not be as rare as previously believed [9], and has been reported to occur worldwide and complicate recovery from COVID-19 [10].

However, there is an evidence gap that must be acknowledged – the preponderance of evidence on the risk of severe COVID-19 and death in children and adolescents comes from studies in high-resource settings. One systematic review suggests that there may be a larger impact of pediatric COVID-19-related fatality in low- and middle-income countries (LMICs) versus high-income countries (HICs) [11]. Clearly, we need more research on the direct health and indirect societal impacts of COVID-19 in children and their families in LMICs.

Still another consideration is the increasing availability of COVID-19 vaccine doses and new vaccines released for children under emergency use authorization. Benefit–risk assessments for this age group must be conducted rigorously for each of the COVID-19 vaccines that have received emergency use authorization. As COVID-19 vaccines become more readily available globally and vaccine coverage rates among high-priority use groups increase, there is now a stronger rationale for vaccinating children. Along those lines, some LMICs have begun pediatric or adolescent COVID-19 immunization campaigns, in some cases, such as in India, using locally produced vaccines. With increasing seroprevalence rates reported globally, especially among children and adolescents, vaccine strategies need to be adapted. The number of vaccine doses, inter-dose interval, and need for booster may differ in settings with high seroprevalence [1].

Taking all the above into consideration, the decision to vaccinate healthy children and adolescents must account for prioritization to first fully protect higher priority-use groups (e.g., older adults, adults with comorbidities, health workers and essential workers) through primary vaccination series, and, as vaccine effectiveness declines with time, through booster doses [1]. Although benefit–risk assessments clearly underpin the benefit of vaccinating all age groups, including children and adolescents, the direct health benefit of vaccinating healthy children and adolescents is lower compared with vaccinating older adults due to the lower incidence of severe COVID-19 and deaths in younger persons. While at the patient level, decisions regarding vaccinating a child must take into account individual circumstances and values and local considerations, at a societal and global level, vaccinating children is a less urgent public health priority at a time when many higher priority-use groups have not yet achieved high levels of access and coverage.

Regardless of vaccination, countries' strategies related to COVID-19 control should facilitate children's participation in education and other aspects of social life, and minimize loss of in-person interactions [12].

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