



Guide for the elaboration of monographs **ON VACCINES FOR VETERINARY USE**

the time when the vaccine is to be used after reconstitution.



01/2017:0062

VACCINES FOR VETERINARY USE

Vaccina ad usum veterinarium

In the case of combined vaccines, for each component that is the subject of a monograph in the Pharmacopoeia, the provisions of that monograph apply to that component, modified where necessary as indicated (see chapters 5.2.6. Evaluation of safety of veterinary vaccines and immunosera and 5.2.7. Evaluation of efficacy of veterinary vaccines and immunosera). If an immunological product for veterinary use is intended for minor use, certain tests may be excluded, subject to approval by the competent authority⁽¹⁾.

1. DEFINITION

Vaccines for veterinary use are preparations containing antigenic substances and are administered for the purpose of inducing a specific and active immunity against disease provoked by bacteria, toxins, viruses, fungi or parasites. The vaccines, live or inactivated, confer active immunity that may be transferred passively via maternal antibodies against the immunogens they contain and sometimes also against antigenically related organisms. Vaccines may contain living or inactivated micro-organisms (bacteria, viruses or fungi), parasites, or antigenic fractions or substances produced by these organisms (e.g. toxins), rendered harmless whilst retaining all or part of their antigenic properties; vaccines may also contain combinations of these constituents. The antigens may be produced by recombinant DNA technology. Suitable

Unless otherwise indicated given below for bacterial vaccines, bacterial toxoid combination of bacterial

1-2. VIRAL VACCINES
Viral vaccines are prepared (5.2.4), in tissues, in micro where no other possibility by other suitable means. been modified by genetic freeze-dried preparation subunits or peptides.

Live viral vaccines are pr virulence or of natural lo Inactivated viral vaccine for inactivation of the vi concentrated.

1-3. VECTOR VACCIN
Vector vaccines are liqu one or more types of liv or fungi) that are non-p for the target species an or more genes encoding response protective aga

2. PRODUCTION

General provisions. P a finished product that requirements. Compli demonstrated by safety batches during develop The tests to be applied monographs. In accor performance of all the a prerequisite for a ma the Pharmacopoeia be

European Pharmacopoeia

EDQM
1st Revision
Edition 2016

Guide for the elaboration of monographs
ON VACCINES FOR VETERINARY USE

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1 **GUIDE FOR THE ELABORATION OF MONOGRAPHS ON**
2 **VACCINES FOR VETERINARY USE**

3
4 1. STANDARDISED TEXT FOR THE ELABORATION OF MONOGRAPHS

5 The sections of this guide provide the main examples of the structure and phrases and terms that
6 should be used by rapporteurs when drafting monographs. Examples are given for monographs for
7 different types of products but which are drafted to suit the bulk of the products of that type (e.g.
8 live viral vaccines). The standard layout and wording given in these examples should be used as far as
9 possible when drafting a monograph. It is, however, accepted and expected that, in some cases,
10 there will be reasons for adopting a different approach, adding or deleting sections to reflect
11 requirements that are different from the norm and which reflect the particular characteristics of a
12 product type.

13 2. TITLE OF MONOGRAPHS

14 **Recommended format:** Vaccine + type (live, inactivated, etc.) + name of the disease + for veterinary
15 use (where the vaccine for human use also exists) + target species where necessary.

16 Examples:

17 Egg drop syndrome '76 vaccine (inactivated)

18 Avian infectious bronchitis vaccine (inactivated),

19 Anthrax spore vaccine (live) for veterinary use

20 Canine parvovirus vaccine (live)

21 *Note: the recommended format is not always appropriate. Vaccines intended to protect against*
22 *canine parainfluenza virus are not called kennel cough vaccines since this disease may be caused by*
23 *several agents. In such a case, the title includes the name of the micro-organism: Canine*
24 *parainfluenza virus vaccine (live).*

25 A given micro-organism may cause several distinct diseases and the title is then usually based on the
26 scientific name of the causative agent (for example *Clostridium perfringens* vaccine for veterinary
27 use).

1 3. STRUCTURE AND CONTENTS OF MONOGRAPHS

2 The information in [brackets] are to be replaced in the monographs by the appropriate information,
3 such as the name of the animal.

4 The information presented *in italics* corresponds to the parts of sections that may vary from one
5 monograph to another.

1 3.1. VIRAL VACCINES (LIVE) – TYPICAL PARAGRAPHS

2 *Note: the numbering presented in this section corresponds to that which is to be used during*
 3 *elaboration of the monograph and is independent of the layout of this guide.*

4 1. DEFINITION

5 ... vaccine (live) is a preparation of *a/one or more* suitable strains of [virus]. This monograph applies
 6 to vaccines intended for the *active immunisation* of [animals] *and/or for passive protection of their*
 7 *progeny against [disease] caused by [virus].*

8 2. PRODUCTION

9 2-1. PREPARATION OF THE VACCINE

10 The vaccine virus is *grown in embryonated hens' eggs or in cell culture. ...The vaccine may be*
 11 *adjuvanted.*

12 2-2. SUBSTRATE FOR VIRUS PROPAGATION

13 2-2-1. **Embryonated hens' eggs.** If the vaccine virus is grown in embryonated hens' eggs, they are
 14 obtained from flocks free from specified pathogens (SPF) (5.2.2).

15 2-2-2. **Cell cultures.** If the vaccine virus is grown in cell cultures, they comply with the requirements
 16 for cell cultures for production of veterinary vaccines (5.2.4).

17 2-3. SEED LOTS (AVIAN VACCINES)

18 2-3-1. **Extraneous agents.** The master seed lot complies with the test for extraneous agents in seed
 19 lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages
 20 from the master seed lot at the start of the tests.

21 2-4. CHOICE OF THE VACCINE VIRUS

22 The vaccine virus is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the
 23 [animals] for which it is intended.

24 The following tests for [list of all tests of this section: name (section 2-4-x)] may be used during the
 25 demonstration of safety and efficacy.

26 2-4-1. **Safety.** Carry out the test for each route and method of administration to be recommended for
 27 vaccination *and (where applicable) in [animals] of each category for which the vaccine is intended,*
 28 *(using in each case [animals] not older than the minimum age to be recommended for vaccination).*
 29 Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine.

30 2-4-1-1. **General safety.** For each test, use not fewer than x [animals] *not older than the minimum age*
 31 *to be recommended for vaccination and from a SPF flock (5.2.2.)/that do not have antibodies against*
 32 *[virus].* Administer to each [animal] a quantity of the vaccine virus equivalent to not less than 10
 33 times the maximum virus titre likely to be contained in one dose of the vaccine. Use each
 34 recommended route of administration, unless one route has been shown to cause the most severe

1 effects. Observe the [animals] at least daily for x days (21 days for birds and 14 days for other
2 animals).

3 *The test is not valid if more than x per cent of the [animals] show abnormal signs or die from causes
4 not attributable to the vaccine virus.* The vaccine virus complies with the test if no [animal] shows
5 abnormal local or systemic reactions, signs of disease or dies from causes attributable to the vaccine
6 virus.

7 *2-4-1-2. Safety in pregnant [animals].* If the vaccine is intended for use/may be used/is not to be
8 contraindicated for use in pregnant [animals], use for the test not fewer than x pregnant [animals] at
9 the stage or at different stages of pregnancy according to the schedule to be recommended.
10 Administer to each [animal] a quantity of the vaccine virus equivalent to not less than x times the
11 maximum virus titre likely to be contained in one dose of the vaccine. Observe the [animals] at least
12 daily until 1 day after [whelping/farrowing/calving... if a specific word exists, otherwise parturition].

13 The vaccine complies with the test if no [animal] shows abnormal local or systemic reactions, signs of
14 disease or dies from causes attributable to the vaccine and if no adverse effects on the pregnancy or
15 the offspring are noted.

16 *Note: The general safety test is carried out using pregnant animals only when the vaccine is
17 specifically intended for pregnant animals. –A minimum of 8 animals are used. (In some particular
18 cases only 5 animals have been required in existing monographs. This is where the products have a
19 well-established history of use. Examples are Bovine parainfluenzavirus vaccine (live) (1176), Bovine
20 respiratory syncytial virus vaccine (live) (1177), Canine adenovirus vaccine (live) (1951), Canine
21 parainfluenza virus vaccine (live) (1955), Canine distemper vaccine (live) (0448), Canine parvovirus
22 vaccine (live) (0964), Distemper vaccine (live) for mustelids (0449), Feline infectious enteritis (feline
23 panleucopenia) vaccine (live) (0251), Infectious bovine rhinotracheitis vaccine (live) (0696).*

24 **2-4-2. Increase in virulence.** Carry out the test according to general chapter 5.2.6 using [animals:
25 number, age], *from an SPF flock (5.2.2) / free from antibodies against [virus].* If the properties of the
26 vaccine virus allow sequential passage through 5 groups via natural spreading, this method may be
27 used, otherwise passage as described below is carried out.

28 Administer to each [animal] of the 1st group by [route] *a quantity of the vaccine virus that will allow
29 recovery of virus for the passages described below. Administer the virus by the route to be
30 recommended for vaccination most likely lead to reversion to virulence. ... [Description of the
31 preparation of the suspension].* Administer x ml of the [suspension] by the [route] to each of
32 [animals] of the next group. Carry out this passage operation not fewer than 4 times; verify the
33 presence of the virus at each passage. If the virus is not found at a passage level, repeat the passage
34 by administration to a group of 10 animals.

35 If the 5th group of animals shows no evidence of an increase in virulence indicative of reversion
36 during the observation period, further testing is not required. Otherwise, carry out an additional
37 safety test and compare the clinical signs and any relevant parameters in a group of at least 8
38 animals receiving the material used for the 1st passage and another similar group receiving the
39 organism at the final passage level.

1 *Carry out the test for ... (safety) (section 2-4-1) using the material used for the 1st passage and the*
 2 *virus at the final passage level.*

3 The vaccine virus complies with the test if no indication of increased virulence of the organism
 4 recovered for the final passage compared with the material used for the 1st passage is observed. If
 5 virus is not recovered after an initial passage in 2 animals/5 birds and a subsequent repeated passage
 6 level in 10 animals/birds, the vaccine virus also complies with the test.

7 **2-4-3 Immunogenicity.** A test is carried out for each route and method of administration to be
 8 recommended for vaccination using in each case [animals] *of/not older than the minimum age to be*
 9 *recommended for vaccination / weighing...* The quantity of vaccine virus to be administered to each
 10 [animal] is not greater than the minimum virus titre to be stated on the label and the virus is at the
 11 most attenuated passage level that will be present in a batch of vaccine.

12 Use for the test not fewer than x [animals] of the same origin *and from an SPF flock (5.2.2)/that do*
 13 *not have antibodies against [virus].* Vaccinate by a recommended route not fewer than x [animals]
 14 according to the schedule to be recommended. Maintain not fewer than x [animals] as controls.
 15 Challenge each [animal] after x days by [route] with a sufficient quantity of a [virulent virus]. Observe
 16 the [animals] at least daily for x days after challenge. ...

17 The test is not valid if ... The vaccine complies with the test if ...

18 *Note: challenge done usually after 21 days for birds / 20-22 days after the last vaccination for other*
 19 *species unless the specific disease model requires an earlier/later time point*

20 3. BATCH TESTS

21 **3-1. Identification.** The *vaccine virus* is identified using appropriate *molecular biology / biochemistry /*
 22 *cell culture techniques. / The vaccine, diluted if necessary and mixed with a monospecific [virus]*
 23 *antiserum, no longer infects embryonated hens' eggs from an SPF flock (5.2.2) or susceptible cell*
 24 *cultures (5.2.4) into which it is inoculated.*

25 **3-2. Bacteria and fungi.** The vaccine, including where applicable the diluent supplied for
 26 reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for*
 27 *veterinary use (0062).*

28 Frozen or freeze-dried avian live viral vaccines produced in embryonated eggs and not intended for
 29 administration by injection either comply with the test for sterility prescribed in the monograph
 30 *Vaccines for veterinary use (0062)* or with the following test: carry out a quantitative test for bacterial
 31 and fungal contamination; carry out identification tests for microorganisms detected in the vaccine;
 32 the vaccine does not contain pathogenic microorganisms and contains not more than 1 non-
 33 pathogenic microorganism per dose.

34 Any diluent supplied for reconstitution of the vaccine complies with the test for sterility prescribed in
 35 the monograph *Vaccines for veterinary use (0062).* (AVIAN VACCINES)

36 *Note: the last sentence is for avian vaccines and for Bordetella bronchiseptica live for dogs (bacterial*
 37 *vaccine).*

- 1 3-3. **Mycoplasmas** (2.6.7). The vaccine complies with the test for mycoplasmas.
- 2 3-4. **Extraneous agents**. Neutralise the vaccine virus with a suitable monospecific antiserum against
3 [virus] and inoculate into cell cultures known for their susceptibility to viruses pathogenic for the
4 [animals]. The vaccine complies with the test if no cytopathic effect develops and there is no sign of
5 haemagglutinating or haemadsorbing agents...
- 6 The vaccine complies with the tests for extraneous agents in batches of finished product (2.6.25).
7 (AVIAN VACCINES)
- 8 3-5. **Virus titre**. Titrate the vaccine virus by inoculation *into embryonated hens' eggs from an SPF*
9 *flock (5.2.2) or into suitable cell cultures (5.2.4)*. The vaccine complies with the test if one dose
10 contains not less than the minimum virus titre stated on the label.
- 11 3-6. **Potency**. The vaccine complies with the requirements of the test prescribed under
12 Immunogenicity (section 2-4-x) when administered by a recommended route and method. It is not
13 necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a
14 representative batch using a vaccinating dose containing not more than the minimum virus titre
15 stated on the label.

1 3.2. INACTIVATED VIRAL VACCINES – TYPICAL PARAGRAPHS

2 *Note: the numbering presented in this section corresponds to that which is to be used during*
 3 *elaboration of the monograph and is independent of the layout of this guide.*

4 1. DEFINITION

5 ... vaccine (inactivated) is a preparation of a suitable strain of [virus], inactivated while maintaining
 6 adequate immunogenic properties. This monograph applies to vaccines intended for the *active*
 7 *immunisation of [animals] and/or for passive protection of their progeny against [disease] caused by*
 8 *[virus].*

9 2. PRODUCTION

10 2-1. PREPARATION OF THE VACCINE

11 The vaccine virus is grown in *embryonated hens' eggs and/or in cell culture*. The virus harvest is
 12 *inactivated. The vaccine may be adjuvanted.*

13 2-2. SUBSTRATE FOR VIRUS PROPAGATION

14 2-2-1. **Embryonated hens' eggs.** If the vaccine virus is grown in embryonated hens' eggs, they are
 15 obtained from a healthy flock (5.2.13) (AVIAN VACCINES) / flock free from specified pathogens (SPF)
 16 (5.2.2).

17 2-2-2. **Cell cultures.** If the vaccine virus is grown in cell cultures, they comply with the requirements
 18 for cell cultures for production of veterinary vaccines (5.2.4).

19 2-3. SEED LOTS (AVIAN VACCINES)

20 2-3-1. **Extraneous agents.** The master seed lot complies with the test for extraneous agents in seed
 21 lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages
 22 from the master seed lot at the start of the tests.

23 2-4. CHOICE OF VACCINE COMPOSITION

24 The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the
 25 [animals] for which it is intended.

26 The following tests for [list of all the tests in the section: name (section 2-4-x)] may be used during
 27 the demonstration of safety and efficacy.

28 2-4-1. **Safety.** Carry out the test(s) for each route and method of administration to be recommended
 29 for vaccination and (*where applicable*) *in each category of [animals] for which the vaccine is intended,*
 30 *(using in each case [animals] not older than the minimum age to be recommended for vaccination).*
 31 Use a batch of vaccine containing not less than the maximum potency that may be expected in a
 32 batch of vaccine.

33 2-4-1-1. *General safety.* For each test, use not fewer than 8* [animals] *that do not have antibodies*
 34 *against [virus]/ not older than the minimum age to be recommended for vaccination and, in the case*

1 *of chickens, use chickens from a flock free from specified pathogens (SPF) (5.2.2) and if the vaccine is*
 2 *used for species other than chickens, they have not been vaccinated and do not have antibodies*
 3 *against [disease]. Administer by a recommended route and method to each [animal] one dose of the*
 4 *vaccine. Observe the [animals] at least daily for x (14) days / after the last administration.*

5 The test is not valid if non-specific mortality occurs. The vaccine complies with the test if no [animal]
 6 shows abnormal local or systemic reactions, signs of disease, or dies from causes attributable to the
 7 vaccine.

8 *2-4-1-2. Safety in pregnant [animals]. If the vaccine is intended for use/may be used/is not to be*
 9 *contraindicated for use in pregnant [animals], use for the test not fewer than 8 pregnant [animals] at*
 10 *the stage or at different stages of pregnancy according to the schedule to be recommended.*
 11 *Administer to each [animal] a quantity of the vaccine virus equivalent to one dose of the vaccine... If*
 12 *the recommended schedule requires a 2nd dose, administer another dose after an interval of at least*
 13 *14 days. Observe the [animals] at least daily until 1 day after [whelping/farrowing/calving... if a*
 14 *specific term exists, otherwise use parturition].*

15 *The vaccine complies with the test if no [animal] shows abnormal local or systemic reactions, signs of*
 16 *disease or dies from causes attributable to the vaccine and if no adverse effects on the pregnancy or*
 17 *the offspring are noted.*

18 **Note: when the number of animals used for the safety test was below 8 (i.e. 5) at the time the*
 19 *monograph had been elaborated, this number has not been revised (for example for vaccines*
 20 *intended for cats, dogs, calves, ferrets, minks).*

21 **2-4-2. Immunogenicity**

22 *2-4-2-1. Vaccines intended for active immunisation. For vaccines with claims for active immunisation*
 23 *against [disease], a test is carried out for each route and method of administration to be*
 24 *recommended using in each case [animals] of the minimum age to be recommended for vaccination /*
 25 *weighing... The vaccine to be administered to each [animal] is of minimum potency.*

26 *Use for the test not fewer than x [animals] of the same origin and from an SPF flock (5.2.2)/that do*
 27 *not have antibodies against [virus]. Vaccinate not fewer than x [animals] according to the schedule to*
 28 *be recommended. Maintain not fewer than x [animals] as controls. Challenge each [animal] after x*
 29 *days by [route] with a sufficient quantity of a [virulent virus]. Observe the [animals] at least daily for*
 30 *x days after challenge. ...*

31 The test is not valid if ... The vaccine complies with the test if ...

32 *2-4-2-2. Vaccines intended for passive protection. For vaccines with claims for passive protection*
 33 *against [disease], a test is carried out for each route and method of administration to be*
 34 *recommended using in each case [animals] of the minimum age to be recommended for vaccination*
 35 *... The vaccine to be administered to each [animal] is of minimum potency.*

36 *Use for the test not fewer than x [animals] of the same origin and from an SPF flock (5.2.2)/that do*
 37 *not have antibodies against [virus]. Vaccinate not fewer than x [animals] according to the schedule to*
 38 *be recommended. Maintain not fewer than x [animals] as controls. Challenge each [animal] after x*

1 *days by [route] with a sufficient quantity of a [virulent virus]. Observe the [animals] at least daily for x*
 2 *days after challenge. ... (At the end of the observation period, ...)*

3 *The test is not valid if ... The vaccine complies with the test if ...*

4

5 2-5. MANUFACTURER'S TESTS

6 2-5-1. **Residual live virus.** The test for residual live virus is carried out... [Description of the substrate,
 7 of the test procedure]. The quantity of inactivated virus harvest used is equivalent to not less than x
 8 doses of the vaccine. The inactivated virus harvest complies with the test if no live virus is detected.

9 Note : it is currently expected that this test is performed on the harvest and on the bulk blend of
 10 antigens (see test 3-4).

11 2-5-2. **Batch potency.** It is not necessary to carry out the Potency test (section 3-x) for each batch of
 12 the vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the
 13 test is not carried out, an alternative validated method is used, the criteria for acceptance being set
 14 with reference to a batch of vaccine that has given satisfactory results in the test described under
 15 Potency. *The following test may be used.*

16 *[Description of the test]*

17 3. BATCH TESTS

18 3-1. **Identification.** The vaccine contains the antigen or antigens stated under Definition.

19 3-2. **Bacteria and fungi.** The vaccine, including where applicable the diluent supplied for
 20 reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for*
 21 *veterinary use (0062).*

22 3-3. **Residual live virus.** This test may be omitted for batch release, as stated in the monograph
 23 *Vaccines for veterinary use (0062).*

24 A test for residual live virus is carried out to confirm inactivation of [virus].

25 *[Description of the test/tests]*

26 **3-4. Specified Extraneous agents.** Use not fewer than 2 [mammals] not older than the minimum age
 27 recommended for vaccination and preferably, that do not have antibodies against [virus]/that do not
 28 have antibodies against [pathogen] or, where justified, use [animals] with a low level of such
 29 antibodies as long as they have not been vaccinated against [disease] and administration of the
 30 vaccine does not cause an anamnestic response. Administer to each [animal] by a recommended
 31 route a double dose of the vaccine, then another dose after 14 days. Observe the [animals] at least
 32 daily until 14 days after the last administration. Take a blood sample at the end of the observation
 33 period. The vaccine complies with the test if it does not stimulate the formation of antibody against
 34 the following agents (a list is given).

- 1 *Note: the test for specified extraneous agents is not requested for avian live vaccines since reference*
- 2 *to chapter 5.2.13 has been added for Embryonated hens' eggs.*
- 3 3-6. **Potency.** The vaccine complies with the requirements of the test mentioned under
- 4 Immunogenicity (section 2-4-x) when administered by a recommended route and method.

1 3.3. LIVE BACTERIAL VACCINES – TYPICAL PARAGRAPHS

2 *Note: the numbering presented in this section corresponds to that which is to be used during*
 3 *elaboration of the monograph and is independent of the layout of this guide.*

4 1. DEFINITION

5 ... vaccine (live) is a preparation of *a / one or more* suitable strain of live [bacteria]. The vaccine
 6 contains not fewer than xx and not more than xx live bacteria per dose. This monograph applies to
 7 vaccines intended for the *active immunisation of [animals] against [disease] caused by [bacteria]*.

8 2. PRODUCTION

9 2-1. PREPARATION OF THE VACCINE

10 The vaccine strain is grown in a suitable medium. ... *The vaccine may be adjuvanted.*

11 2-2. CHOICE OF VACCINE STRAIN

12 The vaccine strain is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the
 13 [animals] for which it is intended.

14 The following tests for [list of all tests of this section: name (section 2-2-x)] may be used during the
 15 demonstration of safety and efficacy.

16 2-2-1. **Safety.** Carry out the test for each route and method of administration to be recommended
 17 for vaccination *and (where applicable) in [animals] of each category for which the vaccine is intended,*
 18 *using in each case [animals] not older than the minimum age to be recommended for vaccination /*
 19 *weighing...* Use vaccine strain at the least attenuated passage level that will be present in a batch of
 20 the vaccine.

21 2-2-1-1. *General safety.* For each test, use not fewer than x [animals] *not older than the minimum age*
 22 *to be recommended for vaccination and that do not have antibodies against [bacteria]*. Administer to
 23 each [animal] a quantity of the vaccine strain equivalent to not less than 10 times the maximum
 24 number of live bacteria likely to be contained in one dose of the vaccine. Use each recommended
 25 route of administration, unless one route has been shown to cause the most severe effects. Observe
 26 the [animals] at least daily for x days.

27 *The test is not valid if more than x per cent of the [animals] show abnormal signs or die from causes*
 28 *not attributable to the vaccine strain.* The vaccine strain complies with the test if no [animal] shows
 29 abnormal local or systemic reactions, signs of disease or dies from causes attributable to the vaccine.

30 2-2-1-2. *Safety in pregnant [animals]. If the vaccine is intended for use/may be used/is not to be*
 31 *contraindicated for use in pregnant [animals], use for the test not fewer than x pregnant [animals] at*
 32 *the stage or at different stages of pregnancy according to the schedule to be recommended.*
 33 *Administer to each [animal] a quantity of the vaccine strain equivalent to not less than x times the*
 34 *maximum number of live bacteria likely to be contained in one dose of the vaccine. Observe the*
 35 *[animals] at least daily until 1 day after [whelping/farrowing/calving... if a specific word exists,*
 36 *otherwise parturition].*

1 *The vaccine complies with the test if no [animal] shows abnormal local or systemic reactions, signs of*
 2 *disease, or dies from causes attributable to the vaccine and if no adverse effects on the pregnancy or*
 3 *the offspring are noted.*

4

5 2-2-2. **Increase in virulence.** Carry out the test according to general chapter 5.2.6 using [animals:
 6 number, age], free from [pathogen] and that do not have antibodies against [pathogen]. If the
 7 properties of the vaccine strain allow sequential passage through 5 groups via natural spreading, this
 8 method may be used, otherwise passage as described below is carried out. Administer to each
 9 [animal] of the 1st group by [route] *a quantity of the vaccine strain that will allow recovery of bacteria*
 10 *for the passages described below. Administer the strain by the route to be recommended for*
 11 *vaccination most likely to lead to reversion to virulence.* ... [Description of the preparation of the
 12 suspension]. Administer x ml of the [suspension] by the [route] to each of x other [animals] of the
 13 next group. Carry out this passage operation not fewer than 4 times; verify the presence of the
 14 bacteria at each passage. If the bacteria are not found at a passage level, repeat the passage by
 15 administration to a group of 10 animals.

16 If the 5th group of animals shows no evidence of an increase in virulence indicative of reversion
 17 during the observation period, further testing is not required. Otherwise, carry out an additional
 18 safety test and compare the clinical signs and any relevant parameters in a group of at least 8
 19 animals receiving the material used for the 1st passage and another similar group receiving the
 20 bacteria at the final passage level.

21 *Carry out the test for ... (safety) (section 2-4-1) using the material used for the 1st passage and the*
 22 *bacteria at the final passage level.*

23 The vaccine strain complies with the test if no indication of increased virulence of the bacteria
 24 recovered for the final passage compared with the material used for the 1st passage is observed. If
 25 bacteria are not recovered after an initial passage in 2 animals/5 birds and a subsequent repeat of
 26 this passage level in 10 animals/birds, the vaccine strain also complies with the test.

27 2-2-3 **Immunogenicity.** A test is carried out for each route and method of administration to be
 28 recommended for vaccination using in each case [animals] *of the minimum age to be recommended*
 29 *for vaccination / weighing...* The quantity of the vaccine strain to be administered to each [animal] is
 30 not greater than the minimum number of live bacteria to be stated on the label and the strain is at
 31 the most attenuated passage level that will be present in a batch of vaccine.

32 Use for the test not fewer than x [animals] of the same origin *and from an SPF flock (5.2.2)/that do*
 33 *not have antibodies against [bacteria].* Vaccinate not fewer than x [animals] according to the
 34 schedule to be recommended. Maintain not fewer than x [animals] as controls. Challenge each
 35 [animal] after x days by [route] with a quantity of a [virulent strain] sufficient to cause typical signs of
 36 [disease] in an [animal] that does not have antibodies against [pathogen]. Observe the [animals] at
 37 least daily for x days after challenge. ...

38 The test is not valid if ... The vaccine complies with the test if ...

39

1 3. BATCH TESTS

2 3-1. **Identification.** *The vaccine strain is identified by suitable methods./Each strain present in the*
3 *vaccine is identified by suitable morphological, serological and biochemical methods, and by culture*
4 *on selective medium.*

5 3-2. **Bacteria and fungi.** Carry out the test by microscopic examination and by inoculation of suitable
6 media, or verify the absence of micro-organisms other than [bacteria] present in the vaccine as
7 described in the test for sterility prescribed in the monograph on *Vaccines for veterinary use (0062)*.

8 The vaccine complies with the test if it does not contain extraneous micro-organisms. Any diluent
9 supplied for reconstitution of the vaccine complies with the test for sterility prescribed in the
10 monograph *Vaccines for veterinary use (0062)*.

11 3-3. **Live bacteria.** Make a count of live bacteria on a solid medium suitable for the culture of
12 [bacteria + strain]. The vaccine complies with the test if one dose contains not less than the minimum
13 number of live [bacteria] stated on the label.

14 3-4. **Potency.** The vaccine complies with the requirements of the test prescribed under
15 Immunogenicity (section 2-2-x) when administered by a recommended route and method. It is not
16 necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a
17 representative batch using a vaccinating dose containing not more than the minimum number of live
18 [bacteria] stated on the label.

1 3.4. INACTIVATED BACTERIAL VACCINES – TYPICAL PARAGRAPHS

2 *Note: the numbering presented in this section corresponds to that which is to be used during*
 3 *elaboration of the monograph and is independent of the layout of this guide.*

4 1. DEFINITION

5 ... vaccine (inactivated) is a preparation of *a/one or more suitable strains of [bacteria]*, inactivated
 6 while maintaining adequate immunogenic properties. This monograph applies to vaccines intended
 7 for the *active immunisation* of [animals] and/or for *passive protection of their progeny* against
 8 [disease] *caused by [bacteria]*.

9 2. PRODUCTION

10 2-1. PREPARATION OF THE VACCINE

11 Production of the vaccine is based on a seed-lot system. The seed material is cultured in a suitable
 12 medium to ensure optimal growth under the chosen incubation conditions ; *each strain is cultivated*
 13 *separately and identity is verified using a suitable method*. During production, various parameters
 14 such as growth rate are monitored by suitable methods; the values are within the limits approved for
 15 the particular product. Purity of the harvest is verified using suitable methods.

16 After cultivation, the bacterial suspensions are collected separately and inactivated by a suitable
 17 method. ... *The vaccine may contain an adjuvant.*

18 2-2. CHOICE OF VACCINE COMPOSITION

19 The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the
 20 [animals] for which it is intended.

21 The following tests for [list of all tests of this section: name (section 2-2-x)] may be used during the
 22 demonstration of safety and efficacy.

23 2-2-1. Safety

24 2-2-1-1. *Laboratory test*. Carry out the test for each route and method of administration to be
 25 recommended for vaccination and *(where applicable) in categories of [animals] of each category for*
 26 *which the vaccine is intended, using in each case [animals] not older than the minimum age to be*
 27 *recommended for vaccination/in each species of fish for which the vaccine is intended, using fish of*
 28 *the minimum body mass to be recommended for vaccination*. Use a batch of vaccine containing not
 29 less than the maximum potency that may be expected in a batch of vaccine.

30 2-2-1-1-1. *General safety*. For each test, use not fewer than 8 [animals] *that do not have antibodies*
 31 *against [bacteria]*. Administer to each [animal] one dose of the vaccine. If the recommended
 32 schedule requires a 2nd dose, administer another dose after an interval of at least 14 days. Observe
 33 the [animals] at least daily for x days after the last administration. Record body temperature the day
 34 before vaccination, at vaccination, 4 h (2 h, 4 h and 6 h in specific cases) later and then daily for 4
 35 days ; note the maximum temperature increase for each [animal].

1 The vaccine complies with the test if no [animal] shows abnormal local or systemic reactions, signs of
 2 disease or dies from causes attributable to the vaccine ([in specific cases:]if the average body
 3 temperature increase for all [animal] does not exceed 1.5°C and no [animal] shows a rise greater
 4 than 2.0°C).

5 *2-2-1-1-2. Safety in pregnant [animals].* If the vaccine *is intended for use/may be used/is not to be*
 6 *contraindicated for use in pregnant [animals],* use for the test not fewer than 8 pregnant [animals] at
 7 the relevant stages of pregnancy. Administer to each [animal] a quantity of the vaccine strain
 8 equivalent to not less than x times the maximum number of bacteria likely to be contained in one
 9 dose of the vaccine. Observe the [animals] at least daily until 1 day after
 10 [whelping/farrowing/calving... if a specific word exists, otherwise parturition]. Record body
 11 temperature the day before each vaccination, at vaccination, 4 h (2 h, 4 h and 6 h in specific cases)
 12 later and then daily for 4 days; note the maximum temperature increase for each [animal].

13 The vaccine complies with the test if:

- 14 ✓ no [animal] shows abnormal local or systemic reactions or dies from causes attributable to
 15 the vaccine;
- 16 ✓ [in specific cases :]the average body temperature increase for all [animal] does not exceed
 17 1.5°C and no [animal] shows a rise greater than 2.0°C;
- 18 ✓ no adverse effects on gestation or the offspring are noted.

19 *Note: The general safety test is carried out using pregnant animals only when the vaccine is*
 20 *specifically intended for pregnant animals. Usually 8 animals are used (in some particular cases only 5*
 21 *animals may be required, but only for well-established products).*

22 *2-2-1-2. Field studies.* The [animal] used for field trials are also used to evaluate safety. Carry out a
 23 test in each category of [animal] for which the vaccine is intended. Record body temperature the day
 24 before vaccination, at vaccination and daily during the 2 days following vaccination; note the
 25 maximum temperature increase for each [animal].

26 The vaccine complies with the test if no [animal] shows abnormal local or systemic reactions, signs of
 27 disease, or dies from causes attributable to the vaccine and if the average temperature increase for
 28 all [animals] does not exceed 1.5 °C and no [animal] shows a rise greater than 2.0°C.

29 **2-2-2 Immunogenicity**

30 *2-2-2-1. Vaccines intended for active immunisation.* For vaccines with claims for active immunisation
 31 against [disease], a test is carried out for each route and method of administration to be
 32 recommended using in each case [animals] *of the minimum age to be recommended for vaccination /*
 33 *weighing...* The vaccine to be administered to each [animal] is of minimum potency.

34 Use for the test not fewer than x [animals] *that do not have antibodies against [bacteria].* Vaccinate
 35 not fewer than x [animals] according to the schedule to be recommended. Maintain not fewer than x
 36 [animals] as controls. Challenge each [animal] after x days by [route] with a sufficient quantity of a
 37 [virulent strain]. Observe the [animals] at least daily for x days after challenge. ...

38 The test is not valid if ... The vaccine complies with the test if ...

1 2-2-2-2. *Vaccines intended for passive protection. For vaccines with claims for passive protection*
 2 *against [disease], a test is carried out for each route and method of administration to be*
 3 *recommended using in each case [animals] of the minimum age to be recommended for vaccination /*
 4 *weighing... The vaccine to be administered to each [animal] is of minimum potency.*

5 *Use for the test not fewer than x [animals] that do not have antibodies against [bacteria]. Vaccinate*
 6 *not fewer than x [animals] according to the schedule to be recommended. Maintain not fewer than x*
 7 *[animals] as controls. Challenge each [animal] after x days by [route] with a sufficient quantity of a*
 8 *[virulent strain]. Observe the [animals] at least daily for x days after challenge. ...*

9 *The test is not valid if ... The vaccine complies with the test if ...*

10 2-3. MANUFACTURER'S TESTS

11 2-3-1. **Batch potency test.** It is not necessary to carry out the relevant Potency test or tests (section
 12 3-x) for each batch of the vaccine *if it has/they have been carried out* using a batch of vaccine with a
 13 minimum potency. Where the relevant test or tests is/are not carried out, an alternative validated
 14 method is used, the criteria for acceptance being set with reference to a batch of vaccine that has
 15 given satisfactory results in the test(s) described under Potency.

16 2-3-2. **Bacterial endotoxins.** *A test for bacterial endotoxins (2.6.14) is carried out on the final lot or,*
 17 *where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or*
 18 *the mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable*
 19 *amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory*
 20 *in safety tests 2-x given under Choice of vaccine composition.*

21 *The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch*
 22 *used in the safety test for determining the maximum acceptable level of endotoxin is used*
 23 *subsequently for testing of each batch.*

24 3. BATCH TESTS

25 3-1. **Identification.** The vaccine contains the antigen or antigens stated under Definition

26 3-2. **Bacteria and fungi.** The vaccine, including where applicable the diluent supplied for
 27 reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for*
 28 *veterinary use (0062).*

29 3-3. **Residual live bacteria.** Describe a test to detect residual live bacteria when the test 3-2 Bacteria
 30 and fungi above is not able to detect them.

31 3-4. **Potency.** The vaccine complies with the requirements of the test or test(s) mentioned under
 32 Immunogenicity (section 2-2-x) when administered by a recommended route and method.

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