

GUIDANCE FOR INDUSTRY

FOR THE EVALUATION OF COMBINATION VACCINES
FOR PREVENTABLE DISEASES:

PRODUCTION, TESTING AND CLINICAL STUDIES

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Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Diseases:
Production, Testing and Clinical Studies

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I. INTRODUCTION

A. Scope

The Center for Biologics Evaluation and Research (CBER) is providing this document to provide further guidance and advice on the production, testing and clinical study of combination vaccines. A separate section will deal with issues related to simultaneous administration of vaccines. This document does not cover therapeutic combination vaccines. Not all issues outlined in this document will pertain to all types of combination vaccines. For example, some issues related to live vaccines may not apply to inactivated vaccines and vice versa. As with other guidance documents, FDA does not intend this document to be all-inclusive and cautions that not all information may be applicable to all situations. This document is intended to provide information and does not set forth requirements. FDA anticipates that manufacturers may develop alternative methods and procedures, and discuss them with FDA. This guidance document represent the agency's current thinking regarding issues related to combination vaccines. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

B. Defining "Combination Vaccine"

For this document, a combination vaccine consists of two or more live organisms, inactivated organisms or purified antigens combined either by the manufacturer or mixed immediately before administration and intended to:

- (1) prevent multiple diseases, or
- (2) prevent one disease caused by different strains or serotypes of the same organism.

Vectored vaccines and conjugated vaccines are combination vaccines if the prevention of the disease caused by the vector organism or the carrier moiety is to be one of the combination's indications.

C. Applicable Regulations

The regulations in Title 21 of the Code of Federal Regulations (21 CFR) Parts 600-680 pertain to biological products in general. Particularly important is 21 CFR 610.17, Permissible combinations, which states that licensed products may not be combined with other licensed or unlicensed products either therapeutic, prophylactic, or diagnostic, except as a license is obtained for the combined product. 21 CFR 601.2(a) describes the information that is required in a Product License Application (PLA). When filing the PLA, submit completed forms FDA 3211, 3212, or a combination of both. The "Center for Biologics Evaluation and Research: Refusal to File Guidance for Product License Applications and Establishment License

Applications" dated July 12, 1993, (see the Federal Register of July 20, 1993 (58 FR 38770)) explains how CBER determines a PLA to be judged inadequate for filing.

In addition, certain drug regulations such as 21 CFR 201.56, 201.57, 210, 211, and 312 apply to combination vaccines. Also, the regulations in 21 CFR Parts 50 and 56, which provide protection for persons involved in clinical trials, apply to the use of combination vaccines in clinical trials. Combination vaccines must meet the requirements of these regulations where applicable.

D. Incorporation by Reference

CBER may grant, on a case-by-case basis, requests to incorporate manufacturing information from a manufacturer's approved license application into the same or related manufacturer's new PLA by referring to its date of submission(s) and all of its page numbers. This request should be made in writing at the pre-PLA meeting with CBER. Also, include a summary of the submission in the new PLA.

Before making a request for incorporation by reference, manufacturers should note the following:

- (1) the incorporated information will be reviewed again as part of the new application,
- (2) any changes to the cross-referenced component will require supplements to both the individual component PLA(s) and the combination vaccine PLA, and
- (3) a supplement must be filed to the cross-referenced component PLA(s) to include the component for further manufacturing into a combined product.

E. Joint Ventures

When two or more manufacturers wish to cooperate in the production of a combination vaccine, they should consult FDA's Policy Statement on "Manufacturing Arrangements for Licensed Biologics", (see the Federal Register of November 25, 1992 (57 FR 55544)). In light of the new change in the definition of manufacturer (see the Federal Register of May 14, 1996 (61 FR 24227), "Elimination of Establishment License Application for Specified Biotechnology and Specified Synthetic Biological Products"), FDA intends to revise the policy statement concerning cooperative manufacturing arrangements for licensed biological products to address contract, divided, and shared manufacturing arrangements.

F. Nomenclature

Currently, the proposed proper name for a new combination should link the names of all the component vaccines as they are currently licensed; for example, Diphtheria and Tetanus

Toxoids and Pertussis Vaccine Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria CRM197 Protein Conjugate). Understandably, such a name is difficult to place on the label of a small vial and may be inconvenient for other reasons as well. While CBER is considering changes to this naming scheme, it will review reasonable proposals for other names on a case-by-case basis. Alternate proper names should clearly convey the identity of the product, and not be confusing (see 21 CFR 201.6(b)).

II. MANUFACTURING ISSUES FOR COMBINATION VACCINES

A. Formulation Issues

1. Compatibility of Components

Experience has shown that combining monovalent vaccines may result in a new combination which is less safe or effective than desirable. Sometimes the components of inactivated vaccines may act adversely on one or more of the active components. One such instance occurred when the combining of whole cell pertussis vaccine and inactivated poliovirus vaccine (IPV) resulted in a vaccine with a decreased pertussis potency.

Additionally, immunological interference between vaccine viruses or virus subtypes has been observed when live vaccines are combined. Consequently, the combined components stimulated weaker immune responses than did viruses administered separately. Component cross-reactivity could also occur with a combination of live vaccines where recombinational events may allow attenuated organisms to be reconstituted to virulent forms.

Therefore, it is of utmost importance to validate the compatibility of the combined components before any clinical trials begin. CBER advises that the product be characterized and the integrity of the components be assessed by performing a battery of physicochemical, biochemical and biological assays.

To further demonstrate the compatibility of the components, it is recommended that preclinical studies, in an appropriate animal model, be conducted to determine the consequences of combinations on potency and immunogenicity. (See Section III., Preclinical Studies section for further discussion.) The manufacturer should consider that the components of the product may revert to toxicity or virulence and should quantify any such tendency both with the monovalent and the combined vaccines. Similarly, the physical characteristics, including resuspension and the suitability of the container and closure for the combination product should be assessed.

If the combination of component vaccines results in a volume too large to be safely administered, the manufacturer may investigate dose-reduction of some or all components. For

instance, the manufacturer may restore an optimum final volume by utilizing concentrated intermediate bulks to achieve final concentrations equal to the monovalent component vaccines. The effects of such formulation changes (see Section II.B., Demonstrating Consistency of Manufacture) should be evaluated preclinically.

2. Preservatives

21 CFR 610.15 describes the general requirements for agents like preservatives, adjuvants, and other constituent materials which may be added to a vaccine during or at the end the manufacturing process. In a combination vaccine, the preservative or stabilizer in one monovalent vaccine can alter the potency of the other vaccine(s). For example, thimerosal adversely affected the potency of IPV in its combination with Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed. The use of preservatives can be avoided if vaccines are provided in single dose vials.

Inclusion of a preservative does not obviate the need for the combination vaccine to be evaluated for potency and reversion to toxicity for each of the active components. In addition, the manufacturer should consider doing the following:

- (1) quantifying the levels of constituents or antimicrobials, that remain in the finished vaccine, and
- (2) conducting studies on the preservative's ability to protect the final product from contamination (see the Antimicrobial Preservatives Effectiveness Test prescribed in the United States Pharmacopeia (USP).)

3. Adjuvants

21 CFR 610.15 describes the general requirements for adjuvants which are agents that augment specific immune responses to antigens. Currently, the only adjuvants included in U.S. licensed vaccines are aluminum compounds. An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product. As with other ingredients in the final formulation, the adjuvant should be shown to be compatible with all components in the formulation as described above. If appropriate, the manufacturer should demonstrate how much of each component is being adsorbed to the adjuvant. The Investigational New Drug Application (IND) and PLA should describe:

- (1) changes in manufacture concerning adsorption, such as the stage at which the adsorption takes place for a previously licensed component,
- (2) the efficiency and kinetics of simultaneous adsorption (if applicable),
- (3) the efficiency and kinetics of adsorption of components related to changes in the adjuvant, or relative concentrations,
- (4) the assessment of post-formulation adsorption of components not previously present as adsorbed, and

- (5) the effect of the adjuvant on the ability to assay components that were not previously adsorbed; immunologic identity tests or pyrogenicity tests should also be addressed.

Manufacturers should consider whether the following have an impact on the safety, purity and potency of the new combination in comparison to that of the monovalents:

- (1) whether a non-adsorbed component becomes adsorbed,
- (2) whether de-adsorption of the adsorbed component occurs,
- (3) for a previously licensed vaccine, changing the stage of manufacture at which adsorption occurs,
- (4) chemical forms (e.g., aluminum hydroxide and aluminum phosphate) of the adjuvant and buffers which are different from prior manufacturing,
- (5) the effects of mixing different adjuvants, and
- (6) how time affects the adsorption of antigen(s) to the adjuvant (this should be monitored as part of the stability studies of the combined product.)

4. Inactive Components

During formulation development, a manufacturer should determine the effect of using different buffers, salts, and other chemical factors on the safety, purity and potency of the final combined vaccine. Similarly, the manufacturer should ascertain if the stabilizers, i.e., lactose, gelatin, sorbitol, etc., will interact to the detriment of the safety, purity or potency of the vaccine.

5. Stability/Expiration Dating

Storage of a combination product can be divided into at least three stages:

- (1) storage of each in-process component,
- (2) storage of the combined product before initiation of potency testing, and
- (3) storage of the combined product after initiation of potency testing.

Stages 2 and 3 may include storage in bulk or filled containers or a combination of both. The manufacturer should have validated storage times and conditions for each component and the combination product (up until potency testing begins). "Real time" data should be provided to support the appropriateness of these limits. The dating period for a combined vaccine begins on the date of initiation of the last valid potency test on the first component tested and will be no longer than the dating period of the component with the shortest dating period. The data to support storage times and the dating period should confirm the stability of the product over its entire shelf life, i.e., the maximum length of the storage periods (before potency testing) plus the dating period.

At the time of the PLA submission, it is recommended that stability data supporting the dating period be available from at least three lots of final container product covering the requested

dating period. To have adequate data at the time of PLA submission, applicants should initiate stability studies during clinical development. CBER encourages the periodic submission of interim stability data to the IND. Studies should be conducted according to a stability protocol which outlines the tests to be conducted and the intervals when testing will be performed. The studies should be done as outlined in the FDA "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics (1987)" (see the Federal Register of April 3, 1987 (52 FR 10819)); see also the International Conference on Harmonisation final guideline on "Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (see the Federal Register of July 10, 1996 (61 FR 36466)). While accelerated stability data may be used as supporting data, it should not be used to assign the dating period.

B. Demonstrating Consistency of Manufacture

1. Number and Scale of Consistency Lots

After the final manufacturing procedures are established, consistency of manufacture should be demonstrated. This may be done by producing at least three, preferably consecutive, final bulk lots from which final containers are filled. If consecutive lots are not used, an explanation should be provided.

While CBER recommends that consistency lots be produced in the manufacturing facility for which the corresponding Establishment License Application (ELA) (or supplement) will be submitted, pilot facilities may be used following guidance provided in the "Center for Biologics Evaluation and Research; Use of Pilot Manufacturing Facilities for the Development and Manufacture of Biological Products; Guidance" (see the Federal Register of July 11, 1995 (60 FR 35750)); see also "FDA Guidance Concerning Demonstration of Comparability of Human Biological Products Including Therapeutic Biotechnology-Derived Products" (see the Federal Register of April 26, 1996 (61 FR 18612)). Ideally, the lots will be manufactured using the same equipment intended for full scale production. Generally, the lots need not be full size production runs. However, the comparability testing of at least one full manufacturing scale lot may be requested by CBER if the manufacturing procedures create a situation where the scale of manufacture could affect the final product's safety, purity or potency. An example might be an adsorbed product, or a product that is difficult to resuspend, where the final product may be affected by the efficiency of the adsorption or mixing procedure.

CBER also recommends that at least one lot be submitted for release and be available for distribution at the time of licensure. It may be one of the consistency lots if appropriate. More lots may be requested.

2. Necessary Combinations of Monovalent Components

Each of the manufacturing consistency lots should utilize a different bulk lot of each active component. Thus, there should be at least three different bulk lots for each of the immunogens contained in the combination vaccine. However, if a component is an already licensed immunogen, fewer lots of this component in combination as part of the three consistency lots of final product may be adequate. Manufacturers are encouraged to seek guidance from CBER on such situations.

It is recommended that if the vaccine contains unlicensed product intermediates, then the component lots should also represent at least three, preferably consecutive, production lots. Test results for three lots of each intermediate should also be provided. When the intermediate is also a component of a licensed vaccine, the production of three consecutive component lots may not be needed, if the licensed vaccine has been released by CBER or the manufacturer within the previous twelve months.

It may not be necessary to formulate all possible combinations of consistency lots of vaccine components. However, manufacturing consistency should be shown for each of the antigens present in the final vaccine. Thus, the consistency lots for each of the different components of the combination vaccine should meet the specifications for that particular component. For certain products, such as multivalent polysaccharide vaccines, the testing of combinations using fewer than three monovalent lots of each type may be adequate in some cases, since the testing of all possible combinations for separate lots would be prohibitive and also would not contribute significantly to the evaluation of the product.

It is recommended that a matrix table (such as the one shown below) which explains which combination(s) of monovalent products have been used in laboratory, preclinical and clinical testing be submitted to the IND and PLA. The following is one combination that will, in general, be acceptable to CBER for a combination vaccine.

$$\begin{array}{llllll} X_1 & + & Y_1 & + & Z_1 \dots N_1 & = \text{Final Lot 1} \\ X_2 & + & Y_2 & + & Z_2 \dots N_2 & = \text{Final Lot 2} \\ X_3 & + & Y_3 & + & Z_3 \dots N_3 & = \text{Final Lot 3} \end{array}$$

Manufacturers may propose other combinations. The lots of monovalent product selected for the combination product should represent normal manufacturing lots and should be selected from lots that fall within the normal range of test results for important parameters. For example, if some lots normally fall at the low range of acceptable potency and some fall within the high range, then lots selected only from the middle range would not be considered representative. In all cases, early consultation with CBER staff on the proposed scheme is recommended.

A random sampling procedure such as the following may be used as an aid to obtaining representative selections. Suppose, for example, there are 10 lots of component X, 8 lots of component Y, and 10 lots for each component Z...N. Using a random number generator, a random number from 1 to 10 would select the first X lot, a random digit from 1 to 8 would determine the Y lot, and random numbers from 1 to 10 would select lots Z through N, in turn, to define Lot 1. This procedure is repeated until the desired number of final lots of the combination vaccine is determined.

C. Testing Issues

1. General

21 CFR 610.1 requires the manufacturer to test each lot of product to show conformance to the standards applicable to that product. Pursuant to this regulation, each test shall be done after completion of all processes of manufacture that may affect compliance with the standard. Therefore, a manufacturer must perform tests, under 21 CFR 610.1, for the combined product. If the performance of any of these assays are hindered by the new components, the manufacturer should develop new tests to demonstrate that the applicable standards are met. Upon demonstration that a satisfactory assay cannot be developed, the manufacturer should submit either of the following:

- (1) an explanation of how the safety, purity and/or potency will be assured without such test(s), or
- (2) a proposal to perform the test(s) at an earlier stage, while retaining the objective of the original test and assuring the product's safety, purity and/or potency.

2. Potency

Potency, as defined in 21 CFR 600.3(s), is the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through administration of the product in the manner intended, to effect a given result. For a combination product, the potency of each component for which a claim of efficacy is made should be determined. The potency of each component should comply with the potency requirement for the monovalent product unless it can be determined that any reduction in potency due to interaction with other components of the combination product does not result in a lowering of the efficacy in humans. With CBER concurrence, testing of the final formulated bulk vaccine may be substituted for testing in the final container when additional processing has been shown to have no effect on the potency of the final product. However, in some cases such as a lyophilized product, demonstration of the potency of the product in the final container is necessary. Tests for potency should detect any component interactions that may have a potentiating or interfering effect on any other component.

3. Identity Testing

An identity test shall be performed on labeled final containers of vaccines. An identity test should be performed for each active component present in the combination unless it can be shown that this is not necessary. The purpose of the test is to identify the product as the one designated in the product labeling and to distinguish it from any other product being processed in the same laboratory (21 CFR 610.14). Identity tests should focus on those differences that exist between different final container products to assure mislabeling has not occurred.

4. Sterility

All ingredients formulated into a combination vaccine and the final combined product shall meet the sterility requirements outlined in 21 CFR 610.12. The presence of residual antibiotics, the inclusion of preservative for components that were previously unpreserved, and the inclusion of particulate adjuvants may require validated changes in the performance of bulk and final container sterility testing.

5. Purity

Each bulk component of a combined vaccine should meet purity characteristics appropriate for that component. For example, the Haemophilus b conjugate component should meet the acceptance criteria for pyrogenicity testing even if combined with a vaccine such as DTP which is known to contain endotoxin. In this case, final product will not be pyrogen tested. Similarly, purity of bulk components may be demonstrated by SDS-PAGE; however, this assay may be inappropriate for the final product.

III. PRECLINICAL STUDIES

A. Adjuvants

Vaccines adsorbed with aluminum compounds are in widespread use. Although local reactions occur with these vaccines, their safety has been demonstrated by extensive clinical use. Several adjuvants that are not currently components of licensed vaccines (referred to as investigational adjuvants) have been studied preclinically and clinically with the goal of developing more effective immune stimulants. Since adjuvant-antigen combinations formulated together are approved as a single product, an adjuvant alone would not be considered a licensable product.

If a manufacturer contemplates incorporating an adjuvant other than aluminum compounds into combination vaccine formulations for preventable infectious diseases, discussions with CBER

should be initiated early in product development regarding appropriate preclinical and clinical studies. Potential safety concerns for investigational adjuvants include injection site reactions (e.g., pain, induration, erythema, granuloma formation, sterile abscess formation), fever, other systemic adverse effects (e.g., nausea, malaise, headache), immune mediated events (e.g. anaphylaxis, uveitis, or arthritis), systemic chemical toxicity to tissues or organs, teratogenicity, and carcinogenicity.

If there are limited or no toxicology data for the adjuvant being considered for inclusion in a combination vaccine for preventable disease, it is advisable to perform toxicity studies of the adjuvant alone. In addition, preclinical animal studies to evaluate the safety profile of the adjuvant/combination vaccine should be performed. It is recommended that these studies use the exact adjuvant/antigen combination, formulation, and route of administration intended for human use. Sponsors are encouraged to discuss protocols for preclinical toxicology studies with CBER before initiation.

In addition to toxicity studies, it is recommended that preclinical studies evaluate adjuvant effects on the immune response. These studies should optimally utilize the exact adjuvant/antigen combination planned for human use, and include a control group that receives the antigen(s) alone or the antigen adjuvanted with an aluminum compound to provide evidence that the investigational adjuvant augments the immune response to the antigen(s).

B. Animal Immunogenicity

It is recommended that new combinations be studied for the appropriate immunogenicity parameters in an animal model, if available, before the initiation of studies of human clinical trials. The response to each of the antigens in the vaccine should be assessed as well as the quality of the response. This evaluation may include a characterization of antibody class, avidity, affinity, half-life, or function, e.g. examining the ability to neutralize the target agent or toxin. It is preferable to study the new combination in comparison to the individual antigens in animals to determine if any augmentation or diminution of response occurs. Interference between live vaccine strains also may be studied in animal immunogenicity studies.

C. Animal Challenge Studies

Protection studies are recommended using an animal model, wherever one is available, for new vaccines or combination vaccines with a new antigen that has not been previously studied in humans. Protection should be demonstrated upon challenge with a virulent strain(s) of each organism against which the vaccine is intended to protect. The study should be conducted with statistically and scientifically valid procedures for verifying the results and these should be described. It is advisable to perform such studies early in the product development cycle.

IV. CLINICAL STUDIES TO SUPPORT THE LICENSURE OF COMBINATION VACCINES

This section will discuss the design and statistical considerations for clinical studies to demonstrate the safety, immunogenicity and efficacy of combination vaccines, whether the combination consists of previously licensed or unlicensed vaccines. Generally, such studies should:

- (1) be randomized and controlled, and
- (2) for safety and immunogenicity, include comparisons of the separate but simultaneously administered individual vaccines with the combination.

If the sponsor believes it is not feasible to conduct such studies, it is advisable to discuss this with CBER before beginning alternative types of studies. Each new product should be supported by product specific data. Additional data obtained in uncontrolled studies may provide useful supplemental information.

A. Safety Studies

Studies of a combination vaccine have generally been performed to demonstrate that safety is not decreased in comparison to the safety of separate, but simultaneously administered, individual components. CBER will consider all data in the risk-benefit assessment of a combination vaccine. The primary data set for evaluation of safety will be the actual clinical experience with the combined vaccine.

Ideally, studies of comparative safety should be randomized and controlled. Typically, individuals in the control group receive the separate, simultaneously administered components contained in the combination vaccine (e.g., a group receiving an investigational DTaP-Hib vaccine compared to a control group receiving separate injections of DTaP and Hib vaccines). Follow-up safety assessments should be active and prospectively planned with baseline and specific post-vaccination times of assessment. The specific time frame of assessments should be appropriate for the vaccine under study. Typically, participants should be actively monitored (temperature and reaction sites measured and other specific information recorded) on designated post-vaccination days for up to one week for most killed and recombinant vaccines or for fourteen or more days for most live vaccines. Follow-up should continue through at least thirty days by telephone interview or questionnaire whether for live or for killed vaccines. For example, for a particular killed vaccine, it may be appropriate to monitor subjects at 6-12 hours, and days 1,2,3,7, and 30 postvaccination.

Case report forms should query for specific events. These forms can also accommodate the recording of other events that may occur during the observation period. Typically, local event assessment includes measuring erythema and induration, and grading pain (e.g., mild,

moderate, or severe) and tenderness (e.g., pain on pressure). Information should be recorded concerning the investigational combination vaccine injection site, as well as the injection sites in the control group for the simultaneously administered vaccines containing the same components. The primary analysis for local reactions is usually a comparison of the combination vaccine to the separate individual component antigens using the most reactogenic individual component (see Section IV.D., Statistical Considerations). Systemic events to be recorded include fever and symptoms such as malaise, headache, vomiting, protracted crying, etc. The systemic events assessed should be appropriate for the vaccine and the age of the participants.

Often one or more feasibility studies accumulate data on common local and systemic reactions and then an additional, larger trial(s) with a sample size suitable for the evaluation of less common events is undertaken. CBER will consider simplified trial designs for large trials intended to assess or identify less common serious events as part of a total package to support licensure. For such large trials, it may be appropriate to assess only a subset of subjects for the more common events. For example, to assess local injection site events in large safety trials, the active surveillance could include recording protocol-defined significant local events in all vaccines as well as detailed local injection site evaluation in a randomly selected subset. Also, using fever as an example of a more common event, the following strategy may be appropriate: measure the temperature for each subject in a randomly selected subset as well as for any trial participant for whom fever was clinically suspected. A valuable method of capturing a high proportion of serious adverse events is to develop databases linked to those of HMOs, etc. which can then detect the entry of a test subject into the medical care system after vaccination. Uncontrolled prelicensure studies may also provide additional safety information. Also, postapproval studies may be required to assess the potential for rare but serious reactions.

B. Immunogenicity

Studies of the immunogenicity of all vaccine components in the combination should be performed. Again, ideally, the immunogenicity induced by the combination vaccine should be compared with that induced by the separate but simultaneously administered individual vaccines. If vaccine components are already included in a licensed combination, then the current licensed formulation could be used in the control group for comparisons of immunogenicity (see example, Section IV.A., Safety Studies). Each serotype or component of a combination vaccine should show immunogenicity in the combination. Information about immunological correlates of protection for individual components should be submitted with the protocol. Also, information about each assay used to evaluate immunogenicity should be submitted to the IND no later than the clinical protocols utilizing these techniques.

The assessment of appropriate immunological parameter(s) for each component is critical. The selection of such criteria should be discussed with CBER. In general, it is recognized that

factors other than antibody levels and/or "seroconversion" can be important for protection. The quality and not simply the quantity of antibody should be considered. For example, affinity, functionality, epitope recognition and other defining parameters are important in determining the quality of an antibody response.

The objective of comparative immunogenicity studies should be to rule out important differences between the response to the combined vaccine compared to the separate but simultaneously administered antigens. These studies need not be designed to show superiority of the new vaccine. Such studies should have sufficient power to rule out clinically meaningful differences in geometric mean titers (GMTs) and/or seroconversion rates, and should consider the intrinsic variability in assays and subjects. A clinically meaningful difference for each response should be defined prospectively in the clinical protocol. The difference to be ruled out should be clearly stated in the study hypothesis and this difference used in calculating the sample size. Any differences observed should be evaluated for clinical relevance. Changes in dose or schedule of doses for individual components should be supported by the clinical data.

The physical combination of vaccine components is a process for which consistency of manufacture should be demonstrated physicochemically, biologically, and clinically, as would be done for new single component vaccines. The number of lots that should be evaluated clinically may be less than the entire lot series for consistency of manufacture. However, clinical studies of more lots may be needed in some situations, e.g., if the product contains previously unlicensed components. CBER recommends that:

- (1) at least one of the manufacturing consistency lots be included in the clinical studies,
- (2) the same lots used to prepare the combination vaccine be used for the control lots of separately administered vaccines, to rule out differences in lots being the cause of differences in immunogenicity, and
- (3) analysis for differences between lots be performed.

C. Efficacy

The efficacy of each component should be demonstrated in clinical studies. Ideally, clinical trials will be prospective, randomized and controlled. Endpoints used to evaluate efficacy in these trials can range from disease incidence to a well-established correlate of protection. A correlate of protection in vaccine efficacy trials is generally a laboratory parameter that has been shown from adequate and well-controlled trials to be associated with protection from clinical disease. An immunological correlate of protection is most useful if clear qualitative and quantitative relationships can be determined, e.g., a certain type and level of antibody correlate with protection. In some cases, an immunological correlate of protection may have been inferred by serological surveys in an immunized population. Such survey data, however, are frequently difficult to interpret.

For some well-studied vaccines there is ample evidence that a certain level of a defined antibody response correlates with protection. Attainment of such antibody levels in a significant proportion of the target population following immunization could be the basis for licensure in the absence of additional efficacy studies.

If the combined vaccine consists of components that have been licensed based upon well-controlled studies of protective efficacy, such studies may be incorporated by reference in support of the combination product. Immunogenicity data may be used to “bridge” the existing efficacy data where possible. In some cases, CBER may accept the use of comparative bridging immunogenicity data without a well-established correlate of protection, e.g., to compare the immune response of a component in a new combination vaccine to that observed for the component in the efficacy trial formulation.

If antibody levels induced by the combination vaccine are lower than those induced by the component vaccines, a "protective" antibody level might still be attained. In such cases, the manufacturer should provide data or information to support the premise that the lower response will not affect the protective efficacy of the product.

Although case-control studies are not the preferred method of assessing efficacy, there may be situations where they will be acceptable. Because such studies are subject to bias in many ways, all aspects of the design and conduct of the study including measures taken specifically to minimize bias should be described in detail in the study protocol.

CBER will give special consideration to alternative proposals for demonstrating the efficacy of multiple serotype combination vaccines, where determining efficacy of the vaccine against each serotype may be difficult. Studies designed to demonstrate efficacy for such vaccines could be based upon epidemiologic data regarding the disease incidence of each serotype in the target population. Thus, while the primary endpoint may be the aggregate of disease with all serotypes included in the vaccine, the study should be of sufficient size to allow meaningful subgroup analysis of protection against at least some individual serotypes. For multiple serotype vaccines where clinical efficacy can not be demonstrated due to an insufficient number of homologous cases, efficacy may sometimes be inferred from immunogenicity data. This use of immunogenicity data is strengthened if a serological correlate(s) of protection was identified for the serotypes for which clinical efficacy was demonstrated. Supporting immunogenicity data for such less common serotypes should be comparable to that elicited by heterologous serotypes for which clinical efficacy was demonstrated. Functional assays comparing the immune response elicited by the various serotypes may be especially useful in this regard. In all such cases, CBER encourages an early consultation regarding such issues.

D. Statistical Considerations

1. General

Most comparative combination vaccine trials will have the separately administered components or previous combinations of the components as active controls. Subjects should be assigned randomly to vaccine groups. Group assignment by computer-generated random numbers is a typical acceptable randomization procedure. Assignment by subjects' characteristics (e.g., age or day of arrival at the clinic) is not a random mechanism and thus may introduce bias into the analysis. Stratified randomization may be recommended if warranted by the inclusion criteria or known disease risk factors.

The planned randomization procedure should be described in enough detail to allow assessment of the validity of the method. Usually a paragraph or two in the protocol will suffice.

When CBER reviews non-randomized trials, data evaluation will consist primarily of estimation (point estimates and confidence intervals). The sponsor may be asked to demonstrate the reliability and validity of such estimates in the evaluation of the product.

The statistical approach employed in the data analysis may be either hypothesis testing or estimation. The statistical methodology to be used for each endpoint should be presented in detail in the protocol. In addition, the protocol should contain explicit statements regarding the endpoints to be analyzed, the null and alternative hypotheses to be tested, as well as the associated significance level.

A critical point is that failure to reject the conventional null hypothesis of no difference does not necessarily imply equivalence. Therefore, undersized difference-detection trials with nonsignificant results will be insufficient to demonstrate similarity of the combination vaccine to the separately administered components.

Blackwelder [Combined Vaccines and Simultaneous Administration: Current Issues and Perspectives, *Annals of the New York Academy of Sciences*, Vol.754: 321-328, (1995)] has suggested that combination vaccine trials aimed at demonstrating equivalence be designed and analyzed to reject a hypothesis of a difference, rather than the traditional "null" hypothesis of no difference. This approach may be acceptable, depending on the proposed trial design (e.g., aim, error levels, threshold effects, etc.).

Confidence intervals on the difference between the combination vaccine and the separate components should accompany formal hypothesis tests or may themselves comprise the primary analysis. Confidence intervals are particularly useful when testing equivalence hypotheses. To demonstrate equivalence or similarity of rates and proportions, one-sided tests will usually be appropriate, since the comparative trials are aimed at demonstrating that the combined vaccines are "not significantly worse than" the separate components. That is, superiority of the

combination vaccine is not an issue for licensure.

Should the sponsor desire to demonstrate and claim superiority of the combination vaccine compared to the separately administered components, the sponsor should design a difference-detection trial rather than an equivalence trial. In such case, CBER recommends that the sponsor test the conventional null hypothesis of no difference against a two-sided alternative.

Even when one-sided tests are appropriate, two-sided confidence intervals for estimation purposes are recommended, since the entire range of likely differences will be informative. Confidence intervals should be narrow in width and consistent with the significance level specified in planning the trial. For example, a two-sided 90% confidence interval corresponds to a one-sided .05 test, while a two-sided 95% confidence interval provides a one-sided test at the .025 significance level.

2. Immune Response

Analysis of post-vaccination GMTs (geometric mean titers), or GMRs (geometric mean ratios), is customarily aimed at ruling out pre-specified clinically meaningful differences between the combination vaccine and the separately administered components with respect to these endpoints or demonstration that a surrogate level has been met or exceeded. The sample size required to rule out any pre-specified difference should be calculated.

It is desirable that the immune response to the combination vaccine not be significantly lower than to the separate components. Consequently, analysis of GMTs as a two-sided bioequivalence test has merit. Thus, it may be desired that the combined vaccine evoke titers that are neither too low nor too high by some pre-specified amounts.

Comparison of seroconversion rates may accompany the analysis of titers as a supportive analysis. If seroconversion is considered a primary endpoint, clinically meaningful differences in these rates to be ruled out should be specified in the protocol, and the necessary sample size calculations performed. Without established correlates of protection, it will be difficult to evaluate differences in either seroconversion rates or GMTs without joint consideration of both.

Immune surrogates of protection, if established, should be considered when evaluating any differences between the combination vaccine and the separate components.

3. Common Adverse Reactions

Comparisons between the combination vaccine and the separate components regarding common adverse events should assess the by-injection and the by-subject event rates. Since reactions to different injections within the same individual are not independent observations, an additional method of analysis which considers all injections but accounts for the within-individual correlations is encouraged.

The required sample size calculated should refer to the number of subjects needed. The difference in rates to be ruled out should be specified in the protocol, and the appropriate sample size calculations performed.

The comparison of local adverse reactions is problematic. First, combination vaccine studies are not usually double-blinded. This has implications for assessing both local and systemic reactions. Secondly, when local reactions at one injection site for the combination vaccine are compared to local reactions at more than one site for separately but simultaneously administered component vaccines, the analyst must compare seemingly incongruous factors, i.e., one injection site versus two or more injection sites.

Various methods for analyzing local adverse reactions in this context have been considered. One plausible method commonly used is to compare the single site reaction to the worst reaction among the multiple injection sites. All reactions among the multiple injections should be recorded. Determining a method of comparison that is both unbiased and reasonable is a topic that needs further consideration.

4. Less Common Adverse Reactions

It is important that manufacturers monitor less common adverse events carefully, especially when the vaccine is likely to be widely administered. For example, if an adverse event occurs in 1% of vaccinees receiving separate components, and the combination increases this rate to 2%, there will be 10,000 additional adverse events for every million persons receiving the combination vaccine. These rates are cited here for illustrative purposes only and may not be applicable to all trials. The size of the target population should be considered when determining the appropriate size of safety studies.

5. Rare Adverse Events

Sample sizes should be large enough for adequate safety assessment. However, even in a large trial, a rare adverse event associated with vaccination may not be observed. Nonetheless, a large enough sample size is desired so that if no such event is observed, it can be concluded that the event will occur with very low probability, if at all, in the general population.

For example, suppose in a trial with n subjects in the combination vaccine arm, no cases of a

certain rare adverse event are observed. Using the "Rule of 3", (Hanley and Lippman-Hand. JAMA 249(13): 1743-45, 1983) a 95% confidence interval for the rate of occurrence of the event is estimated as $(0, 3/n)$. The sample size in the combination vaccine arm should be large enough to provide a reasonable upper confidence limit. CBER will also consider appropriate use of post-approval studies in the evaluation of rare adverse events. Again, the size of the target population should be considered in determining an acceptable upper limit on the event rate.

6. Sample Size

The protocol should include sample size calculations for each endpoint (immunogenicity, safety, and efficacy if applicable). The largest sample size required for any endpoint should be the one selected for overall trial enrollment. However, immunogenicity studies typically comprise a subsample, randomly selected if possible, from the initially enrolled population.

Sample size calculations should be performed after formulation of the null and alternative hypotheses and should be consistent with those hypotheses. Sample size estimates also may be based upon confidence intervals, if that is the planned analysis approach.

There is no standard for sample size that will be appropriate for all trials of combination vaccines. The criteria for determining an adequate size for a trial will typically be based on statistical, clinical, and basic scientific judgment, and may vary from product to product and from one setting to another.

All assumptions upon which the sample size calculations are based, as well as any special sample size methodology used, should be stated explicitly in the protocol. Enough information should be provided to enable a CBER statistician to verify the sample size estimates.

7. Submission of Data to CBER

When the sponsor is ready to submit a PLA, CBER should be consulted for detailed instructions regarding submission of the data. It is desirable for the data to be submitted to the agency on computer diskettes in a form ready for statistical analysis. Diskettes should be accompanied by a copy of the prospective protocol and a detailed statistical report that describes all data files, the names and locations of variables, and computer program statements used to perform main data analyses. Enough information should be provided to enable a CBER statistician to easily verify the sponsor's analysis results, as well as perform additional analyses as appropriate.

E. Indications and Usage

The development of combination vaccines should be based on rational prevention of the indicated diseases. The data required to support each indication will be the same as for single component vaccines. If the combination vaccine is to be given on a different schedule from that of any previously approved component, data showing adequacy of the proposed schedule should be submitted.

The use of combination vaccines as boosters should also be addressed. If booster use is indicated, it should be supported by safety and immunogenicity data. The labeling should state if booster use is not indicated or was not studied.

V. VACCINES ADMINISTERED SIMULTANEOUSLY WITH THE COMBINATION VACCINE

Immunogenicity and safety data should be obtained in prelicensure studies to support the simultaneous administration of a new vaccine with already licensed vaccines that would be given to the same target population using the same (or overlapping) schedule. The concepts presented in Section IV.D: Statistical Considerations, are also applicable here. With regard to immunogenicity, assessments should be performed to show that subjects still attain an acceptable immune response to both the combination vaccine and the other simultaneously administered vaccines. In some situations, studies of simultaneously administered vaccines compared to vaccines separately administered at different times are useful.

Ideally, the immunogenicity obtained with such simultaneous administration should be evaluated early in clinical development for all components to detect any possible immunological interference. The assessment of immunological interference is particularly valuable before proceeding to a large trial(s) of the investigational vaccine because such trials will typically utilize vaccines for other indications routinely given on the same schedule. Typically, the studies will evaluate safety and interference of the new combination vaccine with one type of simultaneously administered vaccine per indication, e.g., for a new DTaP vaccine, safety and interference will be evaluated in a statistically valid manner with one type of simultaneously administered Haemophilus influenzae type b conjugate vaccine.

The package insert should include descriptions and references for available data concerning simultaneous administration of the new vaccine with licensed vaccines. If no studies have been done, a statement that there are no data regarding safety or immunogenicity of simultaneous administration should also be included.