Manufacturing Issues with Combining Different Antigens: A Regulatory Perspective

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The regulation of biological products is conducted within the framework Title 21 of the US Code of Federal Regulations (CFR). These regulations describe product and clinical testing requirements for drugs and biological products, as well as the requirements for licensure of such products. The requirements outlined in the CFR also apply to combination vaccines. In addition, the Center for Biologics Evaluation and Research has issued a Guidance to Industry document that discusses the manufacturing, testing, and clinical evaluation of combination vaccines. However, as the complexity of mixing the different antigens increases, the challenges associated with product development (e.g., demonstration of comparability of the components and lot consistency) require early interactions with the US Food and Drug Administration. The many areas of difficulty in the arena of combination vaccine development underscore the need for continued reevaluation of current guidance documents in addressing the increasing complexity of vaccines.

A combination vaccine consists of ≥2 immunogens that are physically mixed to form a biologic agent that is intended to prevent multiple diseases or 1 disease caused by different serotypes of the same organism. The “formulation” of the combination vaccine may take place at the manufacturing establishment. Alternatively, some active components may be admixed by a health care provider just before the administration of the combination vaccine [1]. In addressing the regulation of combination vaccines, the Center for Biologics Evaluation and Research (CBER) has considered several specific sections of Title 21 of the US Code of Federal Regulations (CFR) pertaining to combination products [2]. For biological products, 21 CFR 601.25(d)(4) states that safe and effective active components may be combined if (1) each of the active components makes a contribution to the claimed effects; (2) combining the active components does not decrease purity, potency, safety, or effectiveness of the individual components; and (3) when used correctly, the combination provides concurrent prevention or treatment for significant portions of the target population. With the need to develop and license these important products in mind, CBER published a Guidance to Industry document in 1997 that discussed manufacturing, testing, and clinical evaluation of combination vaccines [1]. Here, we comment on general considerations for vaccine product development, as well as on some of the added complications posed by the manufacturing of the complex mixture of antigens and additives that constitute a combination vaccine.

GENERAL CONSIDERATIONS FOR VACCINE TESTING

The regulation of vaccines is conducted within the framework provided by 21 CFR outlining the requirements for conducting clinical studies for investigational products, as well as requirements for licensure of drugs and biological products. The focus of this section will be to highlight those sections of 21 CFR that address the general testing requirements for li-
censed biological products [3]. In this regard, product testing includes assays for identity, purity, potency, sterility, and general safety. In addition to these broadly applicable tests specifically cited in the CFR, other product-specific in-process and release tests for further product characterization may be developed to reinforce assurance that a safe and effective product is being consistently manufactured. Ideally, testing should be performed at the latest manufacturing step compatible with adequate assay performance. Therefore, whereas some testing should be performed on product dispensed into final containers (e.g., identity, sterility, and general safety), some characteristics may be tested upstream prior to vialing of product. For example, although some potency testing may be performed on either the final container or formulated bulk products, some potency-associated characteristics (e.g., polysaccharide/protein ratio for conjugate vaccines) can only be adequately measured at earlier steps (e.g., individual antigen bulks).

Selection of appropriate potency, purity, and identity tests should be an intrinsic part of process validation. Thus, such testing should be initiated early in product development in order to ensure that product of defined quality and consistency is used throughout clinical testing. In addition, all steps taken toward the adoption of a testing procedure, including the rationale, scope, validation, and specifications, should be adequately documented in the license application.

Recently, CBER published a guidance document for vaccine manufacturers discussing documentation for chemistry, manufacturing, and controls [4]. Although this document provides a general overview of the information that should be submitted in a license application, the information can also serve as a framework for product development from before phase 1 through phase 3. The following sections highlight several major areas relevant to documentation of manufacturing and product characterization:

**Description of manufacturing.** Adequately defined manufacturing methods and production controls are important for determining the safety and biological activity profile for a product. These parameters are also key elements in demonstrating manufacturing consistency from lot to lot. The specific product testing and validation/controls may vary, depending on the product. However, clear documentation of the quality and origin of viral seeds or stocks, cell banks, and other starting materials; of production steps and controls; and of appropriate in-process and release tests are requirements common to all vaccines, and these are critical for judging product safety and effectiveness. For combination vaccines, these parameters must be assessed for each of the components in the product.

**Characterization of viral seeds and master bacterial cell banks.** An integral component of characterization of viral seeds and master bacterial cell banks is the documentation of phenotypic and genotypic characteristics of the strains. Important information could include demonstration of biochemical reactions, descriptions of growth and morphologic characteristics, serotype analysis, characterization of virulence markers, or demonstration of purity of cultures. Establishment of a detailed profile for the seeds and banked cells can facilitate changes in seed storage or culture conditions, allowing for an easier evaluation of comparability under the new conditions. For products created using recombinant DNA technology, it is important to evaluate the stability of the construct. For in-depth guidance for characterization of products manufactured by recombinant DNA technology, the Points to Consider [5] and International Conference on Harmonisation [6] documents may be helpful.

**Documentation of cell substrate–related parameters.** An essential aspect of virus and recombinant vaccine production is the documentation of cell substrate–related parameters. Documentation of the identity, source, and passage history of the cells, as well as cell banking procedures, should be provided. One major aspect of cell substrate characterization is adventitious agent testing [7, 8]. It is important to document the type of testing performed, as well as the results of such testing. Because adventitious agent testing is a rapidly developing area, sponsors are encouraged to keep abreast of guidance on current standards for testing and to seek guidance from CBER on proposed novel testing strategies throughout the various stages of product development.

**Quality of starting materials.** Because the purity and quality of raw production materials can greatly affect both the manufacturing process and the final product, the source and characterization of raw materials should also be documented. This may generally require submission of the certificate of analysis and descriptions of any additional or confirmatory acceptance testing performed by the vaccine manufacturer. If materials of animal-derived origin are used in any step of manufacture, from derivation of seeds and cell banks through final formulation and fill, appropriate species-specific adventitious agent testing should be performed (e.g., porcine parvovirus testing of porcine-derived trypsin). The information regarding testing of monoclonal antibodies discussed in the Points to Consider document [5] may provide useful guidance for consideration of adventitious agent testing for components used in the purification of products (e.g., testing of monoclonal antibodies used in purification columns) [9]. Bovine material used in any stage of manufacture must be from countries certified by the US Department of Agriculture (USDA) as being free of bovine spongiform encephalopathy [10]. It is of importance that the USDA list has been periodically updated, and it is the responsibility of the manufacturer to remain current with this information [11]. In instances where human-derived materials (e.g., virus isolates, cells, or blood-derived components) were used in the manufacture or formulation of the vaccine, documen-
ventitious agent testing should be provided.

**Quality control.** The last general consideration pertains to documenting and providing (to the US Food and Drug Administration [FDA]) adequate information about the manufacturing process and its quality control. Specifically, a detailed description of the methods used during manufacture should be provided. A comprehensive flow chart showing each step with its associated critical parameters (e.g., time, temperature, filter size, elution buffer) and control procedures (e.g., pH, sterility, SDS-PAGE, purity check) facilitates FDA review.

In addition, all in-process tests for product quality and safety should be described and ranges of acceptable results established. Tests for viral clearance [12] and verification of detoxification or attenuation are examples of necessary in-process, lot-to-lot tests to support product safety, or they may be used to support validation of manufacturing steps. As more experience is gained through manufacture, in-process and final product acceptance criteria may be adjusted to better reflect process performance.

**SPECIFIC CONSIDERATIONS FOR COMBINATION VACCINES**

As discussed above, the CFR contains specific regulations addressing the combination of active drug and biological products. A key component of these regulations is the issue of compatibility of each active monocomponent with other monocomponents or additives present in the combination. There are at least 3 possible mechanisms that can explain a failure: (1) physiochemical interactions in the product; (2) biological interference among attenuated immunizing agents; and (3) immunological interference, as detected either preclinically or clinically. A number of examples where the administration of combination products have resulted in clinical "interference," where the immune responses to ≥1 of the antigens in the combination vaccine were inferior to those measured when each antigen was individually administered have been described in the literature (reviewed in [13]). We will now discuss several aspects of the manufacturing of combination vaccine, in the context of the 3 mechanisms of incompatibility mentioned, using Guidance to Industry [1] as a framework. The specific areas chosen for comment are representative of the unique situations encountered during product development of combination vaccines.

**Preservatives.** There are several factors to consider when choosing a preservative. In general, the clinical safety and antimicrobial effectiveness of the preservative should be demonstrated during product development. Once licensed, the amount of active preservative should be measured in the final container and should remain within acceptable limits during the dating period. In combination vaccines, it is important to evaluate the impact of the preservative on the potency and stability of all the active components. For example, original attempts to combine diphtheria-tetanus-pertussis (DTP) vaccines with a whole-cell pertussis component (DTwP) and inactivated poliovirus vaccine (IPV) highlighted the sensitivity of the IPV component to the effects of thimerosal [14–16], a preservative used historically for DTP vaccines [17, 18]. It should be noted that compatibility of components is directly related not only to their chemical activity, but also to the time period that those components will be together in combination. Thus, eventually a DTwP-IPV combination vaccine was licensed in Europe by keeping the 2 vaccines separated in individual chambers of a syringe until the actual time of injection.

**Adjuvants.** When developing a combination vaccine, the compatibility of the adjuvant with all components (whether or not they are directly adsorbed to, or originally formulated with, the adjuvant) should be evaluated. It is important to keep in mind that for some products, the degree of adsorption is closely related to their potency. In this regard, attention should be paid not only to the adsorbed component or components of the combination, but also to those components that previously may not have been adsorbed.

In cases where the components were developed as an adjuvanted vaccine, it is important to evaluate any changes in the new combination with regard to adsorption or possible desorption when in combination, as well as the kinetics of simultaneous adsorption. Studies addressing these issues may prompt the manufacturer to change the order of manufacturing steps or make other modifications to the manufacturing process. The combination of monocomponents may also result in a change in the ionic strength of the product. This change may affect the potency of the product immediately or over time. Therefore, careful monitoring of the completeness of adsorption of any component intended to be adsorbed should be incorporated into the product stability program.

An example of a potential effect of the adjuvant normally present in one component on those components in the newly formulated combination that were previously unadsorbed can be found in the DTP-IPV combination. IPV release requires testing for residual mammalian cellular DNA and protein that within preestablished specifications have been associated with a specific safety and effectiveness profile. When combined with components containing adjuvant such as hepatitis B or DTP vaccines, the dynamics of the equilibrium may lead to at least partial adsorption of the poliovirus and residual cell substrate components. The safety and efficacy of the IPV and the associated residual materials in the adsorbed state should be evaluated preclinically and clinically and should not be assumed to reflect that of IPV as an unadsorbed vaccine.

**Potency testing.** “Potency” is defined as the ability of a product to effect a given result in laboratory tests or controlled...
clinical studies [3]. A variety of potency tests may be considered for use; however, one feature common to all tests for potency is that they aim to predict, with more or less accuracy, clinical efficacy [19]. In addition, they provide a tool used in the assessment of product stability. Potency testing may constitute a serological evaluation or a challenge test after vaccination of animals. Alternatively, potency testing may rely on physiochemical characterization, such as composition and molecular weight. The degree of accuracy of a potency test in predicting efficacy in humans will be based on the knowledge of the disease that the vaccine under study will prevent. In cases where a complete understanding of disease pathogenesis and protective mechanisms behind the vaccine efficacy is lacking, potency testing is designed to ensure release of vaccine lots with similar characteristics to those shown previously to be clinically efficacious [20]. In this regard, the limitations in the predictive value of the potency test may be minimized by additional in-process tests for antigen quality and quantity, as well as demonstration of manufacturing consistency. Once a potency test has been established for a vaccine component, it is important to reevaluate the performance of the test in the context of the combination vaccine to ensure that the additional antigens, adjuvants, and excipients do not affect each individual test.

Identity testing. As required by the CFR, identity testing must be performed on the product in its final container. The identity test chosen should be able to distinguish the product being tested from any other product manufactured in the facility. The test can be based on physical or chemical characteristics of the product, inspection of the vials by macroscopic or microscopic techniques, or by culturing or immunologic techniques. In addition to final container identity testing, identity tests for each of the active components in the combination vaccine should be developed. These latter component-specific tests may also be required as final container tests if there is no alternative method for distinguishing the combination product from other products manufactured in the facility.

Stability. The establishment of a dating period for a product is based on real-time stability data. The requirement for real-time stability data also applies to the determination of appropriate storage times for intermediate bulks, if not further processed immediately into formulated final bulk. When establishing dating for a combination vaccine, the date of initiation of the last valid potency test for the component with the shortest dating period will be used as the date of manufacture in the context of the CFR. Depending on the type of product, the dating period in final container may also take into account bulk storage times.

Combination vaccines may pose a unique situation with regard to stability testing and assignment of dating periods. As mentioned earlier, a combination product may be “formulated” at the time of administration by mixing of 2 licensed vaccines by the health care provider at the time of delivery. In this case, the “combination vaccine” does not have a dating period of its own. The only assigned dating periods are associated with the individually licensed products. Thus, the dating period for the admixed combination vaccine is defined by the maximum allowable time between mixing and administration of the vaccine. Because the potency tests for the individual components may not predict clinical effectiveness of the combination vaccine, the maximum allowable time between mixing and administration usually reflects the time frame employed in the clinical studies where efficacy was demonstrated and is included in the package insert. To date, most vaccines of this nature are to be used immediately, within a prespecified maximum time (e.g., 30 min) allowed between mixing and administration.

Manufacturing consistency. When demonstrating manufacturing consistency, the consistency lots are generally derived from at least 3 consecutive final bulk lots. In formulating these bulks, CBER advises that each consistency lot use different antigen bulk lots (or combination of bulk lots). Ideally, these bulk lots should be produced in the facility to be licensed; however, these lots need not be products of full-scale production runs. Importantly, the manufacturing process used to produce the consistency lots should be well established and unchanged between the consistency lot runs and the submission of a license application. Similar considerations regarding number and quantity of manufacturing consistency lots apply when the manufacturing process is scaled up or changed after licensure. As the number of antigens increases in the combination vaccine, manufacturers are encouraged to contact CBER to discuss the approach for demonstrating manufacturing consistency, as well as the formulation of appropriate consistency lots.

CONCLUSIONS

As combination vaccines become more complex, 2 main regulatory challenges become obvious: How can manufacturers meet the requirements for demonstrating lot consistency? Specifically, is it feasible to use multiple lots of each antigen in assessing lot consistency? Can preclinical testing methods be developed that are better predictors of clinical response to allow for more expeditious evaluation of the impact of formulation changes and process scale-up, as well as for assessing compatibility of antigens before use in the clinic?

In summary, combination vaccines present unique challenges for manufacturing and product development. Thus, a well-designed and coordinated product and clinical development program is invaluable. Furthermore, as the complexity of combination vaccines increases, so does the need for frequent interactions with FDA to avoid costly and nonproductive preclinical and clinical studies. The topic of combination vaccines brings to the fore many of the areas of difficulty in the arena...
of combination vaccine development and underscores the need for continued reevaluation of current guidance documents in addressing the increasing complexity of vaccines.

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