Methodological considerations: Potency tests for recombinant adjuvanted RTS,S vaccine

Background

Adjuvanted recombinant RTS,S-based vaccines have been in clinical evaluation since the late 1980s and various adjuvant formulations have been evaluated. The final formulation evaluated in Phase III confirmatory trials is RTS,S adjuvanted with AS01 proprietary adjuvant system.

There is currently no International Standard suitable for the standardization of assays of vaccines from the manufacturer. The manufacturer has therefore established a product-specific reference preparation which is traceable to a lot of vaccine shown to be efficacious in clinical trials. This vaccine will serve as a working standard and will be included in all potency tests. The NRA approves the reference preparation used and the potency limits applied. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters, and it should be replaced when necessary.

Potency tests

Potency tests should be relevant with respect to the hypothesized vaccine mode of action and should be able to distinguish vaccines of low potency which may elicit reduced immunogenicity in humans. For some products, the potency test may also be designed to detect vaccines of high potency (i.e. increased immunogenicity). The potency test is designed to demonstrate consistency between vaccine lots and should not be considered an index of clinical efficacy. The test design and specifications should be defined in consultation with the NRA.
During clinical evaluation of RTS,S adjuvanted vaccine up to and including Phase III clinical material, potency testing for quality control release consisted of in vivo assays performed on the reconstituted RTS,S adjuvanted vaccine. These in vivo potency assays were based on immunogenicity in mice and compared the antibody response against either S or CS induced by the test and the vaccine reference preparation. Specification limits were based on either GMT ranges or relative potency for Phase III clinical trials material. In parallel, an in vitro potency assay consisting of a sandwich S-CS ELISA was carried out on both the final container RTS,S and the reconstituted RTS,S adjuvanted vaccine.

According to the manufacturer, validation studies have been conducted for both assays, and lot-to-lot consistency has been evaluated. Furthermore, for the in vitro test, correlation with in vivo assay results is to be established together with enhanced ability to detect vaccine degradation and differentiate vaccines of low and high potency. Furthermore, compatibility between the RTS,S final container lyophilized product and the liquid adjuvant system was documented. The lack of interaction between the two components was demonstrated using appropriate biophysical methods.

If the in vitro potency assay is used for routine batch release, it must be carried out on each final lot of RTS,S reconstituted in water. However, if the in vivo potency assay is used for routine batch release, the testing should be conducted using the final lot of RTS,S reconstituted with the adjuvant system.

**In vitro potency test**

The manufacturer developed two in vitro potency tests that are complementary: one based on the detection of both HBsAg and a specific portion of the CS protein which assesses RTS,S antigen immunological properties in vaccine, using a sandwich ELISA (ELISA CS-S); and another based only on the detection of HBsAg using an ELISA inhibition assay. The latter follows the same design as the current in vitro potency assay for HepB vaccines and thus uses the same human polyclonal antibodies against HBsAg (NABI anti-HBs) (1), however RTS,S vaccine batches are assessed against a homologous reference standard of RTS,S.
The sandwich ELISA CS-S method is based on binding of RTS,S antigen to specific antibodies against the CS protein and to antibodies against HBsAg. Test samples are incubated in microtiter plates previously coated with a monoclonal antibody directed against the repeat sequence of the CSP protein. In particular, this monoclonal antibody recognizes the NANP immunodominant B cell epitopes (2). Although no immune correlate of protection has yet been identified for RTS,S adjuvanted vaccine, antibody responses specific to this repeat region have been shown to be associated with protection. For instance, polyclonal antibodies raised against the synthetic peptide (NANP)_3 react with the surface of the parasite and neutralize its infectivity (2). Furthermore, antibodies against this specific region of the CS protein have been shown to be associated with protection in human and animal models (3–8).

Bound RTS,S is then detected with a polyclonal antibody directed against the HBSAg (anti-S). Serial dilutions of the standard material are used to establish the standard curve. Appropriate statistical modelling is applied to determine the test vaccine antigen content from the standard curve. Potency of the test vaccine is determined as the ratio of the measured RTS,S antigen content (µg/dose or µg/mL) to the theoretical RTS,S concentration in the final reconstituted vaccine product. The value will be accepted if the parameters of the internal control and the standard are within the appropriate range pre-defined during the assay validation.

Acceptance criteria for the two in vitro assays should be established on the basis of the assay of a suitable number of consecutive final lots. Whichever type of assay is used, the validation studies should show that the assay can verify consistency of production.

In vitro potency tests should be able to identify vaccines of low potency which may elicit reduced immunogenicity in humans (e.g. lots tested during dose-finding clinical studies) as well as vaccine samples with artificially reduced potency as a result of chemical and physical treatments that alter the vaccine. When the manufacturer introduces a change in the test kit or a different in vitro potency test, this must be fully validated according to the ICH guidelines and
the test should be run in parallel with the previous assay so that any potential variations are detected and managed appropriately.

It is important during validation to assess commercial lots and reference vaccine concurrently at optimal dilutions in order to produce dose-response curves that are suitable for quantitative analysis by an appropriate statistical method. The statistical validity of the assay should be assessed.

The vaccine formulation may influence the parameters used in the assay. Therefore, when appropriate it is recommended that optimization of the assay should be carried out when significant changes are made to the vaccine formulation (e.g. a new excipient, etc.). The assay is product-specific and must be validated by demonstrating a correlation with the results of mouse immunogenicity tests. The manufacturer has set a specification for the in vitro test which ensures that vaccines that pass this test would also pass the mouse immunogenicity test.

The validation of an in vitro potency test should be based on ICH principles (9) and should include:

- specificity;
- accuracy;
- precision (including repeatability, intermediate precision and reproducibility);
- linearity;
- range;
- robustness (which should be documented during assay development).

**In vitro assay for antigen quantification of aqueous bulk (purified bulk prior to formulation)**

The same in vitro assay used to determine vaccine potency is generally used to determine RTS,S antigen content of the aqueous bulk. Therefore, it is important to minimize the impact of changes
in commercial kits and to use appropriate reference preparations. The reference material is the same reference batch as that used for in vitro potency testing of the final vaccine lots.

**In vivo potency tests**

The RTS,S adjuvanted malaria vaccine includes two antigenic determinants, S and CS, for which two quantitative in vivo potency assays are performed for characterization purposes. Both of these assays are immunogenicity assays in mice.

A suitable quantitative potency test in mice is as follows. Groups of 10 mice, between six and eight weeks of age, are immunized two times subcutaneously with a 14-day interval with a series of dilutions of the reference and test vaccine using the vaccine reconstituted with the adjuvant system. The strain of mice used for this test must give a suitable dose-response curve with the reference and test vaccine. Terminal bleeds are taken when an adequate antibody response has developed. Individual sera are assayed for either antibodies to S or antibodies to CS using an enzyme immunoassay.

The concentrations of vaccine administered to mice should be selected to permit the calculation of GMTs for anti-S response and of anti-CS GMTs. Then the relative potency of the test material is determined as the ratio of the GMT of the test sample to the GMT of the vaccine reference material. The relative potency should be within limits established by using a suitable statistical approach based on consistency data gathered on both clinical and commercial lots.

Points which should be considered in establishing such an assay include (10):

- The strain and sex of mice used must give a suitable dose response to the reference and test vaccine.
- The number of mice per dilution required to meet the validity criteria of the test.
- The number of dilutions of the reconstituted adjuvanted vaccine and the appropriate selection of doses to be tested.
• The concentrations of vaccine tested should correspond to a dose in the dynamic range of the linearity zone defined using appropriate statistical modeling.

• The assay used to determine the concentration of antibodies to S in sera.

• The assay used to determine the concentration of antibodies to CS in sera.

• The statistical approach used to analyse the results (e.g. probit).

• Interpretation of the results – relative potency.

• The establishment of an in-house mouse reference serum or panel of high-, medium- and low-titre sera for both anti-S and anti-CS could be used to monitor kit performance and to assist in the evaluation of new kits or comparison of results from different laboratories (e.g. manufacturer and national control laboratories).

Establishment of product-specific reference

The vaccine potency (in vivo and in vitro) should be assessed against a product-specific reference preparation. The first (primary) reference preparation should be established using a vaccine lot found to be effective and safe in clinical trials or, alternatively, a vaccine lot that is traceable to a vaccine lot of proven effectiveness and safety. Points to consider when establishing a product-specific reference preparation include:

- source;
- quantity (availability);
- full characterization;
- evaluation in a mouse potency test;
- evaluation in an in vitro potency test;
- stability studies (accelerated degradation and real-time stability).

Replacement of product-specific reference

Standards should be routinely monitored and should be replaced before they begin to show loss of activity. The shelf-life of standards may be longer than the shelf-life of vaccine for routine use.
if data to demonstrate stability of an individual vaccine for this period are available. The shelf-life should be established under the defined storage conditions and maintenance of sterility. Real-time studies on vaccines should be supported by accelerated degradation studies. A replacement working standard should be a typical batch of vaccine that is, preferably, of similar potency to the previous standard.

Points to consider in the establishment and replacement of a reference vaccine are:

- documentation of the procedure for replacing standards;
- information on product/reference stability and establishment of shelf-life;
- the procedure to monitor loss of potency (e.g. trending of relevant values such as changes in dose-response curves, and changes of values compared to an internal reference preparation);
- definition of acceptable limits for values monitored through trend analyses (e.g. mean initial potency minus three standard deviations);
- full characterization of current and new reference vaccines;
- calibration of new reference vaccine against current reference using both in vivo and in vitro potency tests.

References


