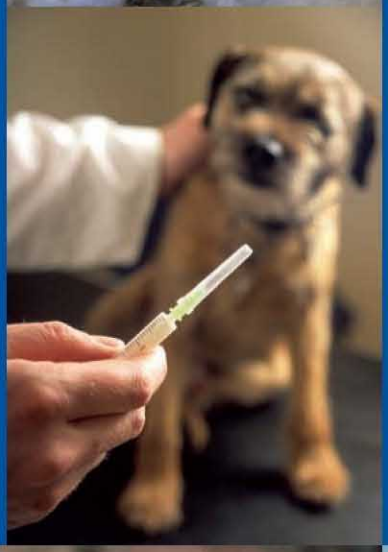

# Advancing animal welfare and the 3Rs in the batch testing of veterinary vaccines

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## Executive summary

Vaccines are of biological origin and have the potential to vary from batch to batch. Consequently, vaccines are tested for batch-to-batch consistency and many of these tests involve animals. Therefore, although veterinary vaccines are used to protect animals, this is at the expense of large numbers of other animals that are used in quality control tests before vaccines are released onto the market.

This report was prepared to provide a summary of the regulatory requirements relating to the use of animals in veterinary vaccine testing. It identifies the tests of most concern from an animal welfare point for view, where refinement of methods and replacement of animals should be a priority. It also highlights inconsistencies in the test requirements. The report explores the problems created by such inconsistencies and shows how the test requirements themselves could serve as a powerful tool in reducing animal use and suffering if they were written in a less ambiguous and more consistent way. In addition, means of reducing the numbers of animals used in quality control testing by avoiding unnecessary testing are considered.

In the European Union (EU), the use of animals in vaccine quality control tests is regulated by two separate sets of regulations. EU Directive 86/609 (under revision at the time of writing) relates to the regulation / control of animal use in testing. EU Directive 2001/82/EC relates to veterinary medicines and specifies that vaccines are tested in accordance with the monographs of the European Pharmacopoeia. Each Directive is the responsibility of a different Directorate-General and the principles of the two sets of regulations are not always complementary. In addition, it should be remembered that the European Pharmacopoeia works under the aegis of the Council of Europe which, having 49 member countries, cannot be expected strictly to align its activities with EU law.

The recommendations arising from this report are summarised here under four headings, but retain the numbering used in the body of the report:

- **Setting priorities for improving animal welfare**: Data on the current use of animals in the quality control of veterinary vaccines are very sparse and should be improved. However, it is possible to identify those tests which cause the most animal suffering and to recommend specific, high priority actions on these tests.
- **Promoting the development and use of alternative methods**: Many opportunities to encourage the development, validation and use of replacement, reduction and refinement alternatives are being missed due to lack of clarity or inconsistency in EP monographs.
- **Deleting requirements for unnecessary tests**: The value of some tests is highly questionable and they should no longer be required.
- **Reducing animal use by optimising study design**: The number of animals used in each test is not apparently based on sound statistical considerations. This may result in excessive use of animals.

### *Setting priorities for improving animal welfare:*

Data relating to animal use in batch testing of veterinary vaccines are not routinely collected and published, making it difficult to determine how animal use fluctuates, or

where efforts to improve animal welfare would be best focused. Ideally, such data should be collected for the whole of the EU, but valuable information could be provided by the Veterinary Medicines Directorate (VMD), an agency of the Department for the Environment, Food and Rural Affairs (DEFRA) in the UK.

Despite the absence of detailed information it is still possible to identify the quality control tests that are of the most concern: potency tests use more animals than any other batch tests and can cause considerable suffering. Among these tests, those relating to inactivated vaccines are of the most concern because they require the testing of every vaccine batch. Although a variety of methods are used to test potency, and most vaccines are assessed using serological methods, some vaccines are routinely tested using a challenge assay that involves infecting animals with disease agents and can cause substantial suffering. It is these tests that should be given the highest priority for efforts to develop alternative methods.

The potency tests that are of the greatest concern are found in monographs for some Clostridial vaccines, canine leptospira vaccines, rabies vaccines and fish vaccines. This is because of the severity of the diseases involved, the number of animals used, and the lack of widespread use of alternative approaches,. There is an urgent need for research efforts to be focused on these tests so that more humane methods of determining potency can be developed, disseminated and implemented.

**Recommendation 1:**

DEFRA should ensure that data relating to the use of animals in the quality control of immunological veterinary medicinal products is collated annually and published in the public domain. The breakdown of statistics should be sufficiently detailed to enable identification of areas where application of the 3Rs is most needed.

**Recommendation 2:**

Funders of research and vaccine manufacturers should focus efforts on the development and validation of alternative methods of assessing batch potency for those vaccines that are routinely tested using challenge assays. Any resulting assays must be formally validated and incorporated into the appropriate monograph as a matter of priority.

**Recommendation 6:**

Serological methods of determining potency of *Clostridium chauvoei* vaccines should be formally validated in Europe so that they can be incorporated into the European monograph.

**Recommendation 7:**

Earlier humane endpoints, which can be applied prior to animals becoming moribund, should be defined and used for potency tests of *C. chauvoei* vaccines. In addition, the monograph should be revised so as to advocate the use of appropriate analgesia to reduce suffering of control animals.

**Recommendation 8:**

Humane endpoints that can reduce the suffering of the hamsters used in batch potency tests of canine leptospira vaccines should be defined, validated and incorporated into the monograph.

**Recommendation 9:**

Any serological or *in vitro* assays that are developed as an alternative to the hamster challenge assay for assessing potency of leptospira vaccines must be validated and incorporated into the monograph as a matter of priority.

**Recommendation 10:**

Humane endpoints need to be developed for batch potency tests of fish vaccines. Research is needed to define clinical signs in fish that may be indicative of disease and impending death.

## *Promoting the development and use of alternative methods*

The European Pharmacopoeia has made considerable progress in revising its monographs to improve welfare over the past 20 years and currently provides much potential for implementing the 3Rs further. However, the Pharmacopoeia could be more active in driving the implementation of the 3Rs. Currently, the wording of several monographs implies that animals can be used in challenge-based tests that cause suffering even when alternative methods may be available. Authorities overseeing the regulation of animal experiments also have a role to play in ensuring that the most humane testing methods are used at all times.

### 1) Improving consistency in the choice of tests

Monographs are extremely inconsistent in the amount of guidance they provide about what is a suitable method for routinely determining potency. Some give no information at all, others suggest a general approach while others provide a relatively detailed protocol. The more information that the monograph provides, the less trial and error is likely to be involved in developing a suitable method. In many instances where monographs lack detail, manufacturers will have developed their own methods regardless. Sharing these methods could help prevent animals being used unnecessarily by others in challenge tests or in test development work.

Most, but not all, monographs indicate that validated alternative testing methods can be used instead of challenge assays for routine potency testing. Such methods are usually serological methods, which avoid causing harmful infections, and measure the response to vaccination in a relatively small number of animals instead. *In vitro* methods, which avoid using animals altogether can also be used in some instances. Many monographs provide examples of serological tests that would be suitable but this is not universal and only two monographs specifically suggest the use of an *in vitro* method.

Even when serological methods have been validated and incorporated into monographs, they may be presented only as an example of a suitable batch potency test and the potential to use a challenge assay routinely may still remain.

**Recommendation 3:**

All veterinary vaccine monographs should state that suitable serological or *in vitro* validated tests might be used for routine use instead of the challenge-based test described under Potency. Examples of suitable batch potency tests, including an *in vitro* test, should be provided.

**Recommendation 13:**

Where serological or *in vitro* methods of determining potency have been formally validated and incorporated into a monograph, the monograph should state that the challenge assay is not used for routine batch testing. Authorities overseeing regulation of animal experiments (e.g. the UK Home Office) should ensure that the validated alternative methods are used where possible.

**Recommendation 16:**

Where monographs do not provide details of suitable batch potency methods, manufacturers that have developed and validated their own methods in-house should publish or otherwise share them. They should also submit their methods for formal validation so that they may be incorporated into the appropriate monograph.

**Recommendation 21:**

Once a serological test method has been formally validated and accepted for incorporation into a monograph, the potential to use challenge assays for routine batch potency tests should be removed, as in the monograph for inactivated feline infectious enteritis vaccine.

### 2) Encouraging the development of alternative methods

The European Pharmacopoeia Commission could do more to ensure that the development of more humane methods is encouraged throughout a vaccine's lifetime. At present, if an alternative method is to be used instead of a challenge assay, it must be developed during vaccine development. If this proves problematic then the challenge assay is routinely used. This provides little incentive to develop novel, more humane methods of testing existing vaccine products that have already been granted marketing authorisation. If a manufacturer were to adopt a new method of assessing potency for an existing product this would require approval from the relevant competent authority which would incur a fee, adding to the disincentive.

**Recommendation 14:**

Where it is not possible to develop a serological or *in vitro* batch potency test during vaccine development, the monograph on Vaccines for Veterinary Use should make it clear that this should not preclude attempts to develop such tests later.

**Recommendation 15:**

Competent authorities should introduce a fee amnesty for processing licensing variations that result in fewer animals being used in quality control tests.

### 3) Accelerating the acceptance of alternatives

Monographs are regularly updated and new, formally validated methods are incorporated during the process. However, the procedure for revision is complex and time consuming. In addition not all researchers developing alternative methods are aware of the mechanisms available for formal validation.

The current validation process requires a correlation to be established between the test described for potency in the monograph (usually a challenge assay) and the alternative method. The results of challenge assays can be variable and so establishing a correlation can be difficult and not necessarily relevant.

**Recommendation 19:**

The Pharmacopoeia Commission should review its procedures for the revision of monographs with a view to accelerating the incorporation of, and acceptance of, more humane testing methods, and deletion of obsolete animal tests.

**Recommendation 20:**

The European Centre for the Validation of Alternative Methods (ECVAM) should promote its role and the existence of its guidelines for submitting test methods for consideration for formal validation more widely within the research community.

**Recommendation 22:**

The European Pharmacopoeia Commission should devise a new strategy for validation that seeks to ensure batch-to-batch consistency of the most relevant parameters rather than relying principally on a correlation with an animal model.

### 4) Consistent application of humane endpoints

Many monographs describe potency tests that can cause substantial suffering in unvaccinated control animals. However the control of suffering through application of humane endpoints is advocated in some monographs but not in others. Even within the same monograph, humane endpoints may be advocated for one species but not for another. This can create the false impression that it is acceptable to control suffering in some tests, and for some species, but not for others.

**Recommendation 4:**

In all monographs that describe tests that have the potential to cause substantial suffering or mortality, the test description should state that those animals showing marked signs of disease should be killed. New monographs should not be incorporated into the Pharmacopoeia until they contain such statements, and existing monographs that do not stipulate the use of humane endpoints in tests that can cause mortality should be revised immediately.

### 5) The need for international harmonisation

If a veterinary vaccine is to be marketed world-wide, it must meet the regulatory requirements of all regions that it is to be used in. Testing requirements differ from region to region which can result in animals being used unnecessarily in different tests that are all designed to measure the same thing but in slightly different ways.

**Recommendation 23:**

The Pharmacopoeial Discussion Group should prioritise harmonisation of monographs that describe challenge assays that are used as routine batch potency tests. This would prevent unnecessary animal use and suffering, and would also permit the use of serological and *in vitro* methods of potency determination in all regulatory regions.

### *Deleting requirements for unnecessary tests*

Generally, the potency testing of live vaccine batches is not as great an animal welfare concern as for inactivated vaccines because an *in vitro* test is usually sufficient and animals are not involved. The majority of live vaccine monographs state that it is not necessary to carry out a potency test on each batch. Unfortunately this is not the case in the monograph for swine fever vaccine, where there is potential for animal tests to be performed routinely.

Batch potency testing is not required for *Brucella melitensis* vaccines but a test to determine the 50% persistence time is routinely performed in mice, the necessity of which has been questioned.

There is considerable scope for reduction of animal use in tests other than the batch potency test. Manufacturers can apply to competent authorities to discontinue the target animal safety test for a particular vaccine if sufficient batches have already passed. However, this test continues to be performed routinely in many instances for a variety of reasons. These include the fees associated with obtaining permission to waive the test and the lack of globally harmonised approach regarding waiving the test.

**Recommendation 11:**

All live vaccine monographs should state that it is not necessary to carry out the test described under Potency for each batch of vaccine.

**Recommendation 12:**

The necessity of the 50% persistence time test as a routine batch test for *Brucella melitensis* vaccines should be reassessed and it should be removed from the monograph as a batch test if it is not essential.

**Recommendation 24:**

Competent authorities and authorities responsible for the regulation of animal experiments (e.g. the UK Home Office) should challenge manufacturers to provide compelling justification for why they continue to perform the batch target animal safety test.

**Recommendation 25:**

The European Medicines Agency should strive for a globally harmonised approach to waiving of the safety test through participation in the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products programme (VICH).

### *Reducing animal use by optimising study design*

Monographs vary greatly in the numbers of animals they require for a particular test and this is frequently species dependent. Where animals that are small, inexpensive and easy to house in a laboratory environment tend to be required in larger numbers than animals that are more “valuable” and problematic to keep in a research facility.

For example, the number of animals required for the batch safety test is species-dependent with more animals being required for tests of fish and bird vaccines than for mammalian vaccines. There is no apparent scientific reason for this discrepancy and the

numbers of animals used in safety tests could be markedly reduced if this were addressed.

Monographs also vary in the experimental designs that they describe for challenge assays and for serological tests. The animals used in unvaccinated control groups during challenge assays are of most concern as these suffer the most. Some, but not all monographs describe experiments where the control group in a challenge assay contains fewer animals than in vaccinated groups. The experimental design of serology assays also shows a lack of consistency, with control groups being required by some monographs and not by others.

The monographs could do more to discourage the excessive use of animals. Test requirements typically require *"not fewer"* than a particular number of a particular species. This use of words implies it is acceptable to use more than the number of animals specified. It does nothing to reinforce the requirement of Directive 86/609 to use the minimum number of animals that are most likely to produce satisfactory results.

There is evidence that animals are sometimes used in excess of test requirements, which in the EU is contrary to the requirements of Directive 86/609, and of the Animals (Scientific Procedures) Act 1986 in the UK.

**Recommendation 5:**

The Pharmacopoeia Commission should establish a panel of statisticians to review the experimental design of tests involving animals to ensure that tests do not require the use of excessive numbers of animals overall, or in control groups. For existing monographs, the numbers of animals required for batch potency tests should be re-evaluated using data that has been generated for previous authorisation purposes. Where potential for reduction is identified, the corresponding monograph should be revised.

**Recommendation 17:**

The general monograph on Vaccines for Veterinary Use should state that the numbers of animals used in tests must be the minimum required for test results to be valid. Competent authorities, and national authorities responsible for regulation of animal experiments, should challenge the methods of vaccine manufacturers who use more animals than the minimum stipulated by a monograph for a particular test.

**Recommendation 18:**

Control groups should only be incorporated into a test design if there are compelling scientific reasons for doing so.

**Recommendation 26:**

The number of animals required for batch safety testing of bird and fish vaccines should be reduced to two, as is the requirement of batch safety tests of vaccines for other species.

**Recommendation 27:**

Within the UK, the Home Office and the VMD should collaborate more actively to ensure that animals are not used in excess of test requirements, taking this report as a starting point.

# 1. Introduction and aims

Veterinary vaccines are routinely used to protect species of companion, farmed and wild animals against a variety of diseases. In addition they are used to prevent zoonotic diseases in humans who may consume, or have contact with, animals or animal products. Such vaccines are developed through programmes of research that use animals to determine the safety and efficacy of the product. Once a vaccine has been developed, each batch must then be subjected to a variety of quality control tests, some of which involve animals, before it can be marketed. These quality control tests are conducted because vaccines are products of biological origin and so have the potential to vary in safety and potency from batch to batch. Consequently, to ensure consistency, each batch is subject to a stringent series of regulatory tests before it can be authorised for use.

The overall numbers of animals used in scientific procedures in the UK each year is published annually by the Home Office, but the number used for developing and/or testing vaccines is not itemised. The same applies to statistics relating to development and testing of vaccines in Europe as a whole. The limited amount of information currently available means it is not possible to determine the scale or pattern of animal use in veterinary vaccine testing on an annual basis. It is known, however, that the scientific procedures involved in this research and testing can cause pain, suffering or distress to the test animals. This can be substantial. In addition to the actual tests, there may also be a variety of other, frequently over-looked, sources of harms to the animals that arise as a result of their life experience in an experimental facility. Potential sources of harm include breeding practices, transport, housing, handling and euthanasia. Thus, there is a conflict between the interests of the target set of animals that veterinary vaccines are intended to protect, and the interests of the animals used in the development and testing of such products.

The aim of the present report is to provide an up-to-date summary of the regulatory requirements relating to veterinary vaccine batch tests that require the use of animals (Sections 2-4). Emphasis is on the potency testing of inactivated vaccines (Section 3), as these are the tests where there is the greatest potential for animal suffering. The report:

i. Summarises where use of alternative methods that have the potential to reduce animal numbers and suffering are suggested within the regulatory requirements;
ii. Summarises where control of suffering is specifically advocated;
iii. Examines the numbers of animals required for the conduct of tests;
iv. Identifies the tests of most concern from an animal welfare point for view, where refinement of methods and replacement of animals should be a priority;
v. Highlights inconsistencies in the test requirements, some of which appear to be species dependent.

Section 5 of the report explores the problems created by such inconsistencies, and shows how the test requirements themselves could serve as a powerful tool in reducing animal use and suffering if they were written in a less ambiguous and more consistent way. In addition, means of reducing the numbers of animals used in quality control testing by avoiding unnecessary testing are considered (Section 6).

# 2. Regulatory framework

Within Europe, two different Directives relate to the use of animals for the testing of veterinary vaccines. One controls the use of animals in research while the other creates the requirement for their use. Each of these Directives is the responsibility of a different EU Directorate-General.

## 2.1 Regulation of scientific procedures on animals

Scientific procedures that may cause animals pain, suffering or distress are regulated in the EU by Directive 86/609/EEC relating to the protection of animals used for scientific purposes[1]. Responsibility for the Directive lies with the Environment Directorate-General. The requirements of the Directive are incorporated into national law in the UK as the Animals (Scientific Procedures) Act 1986 (ASPA). Both pieces of legislation require that:

- experiments using animals protected under the legislation "*should not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available*";

- where there is a choice of experiments, experiments used must be those that "*use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and are most likely to produce satisfactory results*"

In addition ASPA requires that the likely adverse effects on the animals concerned be weighed against the benefit likely to accrue as a result of the research before research projects are authorised.

## 2.2 Regulatory requirements for vaccine testing

European Directive 2001/82/EC relating to veterinary medicinal products requires quality control tests to be conducted in order to ensure batch-to-batch consistency. Responsibility for this Directorate lies with the Enterprise and Industry Directorate-General. The Directive specifies that the tests are conducted in accordance with requirements outlined in the monographs of the European Pharmacopoeia, or in accordance with a National Pharmacopoeia if this covers a substance that does not appear in the European Pharmacopoeia. The monographs are a collection of standardised specifications for researching drugs, ingredients, blood, blood products and vaccines. In the case of immunobiological products, the monographs deal with both the substances used in the preparation of these products and also the means of ensuring the quality standards of the end product. Responsibility for updating and management of the European Pharmacopoeia falls to the Pharmacopoeia Commission, which is within the Council of Europe's Directorate for the Quality of Medicines (EDQM). A panel of experts, 15V, takes responsibility for the monographs relating to veterinary vaccines.

[1] Directive 86/609/EEC is currently under revision

The tests described in the monographs are conducted by, or on behalf of, the vaccine manufacturer, in order for marketing authorisation to be granted by the appropriate regulatory authority (sometimes known as the competent authority). In the UK, the Veterinary Medicines Directorate (VMD)[2] is responsible for granting marketing authorisations on a national level. Alternatively, vaccines can be authorised by the European Medicines Evaluation Agency (EMEA) for use throughout the EU Member States by means of a centralised procedure.

### 2.2.1 The Pharmacopoeia monographs

From the beginning of 2008 there will be 81 monographs in use for the assessment of veterinary vaccines in the UK. One of these is an over-arching monograph, **Vaccines for Veterinary Use**, that dictates, in general terms, requirements that must be met by *all* veterinary vaccines. These relate to issues such as vaccine composition, production methods, demonstration of safety and efficacy, the requirement to test individual batches and the tests that must be performed.

The remaining 80 monographs set out additional and more detailed requirements for each individual vaccine type[3]. Typically these describe the following:

- the nature of the vaccine;
- requirements for vaccine production (including choice of bacterial or viral strain and demonstration of safety and efficacy (referred to as either immunogenicity or potency in the monographs);
- the **quality control tests** that must be conducted on individual vaccine batches to ensure consistency; these typically comprise tests to determine safety and potency, but other tests may also be described;
- whether alternative test methods are expected to be substituted for the test described under potency for routine testing of vaccine batches;
- examples of alternative potency testing approaches that, once validated, can be substituted for the test described under potency for routine batch testing.

### 2.2.2 Repeat testing through OCABR

Quality control tests for release of a vaccine on the EU market are performed by the vaccine manufacturer, or contracted to an approved laboratory, and must meet the specifications of the relevant Marketing Authorisation of the Member State. Article 82 of Directive 2001/82/EC enables Member States to have batches re-tested at an Official Medicines Control Laboratory (OMCL) for the protection of human or animal health. These tests are repeated, even though the same tests have been carried out satisfactorily by the manufacturer. This process is known as 'Official Control Authority Batch Release' (OCABR). The justification for this repeat testing appears to be that government authorities are responsible for ensuring the quality of vaccines. The impact

[2] For a handful of veterinary vaccines (e.g. *Salmonella* Dublin vaccine), authorisation by the VMD is based on monographs that are recognised in Britain, but which are not included in the European Pharmacopoeia.

[3] Samples of monographs for specific vaccines are provided in Appendices 3-6.

of vaccine failure would be particularly high for some vaccines, and Member States may not want to rely on the result of a single test by the manufacturer. Results of OCABR performed by any given Member State must be mutually recognised by all other Member States requiring OCABR for that product. Consequently, a Member State or OMCL is not allowed to retest a batch if it has already been tested by another Member State. The vaccines to which OCABR may be applied are agreed by the Member States and the list is to be reviewed on a regular basis. Recent harmonisation of batch release in the EU has involved various stakeholders, and introduced provisions for both re-testing and administrative release (OBPR or Official Batch Protocol Review, under Article 81). Re-testing under Article 82 currently includes vaccines that protect against Aujesky's disease, brucellosis, equine influenza, infectious bovine rhinotracheitis, rabies, Newcastle disease and swine erysipelas.

### 2.2.3 Quality control tests – the species and numbers of animals used

Data relating to animal use in the batch testing of veterinary vaccines in the UK has not been routinely collected and published in the past and so it is impossible to determine how animal use fluctuates in this area. This lack of information also makes it difficult to identify where efforts to improve animal welfare would be best focussed. However, the Department for the Environment, Food and Rural Affairs (DEFRA) recently announced that it had commissioned the collation of data on the regulatory use of animals in vaccine testing to enable the Government to focus attention on priority areas for application of the 3Rs (DEFRA, 2007). It is important that this data is collected and published regularly in the public domain so that all relevant stakeholders are aware of where implementation of the 3Rs is most needed.

The only relevant statistics currently available were released by the VMD in 2005. They show that 31,047 animals were used in quality control tests of veterinary vaccines authorised for use in the UK during 2003 (Brady, 2005; VMD, 2005). A breakdown of the figures from 2003 shows that the most common type of animal was poultry, accounting for 34.6% of the total, followed by mice (28.2%), guinea pigs (8.7%), fish (8.4%), hamsters (7.7%) and rabbits (4.8%). The remainder comprised companion and larger farmed animals (e.g. dogs, cats, cattle, pigs, and horses) (Table 1) (VMD, 2005).

The breakdown of statistics for 2003 also shows that animals were used in seven different types of quality control tests - the majority in tests to demonstrate safety and potency (Table 2) (VMD, 2005). The latter are of most concern with respect to animal welfare because of the numbers of animals used and the levels of suffering that can be involved. Consequently the majority of this report focuses on batch potency tests although the use of animals in safety tests is also considered later.

**Recommendation 1:**
**DEFRA should ensure that data relating to the use of animals in the quality control of immunological veterinary medicinal products is collated annually and published in the public domain. The breakdown of statistics should be sufficiently detailed to enable identification of areas where application of the 3Rs is most needed.**

**Table 1. Species of animal used in quality control tests conducted on veterinary vaccine batches for release in the UK during 2003**

| Species | Numbers used in quality control tests for release of veterinary vaccine batches during 2003 in the UK (percentage of total)* |
|---|---|
| Poultry | 10732 (34.6 %) |
| Mice | 8759 (28.2 %) |
| Guinea pigs | 2694 (8.7 %) |
| Fish | 2620 (8.4 %) |
| Hamsters | 2375 (7.7 %) |
| Rabbits | 1503 (4.8 %) |
| Cattle | 495 (1.6 %) |
| Dogs | 397 (1.3 %) |
| Pigs | 364 (1.2 %) |
| Rats | 348 (1.1 %) |
| Sheep | 313 (1.0 %) |
| Cats | 266 (0.8 %) |
| Horses | 126 (0.4 %) |
| Pigeons | 55 (0.2 %) |
| **TOTAL** | **31047** |

* data from VMD (2005).

### 2.2.4 Potency tests and impact on experimental animals

The monographs describe two different types of potency testing. The first is often referred to as efficacy testing and must be conducted to demonstrate the vaccine's ability to protect against disease during its development. The second type of potency test is one that must be conducted on each finished batch of vaccine as a quality control test.

Demonstration of potency during vaccine development

Evidence of efficacy, gained in well-controlled laboratory conditions must be gained during vaccine development. This is a required component of any vaccine dossier submitted to a competent authority for marketing authorisation. The test requirements for demonstration of efficacy are described in the monographs, either in a section that is headed 'Immunogenicity', or in a section that is headed 'Potency'. It is a requirement of

the Pharmacopoeia that "*in principle, demonstration of efficacy is undertaken under well-controlled laboratory conditions by challenge of the target animal under the recommended conditions of use*". This involves vaccinating a group of animals and then infecting this group, and an additional group of unvaccinated animals, with the disease agent that the vaccine is designed to protect against. In order for the vaccine to pass the potency test, the vaccinated animals must remain healthy, whilst the control animals must succumb to the disease.

**Table 2. Animal use in the various types of quality control test conducted on veterinary vaccine batches for release in the UK during 2003**

| Type of quality control test | Number of animals used in different quality control tests for release of veterinary vaccine batches during 2003 in the UK (percentage of total)* |
|---|---|
| **Potency** | **16175 (52.1 %)** |
| **Safety** | **6480 (20.8 %)** |
| **Extraneous agents** | **3111 (10.0 %)** |
| **Toxoid contents** | **3120 (10.0 %)** |
| **Toxicity** | **1653 (5.3 %)** |
| **Identity** | **284 (0.9 %)** |
| **Inactivation and sensitising effect** | **170 (0.5 %)** |
| **Sensitising effect** | **54 (0.2 %)** |
| **TOTAL** | **31047** |

* data from VMD (2005).

Potency testing of vaccines for quality control purposes

Batch potency tests are conducted on each batch of vaccine to confirm its "strength" and to ensure that it offers adequate protection against infection, or against the action of toxins that cause disease

The general monograph on vaccines for veterinary use, to which all vaccines must adhere, makes it clear that "*for most vaccines the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches*". Thus, the challenge assay described in the monograph is not recommended for batch testing of most vaccines. Instead, the aim of the batch potency test is *"to ensure that each batch of vaccine would, if tested, comply with the test described under Potency or Immunogenicity".*

The batch potency of live vaccines is usually determined by a simple *in vitro* method. However the same methods are not appropriate for inactivated vaccines which differ

from live vaccines in the way that they stimulate the immune system[4]. Therefore development of a batch potency test for inactivated vaccines is not always straightforward. Many specific vaccine monographs give an example of a type of test that would be considered suitable for assessing batch potency. Typically such a test is a serological assay where potency is confirmed by determining specific antibody levels in vaccinated animals. However, this is only a suggested approach and manufacturers are under no obligation to adopt it if they wish to use a different method.

Regardless of which method manufacturers adopt, they must show that it will generate results that correlate with those obtained in the challenge-based potency test used to demonstrate efficacy during vaccine development. Consequently, batch potency tests are developed and correlation established with efficacy tests based on challenge assays during the development phase of a vaccine. Where it is not possible to establish a correlation between the challenge test (either described as Immunogenicity or Potency in the monograph) and the proposed batch potency test, then the challenge test used during vaccine development must be used routinely.

This approach to confirming batch potency on a routine basis means that there are a variety of test methods that may be utilised. These fall into the following broad categories:

- challenge assays
- toxin neutralisation assays
- serological assays
- vaccine response tests
- *in vitro* assays

**Challenge assays** involve infecting both vaccinated and unvaccinated animals with the disease agent that the vaccine is designed to protect against. In order for potency of the vaccine to be judged as adequate the control animals must show significant signs of infection whilst vaccinated animals remain healthy. Control animals may suffer substantially in such tests. Some monographs even state that control animals should die. A small number of challenge assays involve infection with bacteria or viruses whose effects are likely to have a less pronounced effect on animal welfare, mainly producing changes in reproductive parameters (e.g. egg quality in some avian vaccine monographs). Regardless of the severity of the disease, all testing involving infectious agents is performed in bio-secure research facilities where husbandry systems may not always allow animals to express their natural behaviours. This in itself can have a negative impact on welfare.

[4] **Live vaccines** consist of viable micro-organisms that have been modified to make them less harmful. The replication of these organisms within the body, provides a powerful stimulus to the immune system, without causing a significant illness in immune-competent individuals. **Inactivated vaccines** consist of either killed micro-organisms or purified fractions of micro-organisms that cannot replicate in the host. They are less able to cause an immune response than live vaccines and potency must be determined for every batch.

**Toxin neutralisation assays** are used to test the potency of some clostridial vaccines. The test requires the vaccination of laboratory rabbits so that they develop antibodies. Blood is then taken from the rabbits to collect serum, which is then mixed with clostridial toxins. The mixtures are injected into mice to determine the concentration of toxin that causes death and/or clinical signs of disease. This test method causes substantial suffering to those mice developing disease.

During 2003, 9261 animals were used in challenge or toxin neutralisation assays for the release of veterinary vaccines in the UK. This is equivalent to approximately 57% of animals used in potency tests. Of the animals used, 4021 were controls that would be expected to show some sign of disease (Immunologicals Team, VMD, personal communication).

**Serology assays** involve vaccination of a group of animals from which blood is later sampled to establish the presence of circulating antibodies. The vaccine passes the test if the level of antibodies is found to be equivalent or greater than a threshold value. Serology assays are marked refinements of the potency assays because they involve minimal distress compared to challenge assays or toxin neutralisation tests. Serology assays also offer scope for reducing the number of animals used in each test. For example, monographs relating to fish vaccines describe a challenge assay that requires a minimum of 60 animals per test, whereas the alternative serological assays described in the monographs require a minimum of 35 animals. During 2003, 6941 animals were used in serology assays for the release of veterinary vaccines in the UK, equivalent to approximately 43% of the total used in potency tests (Immunologicals Team, VMD, personal communication).

**Vaccine response tests** involve checking the site of vaccination for the presence of skin lesions which indicate that the vaccine is adequately potent. This test is only described in one monograph used in the UK, contagious pustular dermatitis, where the test is performed on the target species, sheep.

***In vitro* assays**, which do not involve animals, are routinely used to assess the potency of live vaccines. The assay involves determining the bacterial or viral titre of the vaccine. Validated *in vitro* assays, which involve the quantification of antigens, are permitted for batch potency testing of inactivated vaccines. However development and validation can be difficult to achieve, particularly for adjuvanted vaccines where the relationship between antigen load and protection may not be clear. Consequently use of *in vitro* assays in routine batch potency tests of these vaccines is not widespread.

Of these five methods of determining potency, challenge assays and toxin neutralisation assays have the potential to cause the most suffering and distress to experimental animals.

### 2.2.5 Batch potency testing requirements for live and inactivated vaccines.

Of the 80 monographs relating to specific types of vaccine, 47 are for inactivated vaccines and 33 are for live vaccines. The nature of the vaccine, whether it is live or inactivated, determines the type of test routinely used to assess batch potency.

In the case of live vaccines, it is usually only necessary to demonstrate the potency of a *representative* batch using a challenge assay during development of the vaccine. Thereafter batch potency is confirmed by an *in vitro* bacterial count or determination of virus titre. For inactivated vaccines, potency tests must be conducted on *every* batch.

Thus, the greatest concern from an animal welfare perspective, in terms of both numbers of animals and levels of suffering, are potency tests for inactivated vaccines. This is where efforts to implement the 3Rs should be increased.

**Recommendation 2:**
**Funders of research and vaccine manufacturers should focus efforts on the development and validation of alternative methods of assessing batch potency for those vaccines that are routinely tested using challenge assays. Any resulting assays must be formally validated and incorporated into the appropriate monograph as a matter of priority.**

## 3. Potency testing requirements for inactivated vaccines

From January 2008, monographs for 47 inactivated veterinary vaccines will be used to assess vaccine quality for marketing authorisation in the UK.

The potency testing requirements in the monographs for inactivated vaccines are summarised in Appendices 1 and 2. In Appendix 1 the monographs are arranged in tables according to the target animal species. An additional set of tables is dedicated to Clostridial vaccines, where the target animal may be one of a number of species. The tables summarise:

- each monograph's position regarding routine use of the challenge-based potency test, used to assess efficacy during vaccine development, as a batch potency test
- each monograph's suggestions for a suitable approach to batch potency testing
- whether each monograph provides an example of a suitable batch potency test, including which species is recommended for use in the example test
- where there is the potential for a challenge assay to be used for batch potency tests, whether such a test has the potential to cause marked clinical signs and mortality and hence animal suffering
- whether the demonstration of marked clinical signs or mortality is a requirement of the challenge-based test

- whether those monographs that provide potential for challenge assays to be used for batch potency make specific reference to the use of humane endpoints to control suffering

In Appendix 2 the tables show the minimum number of animals that are required for each test where this is specifically stated. The data are grouped in tables according to the species that is used in the test.

## 3.1 Monograph position regarding challenge-based assays for routine batch potency testing

Tests described in the monographs under the headings of Potency or Immunogenicity are typically challenge-based. The general monograph on Vaccines for Veterinary Use states that "*for most vaccines, the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches*." The caveat phrase "*most vaccines*" permits exceptions to this general principle and these exceptions can be identified by looking at the monographs for specific vaccines.

This principle conveyed in the general monograph is reinforced in many of the specific vaccine monographs by the statement *"the test described under Potency is not carried out for routine testing of batches of vaccine".* However some monographs only go as far as to say that "*the test described under Potency is not necessarily carried out*" or that "*it is not necessary to carry out the potency test for each batch of the vaccine*". Other monographs make no statement at all regarding suitability of tests for batch potency. Consequently the challenge assays described under Potency can be used routinely for batch potency determination while still being fully compliant with the requirements of the Pharmacopoeia. This does not mean that challenge tests are necessarily used by manufacturers for all such monographs, but that there is potential for this to occur. The monographs where challenge assays could be used to routinely determine potency are shown in Table 3.

## 3.2 Approaches to batch potency testing suggested by monographs

The majority of monographs for inactivated vaccines suggest approaches for demonstration of batch potency that avoid routine use of challenge assays. The tables in Appendix 1 show those monographs where serological or *in vitro* approaches are suggested for routine batch potency testing. Appendix 1 also shows that nine monographs do not provide any suggestions regarding suitable batch potency tests. Instead they either simply say that a "*suitable validated test*" should be used, or do not mention batch potency testing at all (*Clostridium botulinum* and *Clostridium chauvoei*). By not referring to a batch potency test, it may be inferred that it is acceptable to use the challenge test described under Potency for routine batch testing. Indeed, in the case of potency tests for *C. chauvoei* vaccines this is what happens and the challenge assay is routinely used (VMD, 2005).

**Table 3. Inactivated vaccine monographs in which there is potential for a challenge assay to be used as a routine batch potency test**

| Vaccine monograph | Animal species used in the challenge assay |
|---|---|
| Aujesky's disease[a] | Pigs |
| Avian Paramyxovirus 3 | Turkeys |
| Canine leptospirosis[a] | Hamsters |
| *Clostridium botulinum*[a] | Mice |
| *Clostridium chauvoei*[a] | Guinea pigs |
| *Clostridium noyvi*[ab] | Mice[b] |
| *Clostridium perfringens*[ab] | Mice[b] |
| *Clostridium septicum*[ab] | Mice[b] |
| Egg Drop Syndrome | Chickens |
| Feline chlamydiosis | Cats |
| Feline rhinotracheitis[a] | Cats |
| Foot and Mouth disease | Cattle |
| Fowl Cholera[a] | Chickens, turkeys geese or ducks (depends on target species) |
| Furunculosis for salmonids[a] | Salmon or trout |
| *Mycoplasma gallisepticum*[a] | Chickens, turkeys |
| Newcastle Disease[a] | Chickens |
| Rabies[a] | Mice |
| Rabbit haemorrhagic disease[a] | Rabbits |
| *Salmonella enteritidis* | Chickens |
| *Salmonella typhimurium* | Chickens |
| Vibriosis for salmonids – cold water[a] | Salmon or trout |
| Vibriosis for salmonids[a] | Salmon or trout |

[a] Challenge assays have the potential to result in mortality if animals are infected with a sufficiently virulent and pathogenic strain and suffering is not controlled

[b] The potency of these vaccines is assessed using a toxin neutralisation test that involves both rabbits and mice. Rabbits are vaccinated to produce sera, but mice undergo lethal tests that involve the administration of bacterial toxins.

Of the nine monographs that provide no guidance on suitable batch potency tests, five state that the challenge assay described in the monograph is unsuitable for routine use. However the wording of the remaining four creates potential for batch potency to be assessed by challenge assay (see Section 3.1). These monographs are:

- Avian Paramyxovirus 3
- *Clostridium* botulinum
- *Clostridium* chauvoei
- Aujesky's Disease

This lack of guidance on routine potency assessment does not encourage the development and validation of more humane test methods.

Most monographs that suggest an approach to routine batch potency determination advocate serological methods. The monographs for rabies and canine leptospira vaccines also suggest an *in vitro* test as a potential approach. The general monograph on Vaccines for Veterinary Use encourages the use of alternative methods to demonstrate compliance with the monograph when this leads to replacement of animals. Consequently, although *in vitro* test methods for batch potency are only specifically mentioned in these two monographs they can be used for the assessment of any vaccine providing the methods can be validated. Unfortunately, developing and validating a suitable *in vitro* assay is not always straightforward. This is because *in vitro* assays for inactivated vaccines involve quantification of antigens, but the action of many inactivated vaccines is dependent not only on antigen load, but also on the action of adjuvants contained in the vaccine formulation. Therefore, merely quantifying the antigen rarely gives a valid indication of the strength of the vaccine, and so this approach is not widely used to routinely assess batch potency. However, difficulties associated with assessing potency *in vitro* should not prevent inactivated vaccine monographs from suggesting *in vitro* approaches because there may be circumstances where their use is appropriate.

## 3.3 Examples of batch potency tests suggested by the monographs

Where monographs indicate that routine batch potency should be determined using a validated alternative to the challenge assay they often describe an example of the type of test that might be used instead. The level of detail relating to methodology provided by such examples varies from highly specific to very vague. In a number of cases, even the species to be used is left open to interpretation with monographs requiring only "*experimental animals*" or "*laboratory animals*" or "*suitable species*". On the one hand this approach provides considerable flexibility to manufacturers when developing potency tests by not being overly prescriptive. On the other hand, it provides little guidance and, at times, may highlight a need for a validated test to be incorporated into the monograph. This range in the level of detail provided about appropriate methods is illustrated in the sample monographs given in Appendices 3-5 which are summarised below:

- the monograph for canine adenovirus vaccine recommends the use of a "*suitable validated alternative method*" but provides no guidance at all regarding appropriate methodology (see Appendix 3).
- the monograph for canine leptospirosis vaccine suggests a serological or an *in-vitro* method can be used, but lacks detail about how these might be conducted. For example, the description of a serology assay does not suggest a suitable species (see Appendix 4);
- the monograph for porcine *actinobacillosis* vaccine suggests a serological method and provides considerable detail regarding suitable methodology (see Appendix 5);

Typically, if vaccines are intended for use in ruminants, pigs, companion animals or horses then a surrogate species (typically mice, guinea pigs or rabbits) is recommended for use in the suggested serology assay. Where the target species is a fish or bird, then the target species continues to be used in the serology assay (Appendix 1).

**Recommendation 3:**
**All veterinary vaccine monographs should state that suitable serological or *in vitro* validated tests might be used for routine use instead of the challenge-based test described under Potency. Examples of suitable batch potency tests, including an *in vitro* test, should be provided.**

## 3.4 Severity of challenge-based batch potency tests and the control of suffering

Data in Appendix 1 shows that where there is potential for a challenge assay to be used for routine batch testing, the assay typically involves infection with disease agents that have the potential to produce marked clinical signs, and hence suffering, in unvaccinated control animals.

It is a requirement of the European Directive 86/609 and the ASPA that:

*"If anaesthesia is not possible, analgesics or other appropriate methods should be used in order to ensure as far as possible that pain, suffering, distress or harm are limited and that in any event the animal is not subject to severe pain, distress or suffering"*

In accordance with this requirement, the general, over-arching monograph on Vaccines for Veterinary Use contains the following text:

"*if it is indicated that an animal is considered to be positive, infected etc. when typical clinical signs occur then as soon as it is clear that the result will not be affected the animal in question shall be either humanely killed or given suitable treatment to prevent unnecessary suffering*."

Hence, it is a clear requirement of the Pharmacopoeia that humane endpoints should be implemented throughout all of the animal tests described in the monographs where this would not affect the results.

Of the 22 inactivated vaccine monographs where the challenge assay can potentially be used to test batch potency, 16 involve infections that have the potential to result in mortality if suffering is not controlled and a sufficiently virulent and pathogenic strain is used to induce disease (Table 3). Indeed, the expected severity of disease is such that 13 of these monographs require unvaccinated control animals to show severe clinical signs of disease, be killed on welfare grounds, or even die, if the vaccine is to meet the requirements of the standard potency test (Appendix 1).

Despite this, most monographs that describe potentially lethal infections that may be used to determine batch potency do not mention humane endpoints. Only one monograph currently includes a statement to the effect that animals showing marked signs of disease should be killed during tests used to confirm batch potency (*Clostridium chauvoei* vaccine). Ironically, humane endpoints are sporadically mentioned in monographs when describing tests to demonstrate efficacy during vaccine development even though these tests are not performed on a routine basis. In two of these monographs use of humane endpoints is specifically indicated even though severe disease is not a stated requirement of the test (Mannheimia for cattle and Mannheimia for sheep vaccines).

The monograph for canine leptospira is worthy of particular attention (see Appendix 4). Here, within the same monograph, humane endpoints are referred to inconsistently and appear to be species dependent. The test conducted to demonstrate efficacy during vaccine development is a challenge assay in dogs where "*if an animal shows marked signs of disease, it is killed*". The death of control animals is not required (although there is the potential for this to occur). For routine batch potency testing a challenge assay in hamsters is used. The requirements of the hamster assay are *"not fewer than 4 of the 5 control animals die showing typical signs of leptospira infection"*. There is no reference to the need to control suffering in this particular test.

This "species effect" is apparent in other monographs. Some monographs specifically mention humane endpoints in development studies to demonstrate efficacy. However reference to humane endpoints tends to be restricted to those tests that require the use of dogs, ruminants or other relatively "valuable" species. The only exception is the monograph for *Clostridium chauvoei* vaccines where the monograph requires moribund guinea pigs to be killed.

**Recommendation 4:**
**In all monographs that describe tests that have the potential to cause substantial suffering or mortality, the test description should state that those animals showing marked signs of disease should be killed. New monographs should not be incorporated into the Pharmacopoeia until they contain such statements, and existing monographs that do not stipulate the use of humane endpoints in tests that can cause mortality should be revised immediately.**

### 3.5 Numbers of animals required and experimental design of batch potency tests

The majority of monographs specify a *minimum* number of animals that must be used if challenge assays are used to assess batch potency. These figures are presented in Appendix 2, arranged according to the nature of the test and the species that is used. Where monographs provide examples of a serological assay that could be used as a batch potency test, the numbers of animals suggested for use in the assay are also given. This data is summarised in Tables 5 and 6. Table 5 shows the average minimum number of a particular type, or species, of animal that must be used if batch potency is assessed using a challenge assay. Table 6 shows the suggested number of animals for use in serological assays, where these are given.

**Table 5. Minimum animal numbers required in challenge assays that can be used to routinely assess batch potency. Mean values calculated by averaging across the tests that specify use of a particular animal.**

| Animal | Number of tests that specify use of the animal | Number of vaccinated animals | | Number of unvaccinated control animals | | Total number of animals required for the test | |
|---|---|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean | Range | Mean |
| **Mouse** | 5 (2*) | 20-60 | **40.0** | 10-40 | **25.0** | 30-100 | **65.0** |
| **Fish** | 3 | 30-30 | **30.0** | 30-30 | **30.0** | 60-60 | **60.0** |
| **Avian species** | 7 | 20-60 | **34.3** | 10-40 | **22.9** | 30-100 | **57.1** |
| **Cat** | 2 | 10-10 | **10.0** | 10-10 | **10.0** | 20-20 | **20.0** |
| **Cattle** | 1 | 15-15 | **15.0** | 2-2 | **2.0** | 17-17 | **17.0** |
| **Guinea pig** | 1 | 10-10 | **10.0** | 5-5 | **5.0** | 15-15 | **15.0** |
| **Rabbit** | 1 | 10-10 | **10.0** | 5-5 | **5.0** | 15-15 | **15.0** |
| **Pig** | 1 | 5-5 | **5.0** | 5-5 | **5.0** | 10-10 | **10.0** |
| **Hamster** | 1 | 5-5 | **5.0** | 5-5 | **5.0** | 10-10 | **10.0** |

* Five potency tests in the monographs involve infection of mice with disease agents. However, three of these are toxin neutralisation assays where the minimum number of animals required is not specified. Hence means for mice have been calculated from the two monographs that do specify minimum numbers.

#### 3.5.1 Challenge assays

The summary statistics in Table 5 show that the average numbers of animals required for assessing batch potency with a challenge assay varies considerably depending on the species concerned. Tests that use mice, birds or fish require much larger numbers of animals than those that use other species suggesting that the number of animals required is sometimes based on cost or availability, rather than scientific considerations.

This table summarises the minimum number of animals required per test where this is stated in the monographs. In actuality, the average number of mice required in infection-based assays is likely to be considerably higher than the numbers shown. This is because three Clostridial vaccine monographs require a toxin neutralisation test where the number of mice used varies depending on the methodology adopted. The minimum number required for this test is not stated in the monograph, although large numbers are used. Indeed, Weisser & Hechler (1997) reported that the testing of vaccines that contained *Clostridium novyi* and *C. perfringens* components used an average of 410 and 400 mice per batch respectively.

Of particular concern in challenge assays are the number of animals in unvaccinated infected control groups as these animals are expected to suffer most. Once again the numbers required appear to be species related. The number of control animals required in these tests ranges from a minimum of 40 mice in potency tests for rabies vaccines to two cows in the potency test for Foot and Mouth Disease vaccines.

There is also considerable inconsistency between the monographs with respect to experimental design and the numbers of animals required in control and vaccinated groups. In some monographs the number required in each group is equal. In other instances monographs require fewer animals in the control group than in the vaccinated group (see Table 5 & Appendix 2). For example, all fish vaccine monographs require a minimum of 30 animals in each group, whilst in the monograph for rabbit haemorrhagic disease vaccines 10 animals are required in the vaccinated group and only five in the control group.

### 3.5.2 Serology assays

Table 6 shows that even in serology assays there is considerable variation in numbers of animals required for a test, and this is also dependent on the species used. Tests involving fish and birds require far greater numbers of animals than tests that use other species. However, this variation is not as pronounced as for challenge assays.

Data in Table 6 and Appendix 2 reveal several inconsistencies between the monographs with respect to the suggested experimental design:

- Some monographs suggest an assay that uses a single group of animals that are vaccinated and then blood sampled for serological analysis (e.g. Canine Parvovirus). Other monographs require an additional group of control animals (e.g. Ruminant *E.coli*).

- Control groups may be either mock-vaccinated (e.g. all fish vaccines) with a placebo product prior to being blood sampled, or they may simply be blood sampled with no requirement for mock-vaccination (e.g Fowl Cholera).

- Where a control group is required, the number of animals in the group can be equal to the vaccinated group (e.g. Avian Infectious Bursal Disease; 10 animals in each group) or it may be considerably less (e.g. Bovine Leptospirosis; 10 animals in vaccinated group, 2 animals in control group).

- Where an assay can be performed on an alternative species, the numbers of animals required can vary depending on the species used. For example, the test described under Potency for Canine Parvovirus that is performed during vaccine development is a serological assay using two dogs. The monograph suggests that this may be replaced with a serology assay involving five guinea pigs for routine batch testing.

These kinds of monograph inconsistencies are discussed further in Section 5. The numbers of animals suggested for use in serological assays must always be based on scientific considerations rather than the availability, financial cost or desirability of a particular species. Control groups should only be required if there is scientific justification for their inclusion.

**Table 6. Animal numbers used in suggested examples of batch potency tests assessed by serological methods. Mean values calculated by averaging across all serological tests that specify use of a particular animal.**

| Animal | Number of tests that suggest use of the animal | Number of vaccinated animals | | Number of unvaccinated control animals | | Total number of animals required for the test | |
|---|---|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean | Range | Mean |
| **Fish** | 3 | 25-25 | **25.0** | 10-10 | **10.0** | 35-35 | **35.0** |
| **Avian species** | 8 | 10-10 | **10.0** | 5-10 | **5.6** | 15-20 | **15.6** |
| **Mouse** | 7 | 5-15 | **8.6** | 0-2 | **0.3** | 5-15 | **8.9** |
| **Rabbit** | 7 | 5-10 | **7.1** | 0-2 | **0.6** | 5-10 | **7.7** |
| **Pig** | 2 | 5-5 | **5.0** | 2-2 | **2.0** | 7-7 | **7.0** |
| **Rat** | 1 | 5-5 | **5.0** | 2-2 | **2.0** | 7-7 | **7.0** |
| **Unspecified laboratory animals** | 6 (5[a]) | 5-5 | **5.0** | 2-2 | **2.0** | 7-7 | **7.0** |
| **Ruminant (sheep, cattle etc)** | 4 | 5-6 | **5.3** | 0-2 | **1.0** | 5-7 | **6.3** |
| **Guinea pig** | 9 (8[b]) | 5-10 | **5.6** | 0-2 | **0.5** | 5-12 | **6.1** |
| **Cat** | 2 | 2-5 | **3.5** | 0-0 | **0.0** | 2-5 | **3.5** |
| **Dog** | 1 | 2-2 | **2.0** | 0-0 | **0.0** | 2-2 | **2.0** |

[a] Six potency tests in the monographs can involve serological assays in unspecified laboratory species of animal. However, the minimum number of animals required is only specified for five of these.

[b] Nine monographs suggest using serological assays in guinea pigs to assess batch potency. However, the minimum number of animals required is only specified for eight of these. Means for guinea pigs have therefore been calculated from the eight monographs that specify minimum numbers.

**Recommendation 5:**
**The Pharmacopoeia Commission should establish a panel of statisticians to review the experimental design of tests involving animals to ensure that tests do not require the use of excessive numbers of animals overall, or in control groups. For existing monographs, the numbers of animals required for batch potency tests should be re-evaluated using data that has been generated for previous authorisation purposes. Where potential for reduction is identified, the corresponding monograph should be revised.**

## 3.6 Potency tests of most concern with respect to animal welfare

### 3.6.1 Clostridial vaccines

Of greatest immediate concern is the potency assay for *C. chauvoei* vaccines, where the challenge assay is the only method described in the monograph for routine use. Alternative approaches to testing have not been developed or validated. Use of these vaccines is widespread, and batch potency is assessed by means of a challenge assay in guinea pigs that results in substantial suffering for control animals. Test animals are injected with the spores or a culture of *C. chauvoei.* This results in oedema and necrosis in the surrounding tissues of unvaccinated control animals and eventually death (Singh *et al*, 1992). The monograph says that moribund animals are killed to prevent unnecessary suffering. However, moribund is not far from death and animals will have undergone substantial suffering before reaching this point.

The lack of a more humane potency assay for this vaccine has long been a cause for concern. Over a decade ago, a European Centre for the Validation of Alternative Methods (ECVAM) workshop recommended a co-ordinated research effort to establish an alternative serological method (Hendriksen *et al*., 1994). Promising attempts to develop enzyme-linked immunosorbent assays (ELISAs) to replace the challenge test have been reported from both Europe (Roth & Seifert, 1997) and the USA (Hauer, 1997). The USA authority responsible for the regulation and testing of veterinary biologicals has recognised the reliability of this approach and now grants exceptions to the guinea pig challenge assay if a standard ELISA method is used instead (Center for Veterinary Biologics, 2003). Despite this, alternative methods have yet to be incorporated into the European monograph. Consequently vaccines produced and tested for batch potency by ELISA in the USA, could still require testing in guinea pigs before being marketed in Europe. Concerned manufacturers are keen to modify this potency test as is evident from collaborative work that has led to a reduction in the number of animals required to satisfy the challenge test requirements (Redhead *et al.*, 1999). However, validation, acceptance and implementation of a more humane testing method within Europe is urgently required.

The potency assay described for *C. botulinum* vaccines is another test that causes substantial suffering, yet the use of humane endpoints or an alternative test method is not advocated in the monograph. The test involves induction of botulism in mice, the clinical signs of which typically involve dehydration, loss of sensorimotor reflexes and heart or respiratory failure (Luvisetto *et al.*, 2003). These vaccines are not currently used routinely in the EU, but there may be cause to use them in the future. Hence there is a need for research to be conducted to develop more humane methods of determining the potency of these vaccines.

**Recommendation 6:**
**Serological methods of determining potency of *C. chauvoei* vaccines should be formally validated in Europe so that they can be incorporated into the European monograph.**

**Recommendation 7:**
**Earlier humane endpoints, which can be applied prior to animals becoming moribund, should be defined and used for potency tests of *C. chauvoei* vaccines. In addition, the monograph should be revised so as to advocate the use of appropriate analgesia to reduce suffering of control animals.**

### 3.6.2 Canine leptospirosis vaccines

The potency of canine leptospira vaccines is routinely determined using a lethal challenge assay in hamsters (VMD, 2005) despite the monograph suggesting that serological or *in vitro* methods can be used (see Appendix 4). The challenge assay involves substantial suffering and while the general monograph, Vaccines for Veterinary Use, requires suffering to be controlled, this specific vaccine monograph requires the death of control animals and makes no reference to humane endpoints. The monograph is clearly in urgent need of revision, and research efforts are required to define those clinical parameters that could be used as the earliest indicators of impending death.

The monograph does not provide examples of acceptable methods for either serological or *in vitro* assays, reflecting how development of such methods for routine potency assessment of these vaccines is a problem. However, development of more humane alternatives to this challenge assay, be it *in vitro* or serological methods, has been the subject of a workshop organised by the Council of Europe (1999). Unfortunately this produced little in the way of positive outcomes that have been carried forward. More recently, DEFRA (2006) issued a call for research proposals aimed at developing *in vitro* assays for this potency test and the UK NC3Rs has awarded funding to a programme of work to develop alternatives to the hamster challenge assay (Coldham, 2007).

**Recommendation 8:**
**Humane endpoints that can reduce the suffering of the hamsters used in batch potency tests of canine leptospira vaccines should be defined, validated and incorporated into the monograph.**

**Recommendation 9:**
**Any serological or *in vitro* assays that are developed as an alternative to the hamster challenge assay for assessing potency of leptospira vaccines must be validated and incorporated into the monograph as a matter of priority.**

### 3.6.3 Rabies vaccines

The monograph for inactivated rabies vaccine outlines a potency assay that involves infecting mice with rabies via the intra-cerebral route. The test requires a minimum of 100 animals, at least 40 being unvaccinated controls (see Appendix 2). This assay is a major concern due to the levels of suffering associated with the route of infection, the

resulting disease, the numbers of animals used in each test and the potential for repeat testing (see Section 2.2.2). The quality control tests performed on rabies vaccines have been the subject of an ECVAM workshop. The resulting report contained a large number of recommendations for application of the 3Rs in this area, including the use of humane endpoints (Bruckner *et al*., 2003). The monograph is currently being revised and the final version will state that clinical signs, described in Bruckner *et al*. (2003), should be used as humane endpoints (European Pharmacopoeia Forum, 2006a).

This change is welcome, but the assay will continue to cause large numbers of animals to suffer each time it is performed. The monograph also describes a serological approach that could be used instead of the challenge assay to assess batch potency once validated. *In vitro* methods are also suggested in the monograph. The serological method given as an example requires only five mice. Therefore its widespread adoption would significantly reduce the numbers of animals used and the suffering involved.

Unfortunately the mouse challenge model is the only method currently used to assess batch potency in the UK (VMD, personal communication). The nature of this particular vaccine means that it is used globally. Consequently quality control tests must satisfy the requirements of all relevant regulatory agencies and these tend to require the challenge assay to be used. DEFRA recently issued a call for research proposals aimed at developing an *in vitro* assay for determining rabies vaccine potency (DEFRA, 2006). However, whilst development and validation of serological or *in vitro* methods is welcomed, it is unlikely to result in significant improvements in animal welfare unless there is global recognition of more humane testing methods, coupled with harmonisation of test requirements.

### 3.6.4 Fish vaccines

Serological approaches to routine potency testing are described in fish vaccine monographs, but challenge assays are still routinely used. The challenge assays are lethal tests and, as fish have been shown to perceive pain (Sneddon, 2003), these assays are likely to cause suffering. The requirement to use humane endpoints is not mentioned in the monographs for fish vaccines, and indeed there is little or no information available regarding clinical signs that may be predictive of impending death. Consequently, research efforts need to be focussed in this area. In addition, the necessity of using such large numbers of fish in batch potency tests should be questioned. Each test uses a minimum of 30 control fish, with over 1800 animals being used to assess the potency of fish vaccines in the UK during 2003 (VMD, 2005). The large numbers of animals used, and their ability to experience pain in these tests is a concern.

One reason for such widespread use of the challenge assay is that fish vaccine monographs were only recently included in the European Pharmacopoeia. Prior to this, manufacturers performed the potency test described in the European Guideline on Fish Vaccines (European Commission, 1994). The Guideline specifies a challenge assay that requires more test animals than the corresponding monographs. Thus, until the monographs were introduced, the challenge assay was the only recommended potency test method and there was little incentive to develop alternatives. The conflicting requirements in the Guideline and the corresponding monographs with regard to

numbers may encourage manufacturers to use more fish than are necessary rather than risk regulatory rejection. However, the monographs of the Pharmacopoeia take precedent over the Guideline and manufacturers must be reminded of this.

The fish farming industry continues to expand rapidly, creating an ever increasing demand for vaccine products. Indeed, the number of fish vaccines listed in the NOAH Compendium of Data Sheets for Animal Medicines, which lists veterinary medicines marketed in the UK, rose from six available in 2006 to ten available in 2007 (NOAH, 2005; NOAH, 2006). This increase in available products will inevitably result in a corresponding rise in the number of batch tests performed on fish vaccines. The UK Home Office recognises the need for alternative methods for routinely assessing the potency of fish vaccines and has funded a research project to develop suitable serological assays (Animal Procedures Committee, 2005). However, even if validated and adopted, serological assays would still have an impact on fish welfare due to the handling and sampling techniques required, unless samples were collected *post mortem*. In addition, monographs for fish vaccines require a minimum of 35 fish to be used in a serology assay. It is not clear why the Pharmacopoeia specifies such a large number of animals when a suitable test has yet to be developed. It would seem more appropriate to first develop a test and then determine how many animals are needed to ensure a valid result.

The ideal method of assessing the potency of fish vaccines would be an *in vitro* assay. Encouragingly, Siwicki *et al*. (2001) have shown that levels of specific antibody secreting cells are increased, not only following *in vivo* immunisation of fish, but also following *in vitro* immunisation of fish spleens. This suggests that there may be potential for developing and adopting an *in vitro* approach, but this is unlikely to happen in the near future without considerable dedicated research effort.

**Recommendation 10:**
**Humane endpoints need to be developed for batch potency tests of fish vaccines. Research is needed to define clinical signs in fish that may be indicative of disease and impending death.**

## 4. Potency testing requirements for live vaccines

For the majority of live vaccines, potency is demonstrated by means of a challenge study during vaccine development. For routine potency testing, *in vitro* methods that involve determination of bacterial counts or virus titres are used. This is clearly described in the general monograph on Vaccines for Veterinary Use. Thus batch potency testing of live vaccines is not as great a concern on animal welfare grounds as it is for inactivated vaccines. However, the wording of the monograph for swine fever vaccine suggests that animal tests could be used routinely in batch potency tests and this would be a concern. In addition, the monograph for *Brucella melitensis* vaccine requires the effectiveness of the vaccine to be assessed in a test of 50% persistence time. The requirements of these two monographs are outlined below.

Swine Fever vaccine

The potency test described in the monograph is a challenge assay in piglets that must result in the death of control animals for the vaccine to pass the test. The use of humane

endpoints is not indicated. The general, over-arching monograph on Vaccines for Veterinary Use says that determination of virus titre should be used to assess potency of live vaccines on a routine basis. This is reinforced in most individual live vaccine monographs by specifying determination of virus titre as a batch test. These monographs also state that the potency test (i.e. challenge assay) is not necessary for each batch. However, the monograph for Swine Fever vaccine does not mention virus titre determination, nor does it indicate that challenge assays are not necessary for routine potency determination. There is a risk that this could be interpreted to mean that the challenge-based potency test is suitable for routine use.

This monograph is currently under review. The draft released for public consultation includes a statement about the use of humane endpoints and a requirement to routinely establish the virus titre. Unfortunately the same draft shows that a proposal to include a similar statement to those found in other live vaccine monographs about it not being necessary to carry out the potency test for each batch has been rejected (European Pharmacopoeia Forum, 2006b). By omitting such a statement, the authors of the proposed revision appear to indicate that routine use of the challenge-assay is acceptable.

Routine vaccination against swine fever has not been used for more than 10 years in Europe and so animals are not used at present within the EU for batch testing purposes. However, the reason for revising the monograph is to "*bring it into line with current concepts since member states may choose to use the vaccine for disease control if there is a widespread outbreak of disease*" (European Pharmacopoeia Forum, 2006b). "*Current concepts*" should include the principle expressed in other monographs that potency testing is not required for each batch of vaccine. Hence the finalised monograph must be worded in such a way that ensures that animals do not suffer in potency tests unnecessarily.

*Brucella melitensis* vaccines

It is not necessary to routinely determine the potency of *Brucella melitensis* vaccines. However, the monograph requires a test for the 50% persistence time in mice to be performed on each batch. The test is performed to ensure that the vaccine strain persists in the organism for sufficient time to ensure a cellular immune response is induced. It involves vaccinating 32 mice and then killing them at specified time intervals after vaccination to check for the presence of the bacteria used in the vaccine formulation. Weisser & Hechler (1997) argue that this test should not be stipulated as a batch test and that it should only be carried out during the licensing procedure.

**Recommendation 11:**
**All live vaccine monographs should state that it is not necessary to carry out the potency test for each batch of vaccine.**

**Recommendation 12:**
**The necessity of the 50% persistence time test as a routine batch test for *Brucella melitensis* vaccines should be reassessed. It should be removed from the monograph as a batch test if it is not essential.**

# 5. Scope for application of the 3Rs in quality control testing

There is considerable potential to reduce the impact on animals during testing of vaccine batches by greater application of the 3Rs principle of reduction, refinement and replacement (Russell and Burch, 1959). In 1997, Weisser & Hechler published an in-depth, technical and critical review of the animal tests stipulated in the monographs of the European Pharmacopoeia with particular reference to animal welfare. The review comprised a detailed analysis of every animal test, in every monograph, for both human and veterinary vaccines that was included in the Pharmacopoeia at the time. Opportunities for reducing animal numbers and levels of suffering were highlighted throughout the text. More recently, Halder (2001) considered the potential for application of the 3Rs in the Pharmacopoeial monographs for human and veterinary vaccines and highlighted considerable scope for their implementation.

Following the adoption of the European Convention for the Protection of Vertebrate Animals Use for Experimental and Other Scientific Purposes in 1986, the European Pharmacopoeia Commission began a programme of activities to review all animal tests in monographs with a view to applying the principles of the 3Rs. Considerable progress has been made since then and a summary of these achievements has been produced (Castle, 2007). However, there is still considerable scope for further progress to be made.

The Pharmacopoeia currently provides great potential for driving implementation of the 3Rs. The general over-arching monograph on Vaccines for Veterinary Use makes it clear that the principles of the 3Rs are to be applied during tests on animals, advocates the use of humane endpoints and encourages the use of alternative methods of testing when this leads to replacement or reduction of animal use, or reduction in suffering. In addition, many monographs for specific vaccines state that challenge assays are not suitable for routine determination of potency and suggest alternative methods that can be used instead. However, the Pharmacopoeia Commission could do much more to press for adoption of more humane test methods.

The Pharmacopoeia could be more harmonious with the requirements of EU Directive 86/609 with respect to using the minimum number of animals and causing the least pain suffering distress or lasting harm, whilst still ensuring consistency of vaccine batches. In order to do this, the Pharmacopoeia Commission should:

- address the seemingly disparate requirements of the monographs and Directive 86/609;
- address inconsistencies and ambiguity between and within monographs;
- implement processes that would facilitate development, validation and incorporation of more humane testing methods into the monographs;
- harmonise testing requirements with those required by agencies outside of the EU.

## 5.1 Disparate legislation, inconsistencies and ambiguity

### 5.1.1 Ambiguity relating to the methods for routine potency testing

The overarching monograph on Vaccines for Veterinary Use says "*for most vaccines the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches*" and many specific monographs specify that challenge assays should not be used routinely. However, the use of phrases such as "*the* [animal] *test described under Potency is not necessarily carried out*" in other monographs implies that alternatives to challenge-based batch tests are merely options that could be considered. This is inconsistent with the requirement of EU Directive 86/609 to use alternative testing methods where these are reasonably and practically available.

Despite the ambiguous wording of the monographs, serological alternatives to challenge assays are commonly used in the UK (VMD, 2005). In 2003, serology assays were used to assess the potency of 82 products released in the UK compared with challenge assays that were used to assess 50 products. However, as more animals are required for a challenge assay than for a serology assay, the formers accounted for approximately 57% of the animals used in potency testing overall (Immunologicals Team, VMD, personal communication).

The adoption of serological methods to test many veterinary vaccines is driven by a number of factors. These include the requirements of legislation controlling animal experiments, improved animal welfare, increased analytical quality of data, and the significant financial costs associated with animal experiments. Challenge assays are labour intensive and have to be conducted in specialised bio-containment facilities. Adoption of serological assays that often use smaller animals, in smaller numbers, with reduced requirements for bio-containment, labour, and specialised skills in clinical assessment and pathology, results in significant financial savings.

The animals used in challenge assays can in themselves be expensive, and this is particularly the case if the target species is used. Indeed serological methods of potency testing are more likely to be developed and utilised if the animals required in challenge assays are "valuable". So, for example, the only use of dogs and pigs in potency testing for release of vaccines in the UK during 2003 was in serology assays. However mice, hamsters and guinea pigs were commonly used in challenge assays (VMD, 2005) even though in some instances, serology assays are a realistic alternative. Indeed, the VMD report that it is difficult to force companies to adopt the serological method while this is only provided as an example test method (VMD, 2005) rather than being the method required.

The methods used for potency testing of swine erysipelas vaccines illustrate this problem. A number of swine erysipelas vaccines are tested using a mouse challenge assay (VMD, 2005) yet the monograph provides an example of a mouse serological method that can be used once validated. The suggested method is based on an ELISA that has been validated by ECVAM (2002) and for which a standard biological reference preparation is available. Hence universal adoption of this method seems achievable. However, rather than stating that the challenge assay should not be used routinely, as many other monographs do, the monograph for swine erysipelas vaccines merely says that "*it is not necessary*" to carry out the challenge assay for batch testing. Consequently, competent authorities can not insist that serological methods are used.

Although serological methods are routinely used to assess batch potency in many instances in the UK, this may not necessarily reflect practices in all EU Member States. In some States, differing cultural values with respect to animal welfare, reduced labour and animal housing costs, the requirement for high-specification laboratories for serological method development and the guarantee of regulatory acceptance, could make the challenge assay a more favoured option. If the language used in monographs was more consistent with that used in regulations relating to the protection of animals used in research, it would serve as a reminder to both manufacturers and regulatory authorities that alternative approaches must be used where possible. This would leave less opportunity for misinterpretation.

EU Directive 86/609 requires that animals should not be used in experiments if other methods are reasonably and practically available. However the general monograph on Vaccines for Veterinary Use says if alternative methods are to be used, they should be "*established during development of the vaccine*". This means that manufacturers are required to submit details of the methods to the relevant regulatory authority for approval at the time of licensing.

Unfortunately, development of alternative methods is not always possible prior to a vaccine's licensing, especially if the nature of immunological protection is not fully understood. Adopting alternative methods later in a vaccine's lifetime will require a change to the vaccine's licence and hence additional regulatory approval. Depending on the procedure used for licensing the vaccine, approval may be required from the regulatory authorities of all relevant Member States in which the vaccine is marketed. There will be financial costs associated with each of these applications for approval and if only one authority objects, the test methods must remain as they were at the time of licensing. Hence, once a vaccine has been licensed there can be little incentive for manufacturers to devote resources to developing alternatives to methods that have already been granted approval. Clearly, the requirements of Directive 86/609 could be more fully realised if manufacturers were encouraged to develop serological or *in vitro* potency tests at any stage of a vaccine's life span.

**Recommendation 13:**
**Where serological or *in vitro* methods of determining potency have been formally validated and incorporated into a monograph, the monograph should state that the challenge assay is not used for routine batch testing. Authorities overseeing regulation of animal experiments (e.g. the UK Home Office) should ensure that the validated alternative methods are used where possible.**

**Recommendation 14:**
**Where it is not possible to develop a serological or *in vitro* batch potency test during vaccine development, the monograph on Vaccines for Veterinary Use should make it clear that this should not preclude attempts to develop such tests later.**

**Recommendation 15:**
**Competent authorities should introduce a fee amnesty for processing licensing variations that result in fewer animals being used in quality control tests.**

### 5.1.2 Inconsistencies between monographs: use of serological or *in vitro* assays for batch potency

There is a lack of consistency between monographs as to whether serological and/or *in vitro* methods of assessing batch potency are advocated. Most inactivated vaccine monographs require, or at least suggest, that potency should be routinely confirmed using serological methods. However, the use of *in vitro* methods, that allow complete replacement of animal tests, is only suggested in two monographs even though the monograph on Vaccines for Veterinary Use encourages the use of alternatives that can replace animals. In addition, there are a few monographs that fail to suggest either serological or *in vitro* methods. This could be interpreted as meaning that potency should be routinely determined using challenge assays (see Sections 3.6 and 4.4). Consequently it is important that all monographs refer to the potential of using serological or *in vitro* tests for determining batch potency to increase the likelihood of them being developed and used.

### 5.1.3 Inconsistencies between monographs: the amount of guidance provided

Unfortunately, in monographs where serological or *in vitro* methods are suggested for routine potency testing, there are marked inconsistencies in the degree of guidance they provide on suitable methods. The level of detail provided regarding methodology ranges from a suggested general approach, to relatively detailed protocols as was highlighted in Section 3.3. Minimal detail may reflect a lack of previously validated methods whilst allowing manufacturers flexibility to develop their own approach to assays. However, the provision of more detailed guidance relating to suitable alternative methods could serve to stimulate alternative test development and use.

The development and validation of methods for routine potency testing is usually the responsibility of the manufacturer or contracted laboratory. The animal health industry is highly competitive and within the UK vaccines account for about a quarter of the veterinary medicine market. It is estimated that in 2005 veterinary vaccine sales were worth £114 million in the UK alone (Anon, 2007). Consequently, there is always considerable pressure to get a product on the market as quickly as possible. If development of a serological or *in vitro* batch potency test is problematic, it has the potential to delay this process. Development and validation of such tests will typically involve overcoming scientific and technical barriers and can be costly in terms of time, money and other resources. At the end of the process there is no guarantee that all relevant regulatory authorities will accept the new method unless the monograph provides clear guidance to both manufacturers and regulators on which approaches to testing are acceptable.

**Recommendation 16:**
**Where monographs do not provide details of suitable batch potency methods, manufacturers that have developed and validated their own methods in-house should publish or otherwise share them. They should also submit their methods for formal validation so that they may be incorporated into the appropriate monograph.**

### 5.1.4 Inconsistencies in the way humane endpoints are referred to

The general over-arching monograph on Vaccines for Veterinary Use requires humane endpoints or other means of controlling suffering to be used if the test result will not be affected. However, this requirement is not consistently reiterated throughout the monographs for specific vaccines. A small number of monographs specify that animals showing marked signs of disease should be killed on welfare grounds, but this is mainly during challenge assays used in vaccine development and not during routine batch tests. The majority of monographs that describe challenge assays that can be used in routine batch tests do not mention humane endpoints even when they describe tests that can cause severe disease. The only exception to this is the monograph for *Clostridium chauvoei* vaccines.

This lack of consistency is confusing and could be interpreted to mean that use of humane endpoints is more important in potency tests for some vaccines than others. At worst, the lack of a statement saying that humane endpoints should be used could be taken to mean that humane endpoints are inappropriate, or not permitted, in a particular test. Indeed, where monographs specify that control animals must die, a possible interpretation could be that applying humane endpoints would "affect" the test result, and that the disease should be left to take its course instead. The wording of all monographs should leave manufacturers, competent authorities, and those responsible for overseeing the regulation of animal experiments in no doubt as to the necessity of controlling suffering by using humane endpoints.

The level of inconsistency is such that the monograph for inactivated canine leptospira vaccines is even inconsistent within itself. Two challenge-based tests are described in this monograph. One uses dogs to assess potency during vaccine development and the other uses hamsters for batch potency tests (Appendix 4). The monograph advocates the use of humane endpoints in the dog test but not in the hamster assay, where the test requires that control animals must die (Appendix 4). This suggests that controlling suffering is thought more important for some species than for others. Urgent revision of this monograph is required to address this inconsistency.

The lack of reference to humane endpoints in so many monographs does not encourage manufacturers to use them, especially since monitoring animals merely for mortality requires less input in terms of staff time and training. Indeed, where a test specifically requires death of control animals, manufacturers may actively avoid using humane endpoints for fear of regulatory rejection. To be more in line with Directive 86/609 all monographs that describe tests where control animals are expected to show severe signs of disease, or die, should include a statement about the requirement to control suffering through use of humane endpoints. This would reduce the suffering of animals used in the test and prevent misinterpretation of test requirements.

### 5.1.5. Defining humane endpoints in terms of clinical signs

Inclusion of a statement about the need to use humane endpoints is important. Ideally monographs that describe tests that caused substantial suffering should describe objective clinical signs, proven to be predictive of death or severe disease, that could be used to a determine when the endpoint has been reached. This approach would ensure that humane endpoints did not affect test results and so would comply with the

requirements of the general monograph on Vaccines for Veterinary Use. Furthermore, it would facilitate a harmonised and consistent approach to how humane endpoints are used, helping to prevent animals suffering more at one establishment than another due to the differing perceptions of both manufacturers and regulators as to when endpoints should be applied. An example of how this approach can be usefully adopted is in the proposed revision of the monograph for rabies vaccine for veterinary use (European Pharmacopoeia Forum, 2006).

Clinical signs that constitute a more humane endpoint would only be incorporated into a monograph following formal validation. However, in keeping with the principles of the 3Rs, it is important that validation studies in themselves avoid causing animal suffering wherever possible. Consequently, endpoint validation should only involve potency tests that are already being carried out for routine regulatory purposes. It should not entail the suffering of additional animals in studies designed solely for validation purposes.

Inclusion of clinical signs to be used as endpoints has long been advocated for particular potency tests. An ECVAM workshop on animal use in the testing of biologicals recommended that the European Pharmacopoeia Commission consider incorporating the clinical signs of hamsters as an endpoint into the canine leptospira vaccine monograph (Hendriksen *et al.*, 1994). The Pharmacopoeia acknowledges that there is a requirement for humane endpoints in this potency test (Castle, 1999), but over a decade has passed since the ECVAM workshop and the situation remains unchanged

### 5.1.6 Ambiguity and inconsistencies relating to animal numbers and experimental design

Number of animals required for a test

The monographs could do more to reinforce the requirements of Directive 86/609 by discouraging the use of excessive numbers of animals in individual tests. They could also be more consistent between and within monographs with respect to the numbers of animals required for a test, which is currently species, and not science, dependent.

When describing test requirements, monographs specify the minimum number of animals to be used. For example, all monographs for fish vaccines say, "*potency may be carried out using groups of not fewer than thirty fish*". Use of the phrase "*not fewer*" implies that it is acceptable, and even desirable, to use more than the number of animals specified. This is clearly not the case. Directive 86/609 requires the minimum number of animals to be used that are most likely to produce satisfactory results. It is appreciated that in some instances, it may be necessary to use more than the minimum number of animals specified in the monograph to demonstrate the required level of difference between control and vaccinated animals. However, monographs should make it clear that the number of animals used should be the minimum required for test results to be scientifically valid.

Tables 5 and 6 show that the number of animals required for a test varies considerably, and that often the number required is species dependent. Tests involving animals that are costly and require labour-intensive husbandry and housing require fewer animals than smaller, cheaper animals (like mice, fish and birds) that are more easily housed in laboratory environments. This would only be justified if results from mice, birds and fish

were expected to be more variable than from other species. There is no evidence that this is always the case. Indeed variability is likely to be greater in some of the more "valuable" species than in laboratory animals and birds. For example, mice bred from closed colonies are more genetically similar and so variation between individuals is likely to be less than in more out-bred species like horses. In addition, monographs for avian vaccines always require the use of SPF birds. The use of SPF animals can reduce variation as clinical and sub-clinical infections can affect test outcomes in non-SPF animals, thereby increasing variation (de Boo & Hendriksen, 2005).

Even within individual monographs there are species-related inconsistencies in the number of animals required for a particular test. For example, the monograph for canine parvovirus (Appendix 2) suggests two possible serological tests for confirmation of potency. One test requires two dogs while the other requires five guinea pigs. There is no reason why variation between individuals should be greater for guinea pigs than for dogs. Similarly, in the monograph for feline infectious enteritis vaccines (Appendix 2), the standard test for potency is a serological assay in cats. An alternative serological assay in guinea pigs is suggested, but the monograph offers no guidance as to how many guinea pigs are required. As a result, the number of guinea pigs required for the test is open to interpretation, and there is potential for more animals to be used than is necessary.

Thus it seems that the number of animals required for a particular test may be based on scientifically irrelevant issues such as availability, cost or ease of housing in laboratory conditions. The number of animals stipulated in the monograph should reflect the numbers required to satisfy the scientific requirements of the test and should not be dependent on the species used.

Experimental design

The numbers of animals required in unvaccinated control groups in challenge assays are of particular concern because of the potential for these animals to suffer substantially. During 2003, 4021 animals were used as controls in challenge assays for release of veterinary vaccines in the UK (Immunologicals Team, VMD, personal communication). In many monographs, fewer animals are required in control groups than in vaccinated groups (e.g. canine adenovirus, Appendices 2 and 3). Such unbalanced group sizes reduce the number of animals that develop significant disease, but this element of experimental design is not applied consistently throughout the monographs. For example, the potency test for canine leptospira vaccine requires equal numbers of hamsters in vaccinated and control groups (Appendices 2 & 4). All control groups should contain the minimum number of animals required to generate meaningful test results, and wherever possible control groups should contain fewer animals than vaccinated groups.

The design of serology assays described in the monographs as examples of suitable batch potency tests is also inconsistent. The majority of tests require a single group of vaccinated animals but others require an additional group of controls (which in the case of fish vaccines have to undergo the stress of mock vaccination). It is not always clear why control groups are required in some monographs and not in others, but their inclusion often appears to be species dependent. For example, control animals are required in all tests that use chicken and fish. They are not required in tests that use dogs or cats, and are required sporadically in tests that use other species.

**Recommendation 17:**
**The general monograph on Vaccines for Veterinary Use should state that the numbers of animals used in tests must be the minimum required for test results to be scientifically valid. Competent authorities, and national authorities responsible for regulation of animal experiments, should challenge the methods of vaccine manufacturers who use more animals than the minimum stipulated by a monograph for a particular test.**

**Recommendation 18:**
**Control groups should only be incorporated into a test design if there are compelling scientific reasons for doing so.**

## 5.2 Development, validation and incorporation of more humane test methods into the monographs

### 5.2.1 The need for a swifter process for revising and implementing monographs

Monographs and other texts of the Pharmacopoeia are revised following a decision of the Pharmacopoeia Commission and potentially such revisions can incorporate more humane test methods. Interested parties may propose a revision to a monograph through their National Pharmacopoeia Authority if there is justification for a revision and sufficient data to back up the proposal. There are rules governing the revision of monographs (EDQM, 2007) and an outline of the procedures involved in the revision process are published in the Pharmacopoeia's journal, *Pharmeuropa*.

On receiving a reasoned request for a revision, the Commission decides the priority with which the revision should be dealt, based on the justification submitted. Revisions to promote animal welfare are always regarded as high priority (Castle, personal communication).

Requests to revise a monograph to incorporate a more humane test method will only be considered by the Pharmacopoeia if proposals are accompanied with sufficient substantiating data, which will usually be obtained during a programme of formal validation. Then, if acceptance is granted, the monograph is revised to incorporate it. Revision of a monograph typically takes at least two years. The entire process of incorporating new test methods is extremely slow, with the time between development and acceptance of an alternative model being more than 10 years in total (Hendriksen, 2002). The scale of the delay between test development and implementation is unacceptably costly in terms of animal welfare. Formal validation is both essential and time-consuming, but the animal welfare costs could be reduced by adoption of swifter review, acceptance and implementation procedures.

**Recommendation 19:**
**The Pharmacopoeia Commission should review its procedures for the revision of monographs with a view to accelerating the incorporation of, and acceptance of, more humane testing methods, and the deletion of obsolete animal tests.**

### 5.2.2 Validation of more humane test methods

If serological or *in vitro* assays are to be used for routine batch potency determination they are validated in-house during vaccine development. Despite the varying level of guidance on acceptable methods given in the monographs, most manufacturers develop batch potency tests for at least some of their vaccines that meet regulatory approval whilst avoiding routine use of challenge assays. However, these test methods are rarely shared between companies or formally validated externally. Consequently, good practice is not disseminated as well as it could be.

This is unsurprising in light of the competitiveness of the animal health industry that makes manufacturers reluctant to share the output of costly development work. However, manufacturers should be encouraged to publish details of alternative testing methods that have gained regulatory approval, and to submit them for formal external validation, for example to ECVAM. Competent authorities are in a unique position to encourage sharing and formal method validation but a supporting statement in the monograph for Vaccines for Veterinary Use would lend weight to this.

Commercial confidentiality issues may make manufacturers unwilling to share novel test methods. However, more humane test methods are also developed in academic environments and research institutes where there is less reluctance to share, and researchers might be keen to see their methods validated and universally adopted. Unfortunately, the role of ECVAM is not widely known outside of the regulatory and manufacturing sectors. Consequently, researchers who have developed novel methods of routinely evaluating potency may be unaware that there is a process for submitting such methods for formal validation.

**Recommendation 20:**
**ECVAM should promote its role and the existence of its guidelines for submitting test methods for consideration for formal validation more widely within the research community.**

### 5.2.3 Formal validation of suggested alternative test methods

The monographs provide plenty of opportunities for manufacturers to avoid challenge assays for routine potency determination by suggesting alternative test methods. However, the only way to guarantee that challenge assays are only used to demonstrate efficacy during vaccine development, and not for routine batch potency, is to remove all references to assessing potency with challenge assays from the monograph. This approach has been already been adopted in a small number of monographs (e.g. inactivated feline infectious enteritis vaccine, Appendix 6), but needs to be extended across far more. This will only be possible if greater efforts are focussed on developing standardised serological potency tests that can be formally validated and then incorporated into monographs, not merely as a suggested approach, but as the standard test described for potency.

**Recommendation 21:**
**Once a serological test method has been formally validated and accepted for incorporation into a monograph, the potential to use challenge assays for routine**

**batch potency tests should be removed, as in the monograph for inactivated feline infectious enteritis vaccine**

### 5.2.4 Problems with validating a new method against a challenge assay

The over-arching monograph on Vaccines for Veterinary Use encourages the adoption of alternative test methods, but makes it clear that in order to gain regulatory approval they must be validated against the standard test described for potency, which is usually a challenge assay. Thus it states:

*" …alternative test methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animal use or reduction of suffering"*

*"With the agreement of the competent authority, certain of the batch tests may be omitted…… ()… where alternative tests validated with respect to the Pharmacopoeia method have been carried out"*

Establishing a correlation between different types of methods can be overwhelmingly difficult, particularly since challenge assays can be difficult to standardise, and reproducibility may be poor (e.g. Goris *et al*, 2007). This means that development of more reliable, reproducible and humane methods is hampered by the need for them to reflect results obtained from highly variable systems, influenced by a whole range of external factors. The mechanisms governing protection may also be poorly understood. Demonstration of equivalence of an alternative method may not only be problematic, but can also be of limited relevance (Castle, 2007). New validation strategies are therefore required in order to define those parameters most relevant to potency that can be used to demonstrate consistency.

This need to validate alternative test methods against the standard challenge-based potency test in the monograph can be a particular problem when developing alternative test methods for existing vaccine products. Not only can establishing a correlation be difficult, but also the validation process may use animal species in challenge studies that might not be required otherwise. This is because the general monograph on Vaccines for Veterinary Use states:

*"The acceptance criteria for the batch potency test are therefore established by correlation with the test described under Potency. Where a batch potency test is described in a monograph, this is given as an example of a test that is considered suitable, after establishment of correlation with the potency test; other test models can also be used."*

This wording suggests any alternative test would have to be validated against the test described under Potency. For example, the test for canine leptospira vaccines is a dog challenge assay (see Appendix 4). However, hamsters are routinely used to determine the potency of batches of existing vaccines. In accordance with the requirements of the general monograph above, manufacturers using the hamster assay will have already demonstrated a correlation between the dog and hamster test during vaccine development to gain regulatory approval. Unfortunately, these same requirements appear to exclude alternative test methods being validated against the hamster assay

that is already being performed routinely. This creates a requirement to perform a study that may cause substantial suffering to animals perceived as 'valuable' and ethically sensitive, in order to validate a more humane test method. This presents a dilemma that is difficult to resolve while the validation requirements remain as they are.

**Recommendation 22:**
**The European Pharmacopoeia Commission should devise a new strategy for validation that seeks to ensure batch-to-batch consistency of the most relevant parameters rather than relying principally on a correlation with an animal model.**

## 5.3 Harmonisation of Test Requirements

Animal health is a global industry and in order to be marketed on a world-wide basis a vaccine must meet the regulatory requirements of all regions in which it is to be marketed. Unfortunately the testing requirements for a particular vaccine may differ between regulatory regions.

An example is the potency test requirements for *Clostridium chauvoei* vaccines. This involves a challenge assay where at least five guinea pigs are used as unvaccinated controls. For release onto the US market, 80% of the control animals must die (USDA, 2005). However, for release in the EU, all control animals must die. If any control animals survive the test is repeated. Infection does not always result in 100% mortality, so repeat testing is often necessary. This means that more animals are used in tests of substantial severity to satisfy the requirements of EU regulators than are used to satisfy the needs of US regulators. In potency tests for other vaccines this pattern can be reversed. For example, the potency test for canine leptospira vaccines requires more hamsters to be infected to meet the requirements of US regulators than to meet the requirements of the European Pharmacopoeia.

Harmonisation of test requirements could eliminate the need to use several test methods in order to satisfy the regulatory requirements of all control agencies. This would drastically reduce the numbers of animals used to gain global marketing authorisations for veterinary vaccines.

The European Pharmacopoeia Commission works on harmonisation through the Pharmacopoeial Discussion Group (PDG) that also involves the Pharmacopoeias of Japan and the US. The PDG works to a full and varied programme, but unfortunately those monographs that result in the most animal suffering, and where there is the most need for harmonisation, have yet to receive attention.

**Recommendation 23:**
**The PDG should prioritise harmonisation of monographs that describe challenge assays that are used as routine batch potency tests. This would prevent unnecessary animal use and suffering, and would also permit the use of serological and *in vitro* methods of potency determination in all regulatory regions.**

# 6. Reducing animal use in other quality control tests

There are a number of opportunities to reduce the numbers of animals used in other tests conducted on vaccine batches for quality control purposes.

## 6.1 Reduction of animal use in batch safety tests

It is a requirement of every monograph for veterinary vaccines that safety tests are conducted on every batch of vaccine to ensure freedom from non-specific contamination. Generally, these are performed in the target animal. There is huge potential to reduce the number of animals used in this test by deleting the test altogether, by waiving the test for particular vaccine products and by addressing the inconsistencies between the monographs relating to the numbers of animals required for the test

Deletion of the safety test would have a massive impact on the numbers of animals used in quality control tests. There are good grounds for its deletion. The relevance of this test has been widely criticised as there is no evidence to suggest that it contributes to the safety of veterinary medicines. There have been multiple calls for its deletion in the past (Possnecker & Cussler, 1998; Possnecker, 1999; Bruckner *et al*., 2000; Advisory Group on Alternatives to Animal Testing in Immunogiologicals, 2002; Halder *et al*., 2004). Batch safety tests have the potential to cause considerable pain and discomfort to the animals that are used because of the large injection volumes they can involve. The volumes required frequently exceed the maximum recommended in established good practice guidelines, especially for batch safety tests of live vaccines where ten times the recommended dose volume is used (Cooper, in press). This provides additional grounds for discontinuing the test where possible.

The general monograph on Vaccines for Veterinary Use provides considerable scope for reduction by allowing the safety test to be waived for established vaccines under certain conditions:

*"…routine application of the safety test will be waived by the competent authority in the interests of animal welfare when a sufficient number of consecutive production batches have been produced and found to comply with the test, thus demonstrating consistency of the manufacturing process…. …The number of consecutive batches to be tested depends on a number of factors such as the type of vaccine, the frequency of production of batches and experience with the vaccine during development safety testing and during application of the batch safety test. Without prejudice to the decision of the competent authority in the light of information available for a given vaccine, testing of 10 consecutive batches is likely to be sufficient for most products..."*

Thus it is possible to obtain approval from the relevant regulatory authority to waive batch safety testing if consistency between vaccine batches can be demonstrated. Guidelines have been developed on the criteria required to enable these tests to be waived if authorisation has been obtained through the centralised procedure (EMEA, 2005). However, manufacturers wishing to discontinue routine batch safety testing may be discouraged or prevented from doing so for a variety of reasons. These include:

- The fees that must be paid to the relevant regulatory authorities in order to be granted authority to discontinue the test.

- Obtaining authority to discontinue the test is relatively straightforward if the product is only marketed in a single EU Member State, or has been registered by means of the centralised procedure. However, if the product is marketed in a number of EU states or third countries, lack of a co-ordinated approach between all relevant regulatory authorities can be a problem. In order to discontinue the test completely, a manufacturer may have to obtain authority from all relevant regulatory authorities. If the regulatory authority of only one State or country objected to removing the test, then the test would have to continue.

- Companies may not have the resources required to gather the data and submit the application to the relevant regulatory authority.

- Tests may be perceived as providing "back-up" to manufacturers in the event of a suspected adverse reaction occurring in the field, justifying adoption of the "it was fine when we tested it in the target species" approach. This is despite evidence that passing the target animal safety test does not guarantee a safe vaccine (e.g. Falcone *et al*., 1999).

Removal of one or more of the above obstacles would facilitate a reduction in the numbers of animals used.

The batch safety test is inconsistent with respect to the numbers of animals that are required for different vaccines and this is species dependent. Vaccines for mammalian species are tested for safety in two animals, while vaccines for birds or fish are tested in ten animals. There is no apparent scientific reason for this variation, and the number of animals used in safety testing would be reduced significantly if the number required for fish and avian vaccines was brought in line with that required for mammalian vaccines.

**Recommendation 24:**
**Competent authorities and authorities responsible for the regulation of animal experiments (e.g. the UK Home Office) should challenge manufacturers to provide compelling justification for why they continue to perform the batch target animal safety test.**

**Recommendation 25:**
**The EMEA should strive for a globally harmonised approach to waiving of the safety test through participation in the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products programme (VICH).**

**Recommendation 26:**
**The number of animals required for batch safety testing of bird and fish vaccines should be reduced to two, as is the requirement of batch safety tests of vaccines for other species.**

## 6.2 Potential for reducing animals use in other quality control tests

There is evidence that animals are sometimes used in excess of test requirements or where alternative methods could be used instead. For example approximately 10% of the animals used in quality control tests of veterinary vaccine batches in the UK during 2003 were in tests for toxoid contents (VMD, 2005). This test can be performed *in vitro* and so use of the animal test would only be justified if it were necessary to satisfy regulatory requirements for vaccine release outside of the EU. It is not clear if this was always the case during that period that data was collected.

The VMD also reported that toxicity tests are required by a small number of monographs, but are sometimes performed in excess of pharmacopoeial requirements (VMD, 2005). If such animal use is avoidable, it is contrary to the requirements of the ASPA and of Directive 86/609. In addition, in the monograph on Vaccines for Veterinary Use the Pharmacopoeia advocates implementation of the 3Rs and says that "*tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm*". Consequently, in instances where manufacturers propose animal tests which are either not essential for regulatory compliance, or if alternative methods are available, competent authorities have a responsibility to encourage the use of more humane approaches to ensure compliance with the Pharmacopoeia.

**Recommendation 27:**
**Within the UK, the Home Office and the VMD should collaborate more actively to ensure that animals are not used in excess of test requirements, taking this report as a starting point.**

# 7. Concluding comments

Veterinary vaccines protect millions of animals from disease, yet the quality control tests that are conducted on each batch for regulatory purposes can cause considerable animal suffering. As we approach the 50th anniversary of the conception of the 3Rs, the RSPCA believes that it is timely and relevant to address this dilemma and evaluate what more can be done to ensure that the principles of replacement, reduction and refinement are applied to their maximum potential in routine vaccine testing.

This report has shown that there is enormous potential for replacing or refining many of the tests that cause the most suffering, and that there is also scope for discontinuing some tests altogether. The report provides recommendations that are both practical and constructive in helping to attain these goals and the RSPCA would like to see the relevant stakeholders take these forward with the urgency that they deserve.

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## Table A. Batch potency test requirements for inactivated vaccines intended for use in avian species

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Avian Infectious Bronchitis** | Chickens | Chickens | No | Serology | Yes | Chickens |
| **Avian Infectious Bursal Disease** | Chickens | Chickens | No** | Serology | Yes | Chickens |
| **Avian Paramyxovirus 3** | Turkeys | Turkeys | Not stated | Suitable validated test | No | - |
| **Egg Drop Syndrome** | Chickens | Chickens | *"Not necessarily"* | Serology | Yes | Chickens |
| **Fowl Cholera** | Chickens, turkeys, ducks, geese | Target species | *"Not necessary"* | Serology | Yes | Chickens |
| ***Mycoplasma gallisepticum*** | Chickens, turkeys | Target species | *"Not necessary"* | Serology | Yes | Chickens |
| **Newcastle Disease** | Domestic fowl | Chickens | Potentially*** | Serology / challenge | Yes | Chickens |
| | Other bird species | Target species | No | Serology | Yes | Chickens |
| ***Salmonella enteritidis*** | Chickens | Chickens | *"Not necessary"* | Serology | Yes | Chickens |
| ***Salmonella typhimurium*** | Chickens | Chickens | *"Not necessary"* | Serology | Yes | Chickens |

* Monographs all require challenge tests to be performed in the target species during vaccine development to confirm efficacy. The test requirements are either described under Immunogenicity or Potency in the monograph.

** In this monograph a challenge assay is described under Immunogenicity and a serology assay described under Potency. This suggests that the serology assay is used for routine batch potency testing providing a correlation has been established during vaccine development.

*** A challenge assay is only performed in the event that a vaccine fails to comply with the serology assay or the results of the serology assay are invalid.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Avian Infectious Bronchitis** | No | - | - | - | Not applicable |
| **Infectious Bursal Disease** | No | - | - | - | Not applicable |
| **Avian Paramyxovirus 3** | Yes | Yes | No | Not applicable | Not applicable |
| **Egg Drop Syndrome** | Yes | No | - | - | Not applicable |
| **Fowl Cholera** | Yes | Yes | Yes | No | No |
| ***Mycoplasma gallisepticum*** | Yes | Yes | Yes | Yes | No |
| **Newcastle Disease** | Yes | Yes | Yes | Yes | No |
| ***Salmonella enteritidis*** | Yes | Yes | No | No | Not applicable |
| ***Salmonella typhimurium*** | Yes | No | - | Not applicable | Not applicable |

## Table B. Batch potency test requirements for inactivated clostridial vaccines

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| ***Clostridium botulinum*** | Includes sheep, cattle, horses, birds | Mice | Not stated | None | No | - |
| ***Clostridium chauvoei*** | Sheep, cattle | Guinea pigs | Not stated | None | No | - |
| ***Clostridium novyi* Type B** | Sheep, cattle, pigs | Mice** | *"Not necessarily"* | Serology | Yes | Rabbits |
| ***Clostridium perfringens*** | Sheep, cattle, pigs | Mice ** | *"Not necessarily"* | Serology | Yes | Rabbits |
| ***Clostridium septicum*** | Sheep, cattle, pigs | Mice ** | *"Not necessarily"* | Serology | Yes | Rabbits |
| ***Clostridium tetani*** | Sheep, cattle, pigs, horses, dogs, cats | None – Potency test is a serology assay | Not applicable | Serology | Yes | Guinea pigs |

* Monographs all require challenge tests to be performed in the target species during vaccine development to confirm efficacy. These test requirements are either described under Immunogenicity or Potency in the monograph. On occasion the monograph will describe a challenge test under Potency that involves a species other than the target. This test is performed in addition to the demonstration of efficacy in the target species.

** In potency tests for *Clostridium novyi* and *C.perfringens* vaccines both rabbits and mice are used. Rabbits are vaccinated to produce sera but mice undergo lethal tests that involve the administration of bacterial toxins in a toxin neutralisation assay.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| ***Clostridium botulinum*** | Yes | Yes | Yes | Yes | No |
| ***Clostridium chauvoei*** | Yes | Yes | Yes | Yes | Yes |
| ***Clostridium novyi* Type B** | Yes | Yes | Yes | Yes | No |
| ***Clostridium perfringens*** | Yes | Yes | Yes | Yes | No |
| ***Clostridium septicum*** | Yes | Yes | Yes | Yes | No |
| ***Clostridium tetani*** | No | - | - | - | - |

## Table C. Batch potency test requirements for inactivated vaccines intended for use in fish species

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Furunculosis for Salmonids** | Salmon & trout | Target species | Yes | Challenge or serology | Challenge – yes<br>Serology - yes | Fish |
| **Vibrosis for Salmonids – cold-water** | Salmon & trout | Target species | Yes | Challenge or serology | Challenge – yes<br>Serology - yes | Fish |
| **Vibrosis for Salmonids** | Salmon & trout | Target species | Yes | Challenge or serology | Challenge – yes<br>Serology - yes | Fish |

* Monographs all require challenge tests to be performed in the target species during vaccine development to confirm efficacy. These test requirements are either described under Immunogenicity or Potency in the monograph.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Furunculosis for Salmonids** | Yes | Yes | Yes | Yes | No |
| **Vibrosis for Salmonids – cold-water** | Yes | Yes | Yes | Yes | No |
| **Vibrosis for Salmonids** | Yes | Yes | Yes | Yes | No |

**Table D. Batch potency test requirements for inactivated vaccines intended for use in dogs and cats**

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Canine adenovirus** | Dogs | Dogs | No | *"suitable validated alternative test"* | No | - |
| **Canine leptospirosis** | Dogs | Dogs | No | Challenge | Yes | Hamsters |
| | | | | Serology | No | Not specified |
| | | | | *In-vitro* | No | - |
| **Canine parvovirus** | Dogs | Dogs | No** | Serology | Yes | Dogs or Guinea pigs |
| **Feline calicivirus** | Cats | Cats | No | Serology | Yes | Mice |
| **Feline chlamydiosis** | Cats | Cats | *"Not necessary"* | Serology | Yes | Cats |
| **Feline infectious enteritis** | Cats | None – Potency test is a serology assay | - | Serology | Yes | Cats or Guinea pigs |
| **Feline rhinotracheitis** | Cats | Cats | *"Not necessarily"* | Serology | Yes | Mice |
| **Feline leukaemia** | Cats | Cats | No | *"suitable validated alternative method"* | No | - |
| **Rabies** | Dogs, cats | Mice | *"Not necessarily"* | Serology | Yes | Mice |
| | | | | *In vitro* | Yes | - |

*The potency test described in the monograph involves a challenge assay in dogs. For a given vaccine it is carried out on one or more occasion. Thereafter, a challenge assay using hamsters may be used instead or alternatively one of the validated assays in Table 5.2 may be used.

** In this monograph a challenge assay is described under Immunogenicity and a serology assay described under Potency. This suggests that the serology assay is used for routine batch potency testing providing a correlation has been established during vaccine development.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Canine adenovirus** | No | - | - | - | - |
| **Canine leptospirosis** | Yes | Yes | Yes | Yes | No |
| **Canine parvovirus** | No | - | - | - | - |
| **Feline calicivirus** | No | - | - | - | - |
| **Feline chlamydiosis** | Yes | Yes | No | Not applicable | Not applicable |
| **Feline infectious enteritis** | No | - | - | - | - |
| **Feline rhinotracheitis** | Yes | Yes | Yes | No | No |
| **Feline leukaemia** | No | - | - | - | - |
| **Rabies** | Yes | Yes | Yes | Yes | No |

**Table E. Batch potency test requirements for inactivated vaccines intended primarily for use in ruminants**

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Bovine Viral Diarrhoea** | Cattle | Cattle | No | Serology | Yes | Calves or laboratory animals |
| **Calf Coronovirus Diarrhoea** | Cattle | Cattle | No | Serology | Yes | Not specified |
| **Calf Rotavirus Diarrhoea** | Cattle | Cattle | No | Serology | Yes | Not specified |
| **Bovine Leptospirosis** | Cattle | Cattle | No | Serology | Yes | Guinea pigs |
| **Foot and Mouth Disease** | Cattle, sheep, pigs | Cattle | *"Not necessarily"* | Serology | Yes | Cattle |
| **Louping Ill**** | Sheep | Sheep | No** | Serology | Yes | Sheep |
| **Mannheimia for Cattle** | Cattle | Cattle | No | *"suitable validated test"* | No | - |
| **Mannheimia for Sheep** | Sheep | Sheep | No | *"suitable validated batch potency test"* | No | - |
| **Ovine Enzootic Abortion**** | Sheep | Sheep | No** | Serology | Yes | Sheep |
| **Pasteurella for Sheep** | Sheep | Sheep | No | *"suitable validated batch potency test"* | No | - |
| **Ruminant *E.coli*** | Ruminants | Sheep | No | Serology | Yes | *e.g. "rabbits, guinea-pigs, rats or mice"* |

** Not a monograph of the European Pharmacopoeia.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Bovine Viral Diarrhoea** | No | - | - | - | - |
| **Calf Coronovirus Diarrhoea** | No | - | - | - | - |
| **Calf Rotavirus Diarrhoea** | No | - | - | - | - |
| **Bovine Leptospirosis** | No | - | - | - | - |
| **Foot and Mouth Disease** | Yes | Yes | No | No | Not applicable |
| **Louping Ill*** | No | - | - | - | - |
| **Mannheimia for Cattle** | No | - | - | - | - |
| **Mannheimia for Sheep** | No | - | - | - | - |
| **Ovine Enzootic Abortion*** | No | - | - | - | - |
| **Pasteurella for Sheep** | No | - | - | - | - |
| **Ruminant *E.coli*** | No | - | - | - | - |

## Table F. Batch potency test requirements for inactivated vaccines intended for use in pigs

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Aujeskys' Disease** | Pigs | Pigs | *"Not necessarily"* | "suitable validated, alternative test" | No | - |
| **Porcine Actinobacillosis** | Pigs | Pigs | No | Serology | Yes | Mice |
| **Porcine *E.coli*** | Pigs | Pigs | No | Serology | Yes | Pigs or laboratory animals ** |
| **Porcine Parvovirus** | Pig | Pigs | No | Serology | Yes | Guinea pigs |
| **Porcine Progressive Atrophic Rhinitis** | Pigs | Pigs | No | Serology | Yes | Pigs or "susceptible laboratory animals" |
| **Swine Influenza** | Pig | Pigs | No | Serology | Yes | Guinea pigs |
| **Swine Erysipelas** | Pigs | Pigs | No | Serology | Yes | Mice |

** The monograph states *"if the nature of the antigens allows reproducible results to be obtained, a test in laboratory animals (for example, guinea-pigs, mice, rabbits or rats) may be carried out"*.

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Aujeskys' Disease** | Yes | Yes | Yes | No | No |
| **Porcine Actinobacillosis** | No | - | - | - | - |
| **Porcine *E.coli*** | No | - | - | - | - |
| **Porcine Parvovirus** | No | - | - | - | - |
| **Porcine Progressive Atrophic Rhinitis** | No | - | - | - | - |
| **Swine Influenza** | No | - | - | - | - |
| **Swine Erysipelas** | No | - | - | - | - |

## Table G. Batch potency test requirements for inactivated vaccines intended for use in horses

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Equine Herpesvirus** | Horses | Horses | No | Serology | Yes | Rabbits, guinea-pigs or mice |
| **Equine Influenza** | Horses | Horses | No | Serology | Yes | Guinea-pigs |

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Equine Herpesvirus** | No | - | - | - | - |
| **Equine Influenza** | No | - | - | - | - |

## Table H. Batch potency test requirements for inactivated vaccines intended for use in rabbits

| Vaccine | Target species* | Species used in challenge-based Potency tests for vaccine development* | According to the monograph, is the challenge-based Potency test routinely used for batch potency? | Monograph's suggested approach to batch potency testing | Is an example batch potency test described? | Species in example batch potency test |
|---|---|---|---|---|---|---|
| **Rabbit haemorrhagic disease** | Rabbits | Rabbits | *"Not necessary"* | Serology | Yes | Rabbits |

| Vaccine | Potential for challenge assay to be used for routine batch testing? | Potential for infection to produce marked clinical signs of disease? | Potential for infection to result in mortality? | Test requires death or severe clinical signs or euthanasia on humane grounds | Humane endpoints mentioned? |
|---|---|---|---|---|---|
| **Rabbit haemorrhagic disease** | Yes | Yes | Yes | Yes | No |

## APPENDIX 2 – Batch potency tests for inactivated vaccines – animal numbers

**Minimum numbers of animals required to satisfy test requirements (where this information is provided in the monographs).**

**Challenge assays**

### Tests using avian species

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Avian Paramyxovirus 3 | 20 | 20 | 40 |
| Egg Drop Syndrome | 60 | 40 | 100 |
| Fowl Cholera | 20 | 10 | 30 |
| *Mycoplasma gallisepticum* | 20 | 20 | 40 |
| Newcastle Disease (chickens) | 60 | 10 | 70 |
| *Salmonella enteritidis* | 30 | 30 | 60 |
| *Salmonella typhimurium* | 30 | 30 | 60 |
| **Mean** | **34.3** | **22.9** | **57.1** |

### Tests using cats

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Feline chlamydiosis | 10 | 10 | 20 |
| Feline rhinotracheitis | 10 | 10 | 20 |
| **Mean** | **10** | **10** | **20** |

### Tests using cattle

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Foot and Mouth Disease | 15 | 2 | 17 |
| **Mean** | **15** | **2** | **17** |

### Tests using fish

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Furunculosis for Salmonids | 30 | 30 | 60 |
| Vibrosis for Salmonids | 30 | 30 | 60 |
| Vibrosis for Salmonids – cold-water | 30 | 30 | 60 |
| **Mean** | **30** | **30** | **60** |

### Tests using guinea pigs

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| *Clostridium chauvoei* | 10 | 5 | 15 |
| **Mean** | **10** | **5** | **15** |

### Tests using hamsters

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Canine Leptospirosis | 5 | 5 | 10 |
| **Mean** | **5** | **5** | **10** |

### Tests using mice (includes toxin neutralisation assays)

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| *Clostridium botulinum* | 20 | 10 | 30 |
| *Clostridium novyi* Type B* | Not possible to determine from the monographs | | |
| *Clostridium perfringens** | | | |
| *Clostridium septicum** | | | |
| Rabies | 60 | 40 | 100 |
| **Mean** | **40** | **25** | **65** |

* The standard test for potency for these vaccines is a toxin neutralisation assay. The monograph does not specify how many mice should be used in each but typically large numbers are required to satisfy test requirements

### Tests using pigs

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Aujeskys' Disease | 5 | 5 | 10 |
| **Mean** | **5** | **5** | **10** |

### Tests using rabbits

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Rabbit haemorrhagic disease | 10 | 5 | 15 |
| **Mean** | **10** | **5** | **15** |

## Serology assays

### Tests using avian species

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Avian Infectious Bronchitis | 10 | 5 | 15 |
| Egg Drop Syndrome | 10 | 5 | 15 |
| Fowl Cholera | 10 | 5 | 15 |
| Infectious Bursal Disease | 10 | 10 | 20 |
| *Mycoplasma gallisepticum* | 10 | 5 | 15 |
| Newcastle Disease | 10 | 5 | 15 |
| *Salmonella enteritidis* | 10 | 5 | 15 |
| *Salmonella typhimurium* | 10 | 5 | 15 |
| **Mean** | **10** | **5.6** | **15.6** |

### Tests using cats

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Feline chlamydiosis | 5 | 0 | 5 |
| Feline infectious enteritis | 2 | 0 | 2 |
| **Mean** | **3.5** | **0** | **3.5** |

### Tests using dogs

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Canine Parvovirus | 2 | 0 | 2 |
| **Mean** | **2** | **0** | **2** |

### Tests using fish

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Furunculosis for Salmonids | 25 | 10 | 35 |
| Vibrosis for Salmonids | 25 | 10 | 35 |
| Vibrosis for Salmonids – cold-water | 25 | 10 | 35 |
| **Mean** | **25** | **10** | **35** |

### Tests using guinea pigs

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Bovine Leptospirosis | 10 | 2 | 12 |
| Canine Parvovirus | 5 | 0 | 5 |
| *Clostridium tetani* | 5 | 0 | 5 |
| Equine Herpesvirus | 5 | 0 | 5 |
| Equine Influenza | 5 | 0 | 5 |
| Feline Infectious Enteritis | Not stated | Not stated | Not stated |
| Ruminant *E.coli* | 5 | 2 | 7 |
| Porcine Parvovirus | 5 | 0 | 5 |
| Swine Influenza | 5 | 0 | 5 |
| **Mean** | **5.6** | **0.5** | **6.1** |

### Tests using mice

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Equine Herpesvirus | 5 | 0 | 5 |
| Feline Rhinotracheitis | 15 | 0 | 15 |
| Feline Calicivirus | 15 | 0 | 15 |
| Porcine Actinobacillosis | 5 | 0 | 5 |
| Rabies | 5 | 0 | 5 |
| Ruminant *E.coli* | 5 | 2 | 7 |
| Swine Erysipelas | 10 | 0 | 10 |
| **Mean** | **8.6** | **0.3** | **8.9** |

### Tests using pigs

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Porcine *E.coli* | 5 | 2 | 7 |
| Porcine Progressive Atrophic Rhinitis | 5 | 2 | 7 |
| **Mean** | **5** | **2** | **7** |

### Tests using rabbits

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| *Clostridium novyi* Type B | 10 | 0 | 10 |
| *Clostridium perfringens* | 10 | 0 | 10 |
| *Clostridium septicum* | 10 | 0 | 10 |
| *Clostridium tetani* | 5 | 0 | 5 |
| Equine Herpesvirus | 5 | 0 | 5 |
| Rabbit haemorrhagic disease | 5 | 2 | 7 |
| Ruminant *E.coli* | 5 | 2 | 7 |
| **Mean** | **7.1** | **0.6** | **7.7** |

### Tests using rats

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Ruminant *E.coli* | 5 | 2 | 7 |
| **Mean** | **5** | **2** | **7** |

### Tests using ruminant species

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Bovine Viral Diarrhoea | 5 | 2 | 7 |
| Foot and Mouth Disease | 5 | 0 | 5 |
| Louping Ill* | 6 | 0 | 6 |
| Ovine Enzootic Abortion* | 5 | 2 | 7 |
| **Mean** | **5.3** | **1** | **6.3** |

### Tests using undefined laboratory animals

| Vaccine | Minimum number of vaccinated animals | Minimum number of unvaccinated controls | Minimum number required in total |
|---|---|---|---|
| Bovine Viral Diarrhoea | 5 | 2 | 7 |
| Calf Coronovirus Diarrhoea | 5 | 2 | 7 |
| Calf Rotavirus Diarrhoea | 5 | 2 | 7 |
| Canine Leptospira | Not stated | Not stated | Not stated |
| Porcine *E.coli* | 5 | 2 | 7 |
| Porcine Progressive Atrophic Rhinitis | 5 | 2 | 7 |
| **Mean** | **5** | **2** | **7** |

## APPENDIX 3 – Monograph for Canine Adenovirus Vaccine, Inactivated

# DEFINITION

Canine adenovirus vaccine (inactivated) is a suspension of one or more suitable strains of canine adenovirus 1 (canine contagious hepatitis virus) and/or canine adenovirus 2, inactivated in such a way that adequate immunogenicity is maintained.

# PRODUCTION

The test for inactivation is carried out using a quantity of virus equivalent to at least 10 doses of vaccine with 2 passages in cell cultures of the same type as those used for production or in cell cultures shown to be at least as sensitive. No live virus is detected.

The vaccine may contain an adjuvant.

### CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety *(5.2.6)* and efficacy *(5.2.7)*. The following tests may be used during demonstration of safety and immunogenicity.

*Safety* Carry out the test for each recommended route of administration in animals of the minimum age recommended for vaccination. Use a batch of vaccine of the maximum potency likely to be attained.

Use for each test not fewer than 10 dogs that do not have antibodies against canine adenovirus 1 or 2. Administer to each dog a double dose of vaccine. If the recommended schedule requires a second dose, administer one dose after the recommended interval. Observe the dogs for 14 days after the last administration. No abnormal local or systemic reaction occurs.

If the vaccine is intended for use in pregnant bitches, vaccinate bitches at the stage of pregnancy or at different stages of pregnancy according to the recommended schedule. Prolong observation until 1 day after parturition. No abnormal local or systemic reaction occurs. No adverse effects on the pregnancy and offspring are noted.

*Immunogenicity* For vaccines intended to protect against hepatitis, the test described under Potency is suitable for demonstration of immunogenicity. If the vaccine is indicated for protection against respiratory signs, a further test to demonstrate immunogenicity for this indication is also necessary.

### BATCH TESTING

## Batch potency test

The test described under Potency is not carried out for routine testing of batches of vaccine. It is carried out for a given vaccine on one or more occasions as decided by or with the agreement of the competent authority. Where the test is not carried out, a suitable validated alternative test is carried out, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

# IDENTIFICATION

When injected into susceptible animals, the vaccine stimulates the formation of specific antibodies against the type or types of canine adenovirus stated on the label.

# TESTS

## Safety

Use dogs of the minimum age recommended for vaccination and preferably having no canine adenovirus-neutralising antibodies or, where justified, use dogs with a low level of such antibodies as long as they have not been vaccinated against canine adenovirus and administration of the vaccine does not cause an anamnestic response. Administer a double dose of vaccine by a recommended route to each of 2 dogs. Observe the dogs for 14 days. No abnormal local or systemic reaction occurs.

## Inactivation

Carry out a test for residual infectious canine adenovirus using 10 doses of vaccine by inoculation into sensitive cell cultures; make a passage after 6-8 days and maintain the cultures for 14 days. No live virus is detected. If the vaccine contains an adjuvant, separate the adjuvant from the liquid phase by a method that does not inactivate or otherwise interfere with the detection of live virus.

## Sterility

The vaccine complies with the test for sterility prescribed in the monograph on *Vaccines for veterinary use (0062)*.

# POTENCY

Use 7 dogs of the minimum age recommended for vaccination and that do not have antibodies against canine adenovirus. Vaccinate 5 of the animals by a recommended route and according to the recommended schedule. Keep the other 2 dogs as controls. 21 days later inject intravenously into each of the 7 animals a quantity of a virulent strain of canine adenovirus sufficient to cause death or typical signs of the disease in a susceptible dog. Observe the animals for a further 21 days. Dogs displaying typical signs of serious infection with canine adenovirus are killed humanely to avoid unnecessary suffering. The test is invalid and must be repeated if one or both of the controls do not die from or display typical signs of serious infection with canine adenovirus. The vaccine complies with the test if the vaccinated animals remain in good health.

## APPENDIX 4 – Monograph for Canine Leptospirosis Vaccine, Inactivated

# DEFINITION

Canine leptospirosis vaccine (inactivated) is a suspension of inactivated whole organisms and/or antigenic extract(s) of one or more suitable strains of one or more of *Leptospira interrogans* serovar canicola, serovar icterohaemorrhagiae or any other epidemiologically appropriate serovar, inactivated and prepared in such a way that adequate immunogenicity is maintained. This monograph applies to vaccines intended for active immunisation of dogs against leptospirosis.

# PRODUCTION

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial harvests are collected separately and inactivated by a suitable method. The antigen may be concentrated. The vaccine may contain an adjuvant.

### CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety *(5.2.6)* and efficacy *(5.2.7)* in dogs. As part of the studies to demonstrate the suitability of the vaccine with respect to these characteristics the following tests may be carried out.

## Safety

The test is carried out for each route of administration to be stated on the label and in animals of each category for which the vaccine is intended. For each test, use not fewer than 10 dogs that do not have antibodies against the principal *L. interrogans* serovars (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Use a batch of vaccine containing not less than the maximum antigen content and/or potency that may be expected in a batch of vaccine. Administer to each animal a double dose of vaccine. If the recommended schedule requires a second dose, administer 1 dose after the recommended interval. Observe the animals for at least 14 days after the last administration. Record body temperatures the day before each vaccination, at vaccination, 4 h later and daily for 4 days. If the vaccine is intended for use or may be used in pregnant bitches, vaccinate the animals at the recommended stage of pregnancy or at a range of stages of pregnancy and prolong the observation period until 1 day after whelping. The vaccine complies with the test if no animal shows an abnormal local or systemic reaction or clinical signs of disease or dies from a cause attributable to the vaccine. In addition, if the vaccine is for use in pregnant animals, no adverse effects on the pregnancy and offspring are noted.

## Immunogenicity

As part of the studies to demonstrate the suitability of the vaccine with respect to immunogenicity and compliance with the claims to be stated on the label, the test described under Potency may be carried out for each proposed route of administration and using vaccine of minimum antigen content and/or potency.

### BATCH POTENCY TEST

The test described under Potency is not carried out for routine testing of batches of vaccine. It is carried out, for a given vaccine, on one or more occasions, as decided by or with the agreement of the competent authority. Where the test is not carried out, one of the following tests may be used.

A. For vaccines with or without adjuvants

If leptospira from more than 1 serovar (for example *L. interrogans* serovar canicola and serovar icterohaemorrhagiae) has been used to prepare the vaccine, carry out a batch potency test for each serovar against which protective immunity is claimed on the label. Inject 1/40 of the dose for dogs stated on the label subcutaneously into each of 5 healthy hamsters not more than 3 months old, which do not have antibodies to the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis) and which have been obtained from a regularly tested and certified leptospira-free source. After 15-20 days, inoculate intraperitoneally into each of the vaccinated animals and into an equal number of non-vaccinated controls derived from the same certified leptospira-free source, a suitable quantity of a virulent culture of leptospirae of the serovar against which protective immunity is claimed on the label. The vaccine complies with the test if not fewer than 4 of the 5 control animals die showing typical signs of leptospira infection within 14 days of receiving the challenge suspension and if not fewer than 4 of the 5 vaccinated animals remain in good health for 14 days after the death of 4 control animals.

B. For vaccines with or without adjuvants

A suitable validated sero-response test may be carried out. Vaccinate each animal in a group of experimental animals with a suitable dose. Collect blood samples after a suitable, fixed time after vaccination. For each of the serovars present in the vaccine, an *in vitro* test is carried out on individual blood samples to determine the antibody response to one or more antigenic components which are indicators of protection and which are specific for that serovar. The criteria for acceptance are set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

C. For vaccines without adjuvants

For each of the serovars present in the vaccine, a suitable validated *in vitro* test may be carried out to determine the content of one or more antigenic components which are indicators of protection and which are specific for that serovar. The criteria for acceptance are set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

# IDENTIFICATION

When injected into healthy seronegative animals, the vaccine stimulates the production of specific antibodies to the leptospira serovar(s) present in the vaccine. If test C is used for batch potency test, it also serves to identify the vaccine.

# TESTS

## Safety

Use 2 dogs of the minimum age recommended for vaccination and which do not have antibodies to the leptospira serovar(s) present in the vaccine. Administer 2 doses of the vaccine to each dog

by a recommended route. Observe the animals for 14 days. The animals remain in good health and no abnormal local or systemic reaction occurs.

## Inactivation

Carry out a test for live leptospirae by inoculation of a specific medium. Inoculate 1 ml of the vaccine into 100 ml of the medium. Incubate at 30 °C for 14 days, subculture into a further quantity of the medium and incubate both media at 30 °C for 14 days: no growth occurs in either medium. At the same time, carry out a control test by inoculating a further quantity of the medium with the vaccine together with a quantity of a culture containing approximately 100 leptospirae and incubating at 30 °C: growth of leptospirae occurs within 14 days.

## Sterility

The vaccine complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

# POTENCY

For each type of the serovars against which protective immunity is claimed on the label, carry out a separate test with a challenge strain representative of that serovar.

Use not fewer than 12 dogs of the minimum age recommended for vaccination and free from specific antibodies against the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Vaccinate half of the animals by a recommended route and according to the recommended schedule. Keep the remaining animals as controls. 25-28 days after the last vaccination, infect all the animals by the conjunctival and/or intraperitoneal route with a suitable quantity of a virulent strain of the relevant *L. interrogans* serovar. Observe the animals for a further 28 days. Examine the dogs daily and record and score clinical signs observed post-challenge and any deaths that occur. If an animal shows marked signs of disease, it is killed. Monitor body temperatures each day for the first week after challenge. Collect blood samples from each animal on days 0, 2, 3, 4, 5, 8 and 11 post challenge. Collect urine samples from each animal on days 0, 3, 5, 8, 11, 14, 21 and 28 post challenge. Kill surviving animals at the end of the observation period. Carry out post-mortem examination on any animal that dies during the observation period and on the remainder when killed at the end of the observation period. In particular, examine the liver and kidneys for macroscopic and microscopic signs of leptospira infection. A sample of each kidney is collected and each blood, urine and kidney sample is tested for the presence of challenge organisms by re-isolation or by another suitable method. The blood samples are also analysed to detect biochemical and haematological changes indicative of infection and these are also scored.

The test is invalid if: samples give positive results on day 0; *L. interrogans* serovar challenge strain is re-isolated from or demonstrated by another suitable method to be present in fewer than 2 samples on fewer than 2 different days, to show infection has been established in fewer than 80 per cent of the control animals.

The vaccine complies with the test if: at least 80 per cent of the vaccinates show no more than mild signs of disease (for example, transient hyperthermia) and, depending on the *L interrogans* serovar used for the challenge, one or more of the following is also shown:

—where the vaccine is intended to have a beneficial effect against clinical signs, the clinical scores and haematological and biochemical scores are statistically lower for the vaccinates than for the controls,

—where the vaccine is intended to have a beneficial effect against infection, the number of days that the organisms are detected in the blood is statistically lower for the vaccinates than for the controls,
—where the vaccine is intended to have a beneficial effect against urinary tract infection and excretion, the number of days that the organisms are detected in the urine and the number of kidney samples in which the organisms are detected is statistically lower for the vaccinates than for the controls.

## APPENDIX 5 – Monograph for Porcine Actinobacillosis Vaccine, Inactivated

# DEFINITION

Porcine actinobacillosis vaccine (inactivated) is a liquid preparation which has one or more of the following components: inactivated *Actinobacillus pleuropneumoniae* of a suitable strain or strains; toxins, proteins or polysaccharides derived from suitable strains of *A. pleuropneumoniae*, and treated to render them harmless; fractions of toxins derived from suitable strains of *A. pleuropneumoniae* and treated if necessary to render them harmless. This monograph applies to vaccines intended for protection of pigs against actinobacillosis.

# PRODUCTION

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate, protein content and quantity of relevant antigens are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial suspensions are collected separately and inactivated by a suitable method. They may be detoxified, purified and concentrated. The vaccine may contain an adjuvant.

### CHOICE OF VACCINE COMPOSITION

The choice of strains is based on epidemiological data. The vaccine is shown to be satisfactory with respect to safety *(5.2.6)* and efficacy *(5.2.7)* in pigs. The following tests may be used during demonstration of safety and immunogenicity.

*Safety*

A. Carry out a test in each category of animals for which the vaccine is intended and by each of the recommended routes of administration. Use animals that do not have antibodies against the serotypes of *A. pleuropneumoniae* or its toxins present in the vaccine. Administer a double dose of vaccine by a recommended route to each of not fewer than 10 animals. Administer a single dose of vaccine to each of the animals after the interval recommended in the instructions for use. Observe the animals for 14 days after vaccination. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 4 days; note the maximum temperature increase for each animal. No abnormal local or systemic reaction occurs; the average temperature increase for all animals does not exceed 1.5 °C and no animal shows a rise greater than 2 °C. If the vaccine is intended for use in pregnant sows, for the test in this category of animals, prolong the observation period up to farrowing and note any effects on gestation or the offspring.

B. The animals used for field trials are also used to evaluate safety. Carry out a test in each category of animals for which the vaccine is intended. Use not fewer than 3 groups each of not fewer than 20 animals with corresponding groups of not fewer than 10 controls. Examine the injection site for local reactions after vaccination. Record body temperature the day before vaccination, at vaccination, at the time interval after which a rise in temperature, if any, was seen in test A, and daily during the 2 days following vaccination; note the maximum temperature increase for each animal. No abnormal local or systemic reaction occurs; the average temperature increase for all animals does not exceed 1.5 °C and no animal shows a rise greater than 2 °C.

*Immunogenicity* The test described under Potency may be used to demonstrate the immunogenicity of the vaccine.

### BATCH TESTING

## Batch potency test

The test described under Potency is not carried out for routine testing of batches of vaccine. It is carried out, for a given vaccine, on one or more occasions, as decided by or with the agreement of the competent authority; where the test is not carried out, a suitable validated test is carried out, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following test may be used after a satisfactory correlation with the test described under Potency has been established.

Inject a suitable dose subcutaneously into each of 5 seronegative mice, weighing 18-20 g. Where the schedule stated on the label requires a booster injection to be given, a booster vaccination may also be given in this test provided it has been demonstrated that this will still provide a suitably sensitive test system. Before the vaccination and at a given interval within the range of 14-21 days after the last injection, collect blood from each animal and prepare serum samples. Determine individually for each serum the titre of specific antibodies against each antigenic component stated on the label, using a suitable validated test such as enzyme-linked immunosorbent assay *(2.7.1)*. The vaccine complies with the test if the antibody levels are not significantly lower than those obtained for a batch that has given satisfactory results in the test described under Potency.

## Bacterial endotoxins

A test for bacterial endotoxins *(2.6.14)* is carried out on the final bulk or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in safety test A described under Choice of vaccine composition or the safety test described under Tests, carried out using 10 pigs. Where the latter test is used, note the maximum temperature increase for each animal; the average temperature increase for all animals does not exceed 1.5 °C. The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch used in the safety test for determining the maximum acceptable level of endotoxin is used subsequently for batch testing.

# IDENTIFICATION

When injected into healthy seronegative animals, the vaccine stimulates the production of specific antibodies against the antigenic components of *A. pleuropneumoniae* stated on the label.

# TESTS

## Safety

Use 2 pigs of the minimum age stated for vaccination and which do not have antibodies against the serotypes of *A. pleuropneumoniae* or its toxins present in the vaccine. Administer to each pig a double dose of vaccine by a recommended route. Observe the animals for 14 days. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily

for 2 days. No abnormal local or systemic reaction occurs; a transient temperature increase not exceeding 2 °C may occur.

## Sterility

The vaccine complies with the test for sterility prescribed in the monograph on *Vaccines for veterinary use (0062)*.

# POTENCY

*The challenge strain for the potency test is chosen to ensure challenge with each Ap toxin*[1]*produced by the serotypes stated on the label; it may be necessary to carry out more than one test using a different challenge strain for each test.*

Vaccinate according to the recommended schedule not fewer than 7 pigs, of the minimum age recommended for vaccination, which do not have antibodies against *A. pleuropneumoniae* and Ap toxins. Keep not fewer than 7 unvaccinated pigs of the same age as controls. 3 weeks after the last vaccination, challenge all the pigs intranasally or intratracheally or by aerosol with a suitable quantity of a serotype of *A. pleuropneumoniae*. Observe the animals for 7 days; to avoid unnecessary suffering, severely ill control animals are killed and are then considered to have died from the disease. Kill all surviving animals at the end of the observation period. Carry out a post-mortem examination on all animals. Examine the lungs, the tracheobronchial lymph nodes and the tonsils for the presence of *A. pleuropneumoniae*. Evaluate the extent of lung lesions at post-mortem examination. Each of the 7 lobes of the lungs is allotted a maximum possible lesion score[2] of 5. The area showing pneumonia and/or pleuritis of each lobe is assessed and expressed on a scale of 0 to 5 to give the pneumonic score per lobe (the maximum total score possible for each complete lung is 35). Calculate separately for the vaccinated and the control animals the total score (the maximum score per group is 245, if 7 pigs are used per group).

The vaccine complies with the test if the vaccinated animals, when compared with controls, show lower incidence of: mortality; typical clinical signs (dyspnoea, coughing and vomiting); typical lung lesions; re-isolation of *A. pleuropneumoniae* from the lungs, the tracheobronchial lymph nodes and the tonsils. Where possible, the incidence is analysed statistically and shown to be significantly lower for vaccinates.

[2] The system of lung scores is described in detail by P.C.T. Hannan, B.S. Bhogal, J.P. Fish, *Research in Veterinary Science*, 1982, 33, 76-88.

## APPENDIX 6 – Monograph for Feline Infectious Enteritis Vaccine, Inactivated

# DEFINITION

Feline infectious enteritis (feline panleucopenia) vaccine (inactivated) is a liquid or freeze-dried preparation of feline panleucopenia virus or canine parvovirus inactivated by a suitable method.

# PRODUCTION

The virus is propagated in suitable cell cultures *(5.2.4)*. The virus is harvested and may be purified and concentrated.

The test for inactivation is carried out using a quantity of inactivated virus equivalent to not less than 100 doses of the vaccine by a validated method such as the following: inoculate into suitable non-confluent cells and after incubation for 8 days, make a subculture using trypsinised cells. After incubation for a further 8 days, examine the cultures for residual live parvovirus by an immunofluorescence test. The immunofluorescence test may be supplemented by a haemagglutination test or other suitable tests on the supernatant of the cell cultures. No live virus is detected.

The vaccine may contain an adjuvant and may be freeze-dried.

### CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety *(5.2.6)* and efficacy *(5.2.7)* in cats. The following test may be used during demonstration of immunogenicity.

*Immunogenicity* Use 10 susceptible cats, 8 to 12 weeks old. Draw a blood sample from each cat and test individually for antibodies against feline panleucopenia virus and canine parvovirus to determine susceptibility. Vaccinate 5 cats by the recommended schedule. Carry out leucocyte counts 8 days and 4 days before challenge and calculate the mean of the 2 counts to serve as the initial value. 20 to 22 days after the last vaccination, challenge each cat by the intraperitoneal injection of a suspension of pathogenic feline panleucopenia virus. Observe the cats for 14 days. Carry out leucocyte counts on the fourth, sixth, eighth and tenth days after challenge. The test is not valid unless the 5 control cats all show on not fewer than one occasion a diminution in the number of leucocytes of at least 75 per cent of the initial value; these animals may die from panleucopenia. The vaccine complies with the test if the 5 vaccinated cats remain in excellent health and show no sign of leucopenia; that is to say, the diminution in the number of leucocytes does not exceed, in any of the four counts, 50 per cent of the initial value.

### BATCH TESTING

## Batch potency test

For routine testing of batches of vaccine a test based on production of haemagglutination-inhibiting antibodies in guinea-pigs may be used instead of test A or B described under Potency if a satisfactory correlation with the test for immunogenicity has been established.

# IDENTIFICATION

When injected into animals, the vaccine stimulates the production of antibodies against the parvovirus present in the vaccine.

# TESTS

## Safety

Use cats of the minimum age recommended for vaccination and preferably having no antibodies against feline panleucopenia virus or against canine parvovirus or, where justified, use cats with a low level of such antibodies as long as they have not been vaccinated against feline panleucopenia virus or against canine parvovirus, and administration of the vaccine does not cause an anamnestic response. Administer by a recommended route a double dose of vaccine to each of 2 cats. Observe the animals for 14 days. No abnormal local or systemic reaction occurs.

## Sterility

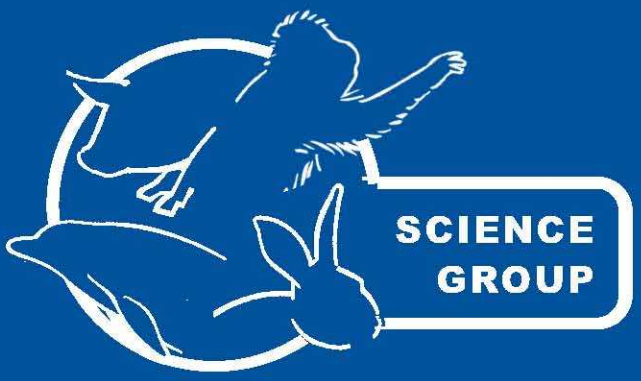
The vaccine complies with the test for sterility prescribed in the monograph on *Vaccines for veterinary use (0062)*.

# POTENCY

*Carry out test A or test B.*

A. Use 4 cats, 8 to 12 weeks old. Draw a blood sample from each cat and test individually for antibodies against feline panleucopenia virus and canine parvovirus to determine susceptibility. Inject by a recommended route one dose of vaccine into each of 2 cats. After 21 days, draw a blood sample from each cat and separate the serum from each sample. Inactivate each serum by heating at 56 °C for 30 min. To 1 volume of each serum add 9 volumes of a 200 g/l suspension of *light kaolin R* in *phosphate buffered saline pH 7.4 R*. Shake each mixture for 20 min. Centrifuge, collect the supernatant liquid and mix with 1 volume of a concentrated suspension of pig erythrocytes. Allow to stand at 4 °C for 60 min and centrifuge. The dilution of the serum obtained is 1:10. Using each serum, prepare a series of twofold dilutions. To 0.025 ml of each of the latter dilutions add 0.025 ml of a suspension of canine parvovirus or feline panleucopenia virus antigen containing 4 haemagglutinating units. Allow to stand at 37 °C for 30 min and add 0.05 ml of a suspension of pig erythrocytes containing $30 \times 10^6$ cells per millilitre. Allow to stand at 4 °C for 90 min and note the last dilution of serum that still completely inhibits haemagglutination. The vaccine complies with the test if both vaccinated cats have developed titres of at least 1:20. The test is not valid if either control cat develops antibodies against canine parvovirus or feline panleucopenia virus.

B. Vaccinate according to the recommended schedule, 2 cats, 8 to 12 weeks old and having antibody titres less than 4 $ND_{50}$ (neutralising dose 50 per cent) per 0.1 ml of serum measured by the method described below. 14 days after vaccination, examine the serum of each animal as follows. Heat the serum at 56 °C for 30 min and prepare serial dilutions using a medium suitable for feline cells. Add to each dilution an equal volume of a virus suspension containing an amount of virus such that when the volume of serum-virus mixture appropriate for the assay system is inoculated into cell cultures, each culture receives approximately $10^4$ $CCID_{50}$. Incubate the mixtures at 37 °C for 1 h and inoculate four feline cell cultures with a suitable volume of each mixture. Incubate the cell cultures at 37 °C for 7 days, passage and incubate for a further 7 days. Examine the cultures for evidence of specific cytopathic effects and calculate the antibody titre. The vaccine complies with the test if the mean titre is not less than 32 $ND_{50}$ per 0.1 ml of serum. If one cat fails to respond, repeat the test using 2 more cats and calculate the result as the mean of the titres obtained from all of the 3 cats that have responded.

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