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Reflection paper on control of the active substance in the finished product for immunological veterinary medicinal products (IVMPs)

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This paper does not open formal consultation but comments are welcome. Comments should be provided by 31 May 2010 using this template to *Vet-guidelines@ema.europa.eu* Fax: +44 20 7418 8447

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1. Introduction

Directive 2001/82/EC as amended requires that a control of the batch titre or potency is carried out on the finished product. It states that a quantification of the active substance shall be carried out on each batch to show that each batch will contain the appropriate potency or titre to ensure safety and efficacy. It should be noted that, while the general aim is to ensure that each batch of vaccine will be equally efficacious, the required test is part of the quality control of the finished product intended to confirm consistency of production and that each batch is formulated equivalent to batches that have been demonstrated to be efficacious.

The biological nature of IVMPs leads to some unavoidable batch to batch variation in production. Manufacturers, therefore, set limits rather than absolute parameters for most processes. The test methods available to assay the product during and at the completion of production are also subject to biological variability. They often only provide indicators of the quality, quantity and the reproducibility of the product batches rather than precise measures. Adequate validation is essential to ensure that the results of the assays accurately reflect the amount, titre, or potency of the active substance measured and to indicate the limitations on the accuracy of the measurements to be expected from the test used. This applies as much to in-process control tests used to measure antigen prior to blending or inactivation as it does to the control of the active substance in the finished product. All these factors will influence the confidence that can be put into the tests for their capacity to determine accurately the titre or potency of the product and the extent to which the value given for the potency reflects the actual or likely potency of the batch.

The aim of this document is to discuss the different methods currently used for control of the active substance in the finished product and their features, difficulties associated with the validation of these tests, problems arising from the use of *in vivo* tests and the possibility of an alternative *in vitro* approach for the batch testing.

2. Current types of control of the active substance in the finished product

Direct measurement of the active substance in the finished product:

- Batch titre: Titration of the living vaccine organism is commonly carried out for live vaccines. Validation of the titration method is required but this usually provides a satisfactory method for the control of the active substance in live vaccines.
- Measurement of inactivated antigens: Direct measurement of vaccine antigens in inactivated vaccines is often difficult because of interference by other components of the vaccine, e.g. adjuvants and preservatives. However, such methods have been satisfactorily employed for a few inactivated vaccines.

Batch potency

Direct measurement of the quantity of the active substance in the finished product may not be easily achieved and other components may interfere with currently available assay methods. Furthermore, the safety and efficacy of an inactivated vaccine may depend on the content and the quality of the active substance (antigen), on the adjuvant, and on the way the two interact together. Applicants may therefore decide to use indirect measures as potency tests for inactivated vaccines. The following approaches have been used:

- Challenge tests: vaccinated animals are challenged with virulent organisms and the observations (e.g. number of diseased or dead animals) are compared to a non-vaccinated challenged group. These tests effectively confirm the efficacy of the batch but often require large numbers of animals for significant results and may be relatively insensitive to small changes in quality or quantity of the active substance. The consequences for animals that succumb to the challenge infection are often severe and humane end points may need to be set to minimise the welfare implications.
- Measurement of a response (e.g. serology) in vaccinated animals: An initial *in vivo* stage may be followed by an *in vitro* test. Both stages need to be validated and while the *in vitro* test may be adequately reproducible the variable responses of vaccinated animals often leads to a wide confidence interval for the test as a whole. The method may therefore be relatively insensitive to small changes in formulation.

  Interpretation of the batch potency test result may depend on the other controls in place to ensure the correct formulation of the vaccine. The following approaches may be considered:

  - Demonstration that the batch being tested is at least as potent as a reference batch that has been shown to be efficacious in the target species. Typically, the reference batch will be a specially formulated 'low potency' batch and standard batches will normally be expected to exceed the potency of this batch.
  - If the vaccine is formulated to contain a fixed quantity of antigen per batch and there are adequate controls in place to ensure correct formulation of each batch, then the potency test can be regarded as a 'confirmatory' test, the result being used to identify batches that are outside the limits established for a 'standard' batch.

3. Expected data for the validation of the control of the active substance in the finished product

As noted above, the control of the active substance in the finished product is an analytical procedure and needs to be validated against the criteria described in VICH guidelines GL1 (Validation of analytical procedures: definition and terminology) and GL2 (Validation of analytical procedures: methodology). These guidelines are particularly applicable to chemical methods applied to pharmaceutical products, with a focus on demonstrating that the method is suitable for the intended purpose, but can in principle also be applied to biological products.

The following sections are a summary of the points that should normally be provided to correctly validate the control of the active substance:

- As a minimum, the test validation needs to be able to demonstrate a dose response (i.e. that it is able to respond to changes in the vaccine that affect its efficacy, such as active substance concentration) and the precision of the test (i.e. repeatability, intermediate precision and repeatability between laboratories if relevant). The control should be sufficiently sensitive to be able to distinguish between a batch containing the correct quantity of active substance and a batch with less active substance.
- If the potency tests for inactivated vaccines involve an initial in vivo stage followed by an in vitro test, validation requires the inherent variation in the in vitro test and also the in vivo stage to be taken into account. Due to the inherent variability in experimental animals this often leads to an unacceptably wide confidence interval, which in turn draws into question the ability of the test as a whole to discriminate a sub-standard batch. For inactivated vaccines, the methods used for the control of the finished product are in general not sufficiently sensitive to detect slight changes of

the antigen amount. Thus, reasonable dilution factors should be applied to the vaccine batch when the dose-response relationship is investigated. Moreover, the biological significance of the statistical analysis of the results should always be examined. For instance, if there is a good dose-response relationship, and a significant difference can be shown between a standard batch and a batch with, for example, half the amount of antigen (thus making possible the detection of a defective batch containing only 50% of antigen), the efficacy of a batch containing half the amount of antigen should be considered.

4. Identification of problems with regard to the control of the active substance by an in vivo potency test

Experience has shown that the data expected for adequate validation of the control of the active substance are not always provided. There is a large degree of inconsistency between companies in the way that control tests of the active substance in the finished product are designed and reproducibility data are interpreted to justify proposed pass criteria. Furthermore, there has also been a degree of inconsistency between member states with respect to the extent of validation data expected in registration dossiers and the way that data is assessed. As a consequence, companies may waste considerable time and also use a large number of animals in developing unsuitable tests and proposing inappropriate limits, and regulatory authorities have difficulty in advising on solutions that are likely to be accepted in other member states.

For the routine batch release potency test, European Pharmacopoeia monographs usually propose an alternative test to that required for immunogenicity testing. For inactivated vaccines, this is usually a test in laboratory animals and is described in some detail. In some cases, it contains suggestions for alternative approaches e.g. different types of animals, number and size of doses administered and a range of days from vaccination to time of collecting blood samples or use of an *in vitro* method. It seems that the applicants are not aware that in all cases, the tests provided are given as examples of the type of test that may be carried out and are not *per se* validated. It is the applicant's responsibility to develop and validate a suitable test to use for batch release and a method that is totally different from the test suggested by the European Pharmacopoeia can be used.

An additional problem that has been encountered, especially in respect to older vaccines or those that have been developed over a period of time, is where potency tests may be changed during vaccine development and several different types of test may have been used for developmental studies and for batch release. While it is understandable that vaccine manufacturers will wish to take account of scientific advances and improvements in technology this can often lead to difficulties in comparing the results of different studies and setting appropriate validated limits for batch release. A particular problem exists when validating alternatives to older "standard" tests, that may not have been fully validated, because it may not be possible to establish a satisfactory correlation.

5. Implementation of *in vitro* methods for inactivated vaccines

In the spirit of reduction, refinement and replacement of animal tests (3Rs) and in accordance with Directive 86/609/EEC, the development of *in vitro* methods as alternatives to *in vivo* potency tests to control the quality of vaccines is encouraged. The revised Annex I of Directive 2001/82/EC as amended refers to the quantification of the active substance in the finished product instead of the previously required assay of biological activity of the active substance(s) in the finished product. Therefore, control of the active substance by an *in vitro* method is considered acceptable to demonstrate the quality of the vaccine batch under test and to show the consistency of production and

is in fact more in line with the requirements of the revised Annex 1 than currently used *in vivo* methods. Furthermore, implementation of *in vitro* instead of *in vivo* tests should result in a considerable reduction in the numbers of animals used for batch control purposes.

The *in vitro* approach is not commonly implemented by applicants at present so there is currently insufficient information to establish a clear position on the future of these methods. Applicants are therefore encouraged to develop *in vitro* tests for the control of active substances in the finished product taking into account the following points:

- Consistency of production: The use of an *in vitro* test can be supported by taking a consistent approach to the manufacturing process. The aim of this concept, which includes GMP, process validation and in-process and finished product tests, is to demonstrate that a manufacturing process produces batches of finished product which reliably fulfil all the specifications laid down in the quality file and which can hence be considered to be as safe and efficacious as the batches used to demonstrate safety and efficacy in the dossier for the marketing authorisation.
- Control of the active substance: The assay of the active substance alone may not be sufficient to demonstrate the consistency of the production process. The Applicant will need to select and justify the antigen(s) to be measured and investigate if a correlation can be established between the quantity of the antigen(s) and the ability of the vaccine to protect. If a satisfactory correlation can be established then it may be possible to base the control on measurement of a component that may not be a protective antigen. The methods of control (in-process and on finished product) should be demonstrated to be able to detect sub-standard batches containing less active substance than standard batches.
- Control of the adjuvant: The properties of adjuvants are important factors in the efficacy of vaccines that contain them. The quality and quantity of the adjuvant should therefore be controlled by validated tests during the production process and, if possible, in the finished product.
- The *in vitro* methods used for in-process and finished product tests should be validated against the criteria described in VICH guidelines GL1 and GL2.

6. Conclusion

It is currently not possible to recommend general solutions to these issues. Vaccine manufacturers are encouraged to discuss possible ways to resolve them with regulators.