Towards Eliminating the Use of Animals for Regulatory Required Vaccine Quality Control

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Summary
Traditionally, regulatory required vaccine quality control relies on the use of laboratory animals. Both batch testing for safety and testing for potency are based on animal models. Quite often these models include procedures that induce severe pain and suffering. This has urged the development of 3Rs methods. Some examples of recent achievements in the quality control of toxoid vaccines are the replacement of challenge procedures by serological methods, the reduction of numbers of animals required by changing from multi-dose to single dose testing, and developments in the area of in vitro models and physicochemical techniques. Central in the progress towards the elimination of animal use is the acceptance of the so-called consistency approach. The essence of the consistency approach is that a new batch of vaccine is no longer seen as a unique product but as only one of a series of batches produced from the same starting material (seed lot). Consequently, the new batch shares many of the characteristics of the previous batches produced from the same seed lot. This allows for a new strategy of vaccine quality control consisting of demonstrating consistency in production, placing emphasis on aspects such as in-process testing, and implementing Good Manufacturing Practice and Quality Assurance (QA). The new strategy particularly focuses on non-animal test models such as physicochemical methods and in vitro models. Implementation of the consistency approach might significantly contribute towards the elimination of the use of animals in regulatory required vaccine quality control.

Keywords: 3R, vaccine, quality control, toxoid, consistency, in vitro

1 Introduction
Most vaccines that are included in the basic immunisation programmes for young children as well as for veterinary use are still produced using technologies that were developed in the early 20th century, i.e. by inactivating/detoxifying or attenuating the virulent micro-organism or toxoid thereof. Owing to the production process, differences in quality might occur between the vaccine batches being produced, resulting in some batches with residual toxicity or insufficient efficacy. Consequently, extensive quality control of conventionally produced vaccines is required by law to demonstrate that each batch of vaccine being produced is both safe and potent. These tests are traditionally based on animal models. Specifications for quality control and criteria for batch release are provided by regulatory authorities. In Europe, these refer to the
monographs issued by the European Pharmacopoeia.

No information is available on the current numbers of laboratory animals being used for this application. However, by extrapolation of data from some EU Member States it might be estimated that in Europe about 10% of all experimental animals are used in the category of vaccines, and 60% of these are used for quality control purposes. With a total use of 10.7 million experimental animals in the EU in 2002 (Anon, 2005), this would mean that vaccine quality control consumes about 600,000 animals a year in Europe alone.

In the next paragraphs information is provided on the use of animals for routine vaccine quality control and on recent 3Rs developments in this area. Finally, a new strategy in vaccine quality control will be discussed which shifts the focus from individual batch control towards demonstrating consistency in production.

2 Vaccine quality control

In terms of numbers, most laboratory animals used in vaccine quality control are required for potency testing of inactivated/detoxified vaccines, such as tetanus and diphtheria toxoid, and to a lesser extent for safety testing. Potency testing of attenuated vaccines is based on in vitro methods such as virus titration in cell cultures or bacterial counts in culture media.

The traditional test for potency of inactivated/detoxified vaccines is based on an immunisation challenge procedure. In general, groups of 3 or 4 of animals are immunised with a dose of the vaccine batch under study or a reference preparation with known potency, respectively. Several weeks after immunisation, all animals are challenged with a lethal dose of virulent micro-organisms or toxin and the number of animals that die or show specific clinical signs within the observation period as a result of the challenge is counted. Thus dose-response curves are obtained both for the batch under study and for the reference preparation. On the condition of linearity (after log transformation) and parallelism of dose-response curves, the potency of the vaccine under study is calculated, including 95% confidence intervals. An alternative approach to this so-called parallel-line assay is the toxin neutralisation assay. In this test animals are immunised with one dose of vaccine. Serial dilutions of serum samples of these animals are mixed with a fixed dose of toxin and each mixture is subsequently administrated to additional animals. Survival or death of these animals is indicative of the level of protective antibodies in the serum sample and consequently of the potency of the vaccine. A graphic representation of the potency tests is shown in Figure 1.

Safety testing of a vaccine is based on administration of a dose of the product to a susceptible animal species and then looking for clinical signs relevant for the specific micro-organism/toxin. Potency testing requires large numbers of animals per test while part of the animals will suffer from the challenge procedure. In general, smaller numbers of animals are needed for safety testing while suffering will only be involved in case of residual virulence/toxicity, which is rarely the case.

3 Recent 3Rs developments

For several reasons in the last two decades attention has been given to the development of methods that could replace, reduce and refine the use of animals (Hendriksen, 2005):

- increasing concern about the extent of animal use and the level of suffering.
- some of the surrogate animal models have questionable relevance for the tar-
get species (e.g. the tetanus vaccine potency test), or have a poor reproducibility within and between laboratories (e.g. the rabies vaccine potency test).

- the fact that animal experiments are time consuming and will shorten the shelf life of vaccines after release.
- the implementation of new developments and strategies in vaccine production such as standardisation of production processes, in-process testing programmes, the introduction of Good Manufacturing Practice (GMP) and Quality Assurance (QA).

Table 1 gives an overview of 3Rs developments for the quality control of inactivated bacterial vaccines for human use that have been incorporated in the monographs of the European Pharmacopoeia (Ph.Eur).

As a result of these 3Rs achievements, animal use in vaccine production facilities has been significantly reduced. Figure 2 provides information from the National Institute of Public Health and the Environment (RIVM)/Netherlands Vaccine Institute (NVI) as an example. As can be seen, numbers of animals used have been reduced by almost 70% since 1989.

4 The consistency approach

Although much has been achieved in vaccine testing, it is at the moment not possible to eliminate animal use altogether without introducing a paradigm shift in the concept of quality control. The current concept is based on the uniqueness of each individual vaccine batch produced. This uniqueness requires extensive batch release testing, giving emphasis to the final product and including endpoints such as immunogenicity/potency and safety. These are complex endpoints that are difficult to copy using relatively simple in vitro models. Therefore, replacing each individual animal test with an in vitro test would be tedious and time consuming and limited progress is to be expected.

However, there is another approach under study that might lead to a total or almost total replacement of animal use for quality control of conventional vaccines. This approach is now generally referred to as ‘demonstration of consistency’ (Metz et al. 2002). The key issue of "consistency" emerged from the category of vaccines that are manufactured using new technologies, such as in the case of rDNA vaccines. These products are produced in a consistent way and the focus of quality control is on in-process monitoring rather than on final batch testing. In-process testing is almost exclusively based on in vitro biochemical, physicochemical and immunochemical tests. The consistency concept has become state-of-the-art for the new generations of vaccines. However, also in the field of conventional vaccines increased knowledge of vaccine antigens and continued advances in production technology have resulted in more defined and thus less variable products. Most production facilities have implemented the principles of Good Manufacturing Practice (GMP) and Quality Assurance (QA), and extensive in-process testing programmes are in place to monitor the production process.
Therefore, it is believed that also for the category of conventionally produced vaccines the extent of batch release testing should reflect the level of consistency obtained within the production facility. Thus, a vaccine manufacturer should perform extensive testing (including animal testing) during the vaccine development phase of a new product and on the first few vaccine batches (lots) produced to fully characterise (fingerprint) the vaccine. This set of information should also include clinical data that demonstrate vaccine efficacy and safety in the target species (the 'clinical lot').

When consistency in production has been shown (e.g. after full testing of 5 consecutive batches), testing of subsequent batches produced from the same working seed lot could rely on a battery of easy-to-use in vitro assays (Tab. 2). The selected in vitro assays should monitor characteristics of the vaccine antigen that are known to reflect key aspects of efficacy and safety. Characterising the vaccine antigen using these in vitro assays and comparing the 'fingerprint' with that of the clinical lot would confirm consistency in production and quality in terms of efficacy and safety. Applying this new approach will allow an almost total replacement of animal use. Animals will only be required for vaccine development and demonstration of consistency. In order to gain experience with the new approach and for a limited time period, it could be suggested that the validity of the consistency approach is confirmed by immunising only a few animals with one dose of the vaccine batch being tested.

5 Further research

At the moment some key elements of the consistency approach are still not fully understood and further research will be needed. One of these is the final production step when vaccine antigen is adsorbed onto the adjuvant. Adjuvants are products that stimulate and/or modulate the immune response to the administered antigen. In humans, alum salts are used for routine immunisations; in veterinary healthcare programmes the adjuvant repertoire is much larger. The efficacy of a vaccine might be affected by the physical properties of the aluminium salts, while in turn the antigen structure might be changed due to adsorption. Many of the physicochemical and immunochemical tests mentioned in Table 2 cannot be performed in the presence of the adjuvants and consequently adsorbed vaccine antigens are difficult to characterise.

Fortunately, however, new developments in analytical techniques were described recently (White and Helm, 2000; Wittayanukullik et al., 2004) that might allow antigen and antigen-adjuvant characterisation. Another hurdle in the acceptance of the consistency approach is antigen – antigen interaction. Most vaccines are now multivalent products, meaning that they are composed of several vaccine antigens. Thus, DWP/IPV vaccines include both tetanus and diphtheria toxoid, inactivated pertussis micro-organisms and inactivated polio virus. It is well known that WP (whole cell pertussis) enhances the immune response to some antigens. This information will be obtained in the potency test but not by using analytical methods. Further study will be needed to demonstrate the application of the principle of consistency to vaccine antigens other than diphtheria and tetanus toxoid alone as well as to the antigen – antigen interactions.

References


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<tr>
<th>Tab. 2: Overview of physico-chemical, immunochemical and in vitro tests (Metz et al., 2003)</th>
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<tr>
<td><strong>Physico-chemical (analytical, spectroscopic)</strong></td>
</tr>
<tr>
<td>• circular dichroism</td>
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<tr>
<td>• tryptic digest analysis</td>
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<td>• fluorescence spectroscopy</td>
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<td>• chromatography</td>
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<tr>
<td><strong>Immunochemical</strong></td>
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<td>• ELISA</td>
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<td>• immunoblotting</td>
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<td>• etc.</td>
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<tr>
<td><strong>In vitro functional</strong></td>
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<tr>
<td>• cytokine production</td>
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<tr>
<td>• in vitro antigen processing</td>
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<tr>
<td>• antibody production</td>
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<tr>
<td>• lymphocyte activation/proliferation</td>
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