Combination vaccines differ from single-component vaccines in composition and in how they are manufactured, which poses significant challenges to implementing effective quality-control tests, including measurement of potency. Because each combination vaccine is unique, existing guidelines often fail to provide sufficient information to overcome the inevitable problems encountered when developing and implementing potency tests. Success depends on careful consideration of scientific and regulatory issues. Significant problems may occur if potential interactions between different components in the vaccine are not taken into account during product and test development. Thorough analysis of critical assay parameters and attention to scientific and statistical justifications for the test increase the likelihood of its acceptance. Practical approaches based on experience include rational design of validation studies, complete evaluation and documentation of the potency tests under the conditions in which they are to be applied, and establishing the relationship between production lots of vaccine and lots used in clinical trials.

Many articles in these proceedings and elsewhere have reviewed the development and application of combination vaccines (for examples, see [1–4]), but relatively few have dealt with laboratory testing of these products. For some combination vaccines, pharmaceutical development has outpaced the development of quality-control (QC) procedures, including potency tests [5]. Potential problems associated with tests of potency and other QC tests of combination vaccines have long been recognized [6], but those have rarely been addressed, although general discussions of QC procedures for vaccines are available in guidance documents and other publications.

Potency testing is performed on vaccine lots to demonstrate the capability of the product to confer protective immunity. From one perspective, potency test methodology has reached a high level of refinement and has proven over time to be very effective in assuring the excellent quality of vaccine products. From other perspectives, the methodology needs further research and development—for example, to establish suitable correlates of vaccine efficacy and to find alternatives to the use of laboratory animals. Current scientific and technical problems in potency testing may defy solution without further research on pathogenic mechanisms of infectious agents and the mechanisms of immune regulation. Application of potency tests to combination vaccines poses a special challenge.

Pharmacopeial requirements for the individual components of combination vaccines provide a starting point to establish relevant and effective potency tests. Test development for combination vaccines has been limited by unanticipated interactions between vaccine components, adjuvants, and excipients; the lack of standardized, generally accepted assays; and, in specific cases, the absence of a surrogate laboratory test that correlates with protection in the target population. Even when a correlation is already established, some potency tests may lose their predictive power for protective efficacy when applied to combination vaccines such as Haemophilus influenzae type b (Hib) vaccine combined with acellular diphtheria-tetanus-pertussis (DTP) vaccine [7]. The potency of the Hib component was adequate when...
measured by an in vitro assay, but the immunogenicity observed in clinical studies was reduced 5-fold to 15-fold.

REGULATIONS AND GUIDANCE DOCUMENTS

Regulatory requirements and guidance documents provide a general framework to approach the validation of potency tests of combination vaccines. Potency of a biological product is defined in Title 21 of the Code of Federal Regulations (21 CFR), section 600, as “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result” [8]. For vaccines, the “given result” is protective immunity. Potency tests are performed to demonstrate that the vaccine will be capable of achieving this result, but the language of the rule allows for various test methods to be used. According to 21 CFR section 610, “Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in §600.3(s) of this chapter” [9]. Equivalent methods may be acceptable, according to 21 CFR section 610 [10]. Specific requirements for bacterial products and viral vaccines contained in 21 CFR sections 620 and 630 [11, 12] were removed from the regulations in 1996, allowing for additional flexibility in potency testing. Center for Biologics Evaluation and Research guidance documents on vaccines and combination vaccines [13, 14] provide a general description of when and how potency testing is to be conducted, but information is not provided for specific components or products.

General guidelines on validation of analytical methods and vaccine potency tests have been provided by the World Health Organization [15, 16] and the International Conference on Harmonisation [17, 18]. However, the lack of specific, practical guidance has been a barrier to the development of new methods [19]. In contrast, guidelines for validating alternative tests for toxicity have existed for several years [20]. The toxicity testing guidelines are useful, in that they describe the steps in method development needed to achieve regulatory acceptance of new tests, but, until very recently, little specific information has been available to aid development of potency tests for combination vaccines.

RELEVANCE

The relevance of a potency test to clinical efficacy is fundamental to its use for a combination vaccine. Different test methods, such as assays of physicochemical properties, antigenicity, immunogenicity, infectivity, and protection against infection or disease, are used to measure potency. Their applicability depends on the nature of the vaccine antigens and the purpose of the test. Physicochemical and antigenicity assays can be used alone or in parallel to control manufacturing consistency and vaccine formulation, but their correlation with protective efficacy is often difficult to establish. In most situations, too little is known about essential immunological characteristics of the antigens and how these relate to mechanisms of pathogenesis and immune defense against disease. One example is the controversy over serologic correlates of protection for acellular pertussis vaccines. Serologic testing in efficacy studies has revealed that highly protective combination vaccines containing acellular pertussis antigens can fail to elicit high titers of human serum agglutinin activity against either of the pertussis toxoid or filamentous hemagglutinin antigen components of the vaccine [21]. It has also been observed that different acellular pertussis-containing vaccines may have similar immunogenicity with regard to the pertussis toxoid and filamentous hemagglutinin antigen components but differ in efficacy [21]. Therefore, serologic data may not reflect the efficacy of pertussis components in combination vaccines. As a result, it is more difficult to demonstrate the relevance of in vivo or in vitro assays, compounding the problems in extrapolating potency data from laboratory tests to humans.

VALIDATION

Careful attention to the essential elements of assay validation can help to avoid common problems with potency tests encountered during the licensing process. Validation includes a complete assessment of the suitability of the method for its intended purpose and a thorough evaluation of a number of assay parameters. Conducting validation studies early in the life cycle of product development may help to establish consistency between the lots used in efficacy trials and actual production lots. For potency tests, the critical assay parameters include accuracy, precision, linearity, range, specificity, and robustness, according to the World Health Organization and International Conference on Harmonisation guidelines [16–18]. In addition, scientific and statistical justifications are needed to set appropriate specifications for QC and product release.

When potency tests of individual components are already approved for use on licensed products, it is necessary to show that they still produce valid information when applied to a new combination vaccine [6]. For newly developed tests that are not yet approved, it is important to establish the relationship between laboratory test results and clinical efficacy of the product. In general, in vivo tests are still needed at some stages of product development [22, 23], particularly in situations where clinical efficacy has no universally accepted in vitro correlate. Immunogenicity and challenge studies should be conducted early in the product development life cycle [14] to show the
adequacy of an alternative potency test for the combination vaccine.

In principle, limited bridging studies may show that an existing assay used on a monovalent formulation is also valid for a combination vaccine, but in practice, stand-alone validation studies of critical assay parameters are often needed. Each combination vaccine is comprised of a unique combination of active components, excipients, and residual substances. Any of these materials might interfere with accurate measurement of the potency of a given component. The “sample matrix” for the potency test consists of all other substances present in the combination vaccine, including the other active components, and the complexity of the sample matrix increases the potential for assay interference. Such matrix effects are difficult to predict and may require a complete validation study of the proposed potency test [24]. One example of this type of interference is the interaction of Hib conjugate vaccine with DTP vaccine. In a vaccine containing the whole-cell pertussis component and Hib polyribosyl ribitol phosphate (PRP) conjugated to tetanus toxoid, a disproportionate increase in the antitetanus response was observed in both in vitro and in vivo laboratory tests of potency but was not observed in the antitetanus responses in clinical studies [25]. Where such interference occurs in a potency assay, the relevance of the test to clinical efficacy must be reevaluated. This emphasizes the importance of initiating validation studies early in product development.

Potential effects of adjuvants on QC testing are also a major concern; adjuvants may selectively desorb an antigen or interfere with a potency assay. Those features may affect not only the measurement of potency but also the stability and toxicity of the product [6, 25, 26]. Antigenicity testing often requires antigen desorption before assay, adding an additional step to the potency test and requiring further validation studies to confirm that measurement of the desorbed antigen is relevant to the efficacy of the vaccine. A desorption step is likely to increase assay variability and complicate the establishment of product specifications and reference materials for the potency test. The possibility that unadsorbed components that are mixed with adsorbed components may bind to adjuvant with potential impact on potency test results must be investigated during product development.

EXPERIENCE WITH POTENCY TESTS

Examples of problems identified during review of potency tests of combination vaccines can help in the development of acceptable tests (table 1).

1. Lack of an accepted experimental model as a correlate of protection. The combination vaccine may include ≥1 components for which there is no test that has been shown to correlate with clinical efficacy; this is the case for acellular pertussis vaccines [21]. There may be no single method that is generally accepted as a substitute test. Then the laboratory tests must be sufficient to demonstrate consistency in the content and quality of vaccine components between the lots used in clinical trials and production lots.

2. Failure to correlate assay results with clinical studies or accepted test methods. Proposed potency tests may be difficult to accept if their results have not been shown to correlate with results of clinical studies or accepted test methods. Failures are often attributable either to inadequate study design or poor evaluation of the potency test. Refinement of the standard operating procedure for the test, revalidation of the method, or further characterization of the test may be needed to demonstrate suitability of the test.

3. Missing validation information. Incomplete testing or documentation may prevent acceptance of the method, especially if critical parameters have not been examined. For example, the specificity of immunological reagents used in antigenicity tests of individual components may not have been demonstrated for the combination vaccine. Validation studies must always include appropriate control samples.

4. Failure to account for possible effects of changes in sample matrix. The reliability of a method to detect a subpotent component may be affected by excipients or residual materials introduced into the combination vaccine by other components. An example of this interference is found in combination vaccines that contain Hib PRP conjugate vaccine and DTP [25]. Validation studies must evaluate matrix effects that can occur as a result of interference with potency measurements by the presence of other substances. Efficient approaches to this problem have been proposed on the basis of statistical experimental designs that incorporate rational selection of the ranges of the matrix components to be evaluated [24].

5. Incorrect or inappropriate use of variance estimates. Assay variability is a critical issue. If the variability increases when an existing test is applied to a combination vaccine, the confidence of the test will be adversely affected. Control limits and product specifications may need to be reevaluated. Assay variability must be established under the conditions in which the test will be applied.

6. Inappropriate or unvalidated reference materials. In

Table 1. Common problems with potency tests of combined vaccines.

- Lack of an accepted experimental model as a correlate of protection
- Failure to correlate results of a proposed test with results of efficacy studies or accepted test methods
- Missing validation information
- Failure to account for possible effects of changes in sample matrix
- Incorrect or inappropriate use of variance estimates
- Inappropriate or unvalidated reference materials
some situations, the use of historical reference materials may be inappropriate. A reference material that performs adequately when used to test licensed single-component vaccines may not indicate the successful performance of the assay as applied to a combined vaccine. It is necessary to demonstrate that incorporation of additional components in the vaccine does not affect any of the conclusions about the assay or the product that are based on the reference material. For example, a combined vaccine that contains an adjuvant in 1 of the components may require additional procedures, such as antigen desorption, before potency testing. The historical reference material may be unsuitable when applied to the new test procedure. It may be necessary to develop and qualify new reference material that is similar in composition to the combined vaccine. Failure to characterize new reference materials under the conditions in which the test will be applied may hinder acceptance of the test. For example, it is necessary to determine the stability of the new reference material in order to show that it will perform adequately over the lifetime of the product for which the assay will be performed as a QC test.

CONCLUSION

Aside from fundamental scientific problems that can only be solved by primary research, most of the difficulties in acceptance of potency tests for combined vaccines arise because basic principles in method development and validation have been overlooked. In deciding how to select, validate, and perform potency tests of combination vaccines, it is useful to keep the following general points in mind.

1. Combining multiple components changes the sample matrix.
2. Any modification of procedures or manipulation of samples can affect the variability of the assay.
3. In validation studies, assay parameters should be evaluated under the conditions in which the test will be applied to vaccine lots.
4. Reference materials and test specifications must be appropriate for the method and the combination vaccine.
5. Product development and test development are interdependent.

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