

Guide

for the elaboration of monographs on
vaccines for veterinary use

European Pharmacopoeia

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GUIDE FOR THE ELABORATION OF MONOGRAPHS ON VACCINES FOR VETERINARY USE

1. STANDARDISED TEXT FOR THE ELABORATION OF MONOGRAPHS

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

2. TITLE OF MONOGRAPHS

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

Examples:

Egg drop syndrome '76 vaccine (inactivated)

Avian infectious bronchitis vaccine (inactivated),

Anthrax spore vaccine (live) for veterinary use

Canine parvovirus vaccine (live)

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

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

1 3. STRUCTURE AND CONTENTS OF MONOGRAPHS

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

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

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3.1. VIRAL VACCINES (LIVE) – TYPICAL PARAGRAPHS

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

1. DEFINITION

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

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

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

2-2. SUBSTRATE FOR VIRUS PROPAGATION

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

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

2-3. SEED LOTS (AVIAN VACCINES)

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

2-4. CHOICE OF THE VACCINE VIRUS

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

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

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

2-4-1-1. *General safety.* For each test, use not fewer than *x* [animals] *not older than the minimum age to be recommended for vaccination and from a SPF flock (5.2.2)/that do not have antibodies against [virus]*. Administer to each [animal] a quantity of the vaccine virus equivalent to not less than 10 times the maximum virus titre likely to be contained in one dose of the vaccine. Observe the [animals] at least daily for *x* days (21 days for birds and 14 days for other animals).

1
2 *The test is invalid if more than x per cent of the [animals] show abnormal signs or die from*
3 *causes not attributable to the vaccine virus. The vaccine virus complies with the test if no*
4 *[animal] shows notable signs of disease or dies from causes attributable to the vaccine virus.*
5

6 *2-4-1-2. Safety in pregnant [animals]. If the vaccine is intended for use/may be used/is not to*
7 *be contraindicated for use in pregnant [animals], use for the test not fewer than x pregnant*
8 *[animals] at the stage or at different stages of pregnancy according to the schedule to be*
9 *recommended. Administer to each [animal] a quantity of the vaccine virus equivalent to not*
10 *less than x times the maximum virus titre likely to be contained in one dose of the vaccine.*
11 *Observe the [animals] at least daily until 1 day after [whelping/farrowing/calving... if a*
12 *specific word exists, otherwise parturition].*
13

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

18 **2-4-2. Increase in virulence.** The test for increase in virulence consists of the administration
19 of the vaccine virus at the least attenuated passage level that will be present between the
20 master seed lot and a batch of the vaccine to [animals: number, age], *from an SPF flock*
21 *(5.2.2) / free from antibodies against [virus], sequential passages, 5 times where possible, to*
22 *further similar groups and testing of the final recovered virus for increase in virulence. If the*
23 *properties of the vaccine virus allow sequential passage to 5 groups via natural spreading, this*
24 *method may be used, otherwise passage as described below is carried out and the maximally*
25 *passaged virus that has been recovered is tested for increase in virulence. Care must be taken*
26 *to avoid contamination by virus from previous passages.*
27

28 Administer to each [animal] by [route] *a quantity of the vaccine virus that will allow recovery*
29 *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 x*
32 *other [animals] of the same age. Carry out this passage operation not fewer than 5 times;*
33 *verify the presence of the virus at each passage. If the virus is not found at a passage level,*
34 *carry out a 2nd series of passages.*
35

36 *Carry out the test for ... (safety) (section 2-4-1) using unpassaged vaccine virus and the*
37 *maximally passaged virus that has been recovered.*
38

39 The vaccine virus complies with the test if no indication of increased virulence of the
40 maximally passaged virus compared with the unpassaged virus is observed. If virus is not
41 recovered at any passage level in the 1st and 2nd series of passages, the vaccine virus also
42 complies with the test.
43

44 **2-4-3 Immunogenicity.** A test is carried out for each route and method of administration to be
45 recommended using in each case [animals] *of/not older than the minimum age to be*
46 *recommended for vaccination / weighing...* The quantity of vaccine virus to be administered
47 to each [animal] is not greater than the minimum virus titre to be stated on the label and the
48 virus is at the most attenuated passage level that will be present in a batch of vaccine.
49

1 Use for the test not fewer than x [animals] of the same origin *and from an SPF flock*
2 *(5.2.2)/that do not have antibodies against [virus]*. Vaccinate by a recommended route not
3 fewer than x [animals] according to the schedule to be recommended. Maintain not fewer than
4 x [animals] as controls. Challenge each [animal] after x days by [route] with a sufficient
5 quantity of a [virulent virus]. Observe the [animals] at least daily for x days after challenge. ...

6 The test is invalid if ... The vaccine complies with the test if ...

7 8 3. BATCH TESTS

9
10 3-1. **Identification.** The *vaccine virus* is identified using appropriate *molecular biology /*
11 *biochemistry / cell culture techniques*.

12
13 3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it
14 comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use*
15 *(0062)*.

16 For avian live viral vaccines, for non-parenteral use only, the requirement for sterility is
17 usually replaced by requirements for absence of pathogenic micro-organisms and for a
18 maximum of 1 non-pathogenic micro-organism per dose.

19
20 3-3. **Mycoplasmas.** The vaccine complies with the test for mycoplasmas (2.6.7).

21
22 3-4. **Extraneous agents.** Neutralise the vaccine virus with a suitable monospecific antiserum
23 against [virus] and inoculate into cell cultures known for their susceptibility to viruses
24 pathogenic for the [animals]. The vaccine complies with the test if no cytopathic effect
25 develops and there is no sign of haemagglutinating or haemadsorbing agents...

26
27 *The vaccine complies with the tests for extraneous agents in batches of finished product*
28 *(2.6.25). (AVIAN VACCINES)*

29
30 3-5. **Safety.** Use 2 [mammals]/10 [SPF birds] *not older than the minimum age recommended*
31 *for vaccination and that do not have antibodies against [virus]/preferably, that do not have*
32 *antibodies against [virus] or, where justified, use [animals] with a low level of such*
33 *antibodies as long as they have not been vaccinated against [disease] and administration of*
34 *the vaccine does not cause an anamnestic response*. Administer to each [animal] by a
35 recommended route 10 doses of the vaccine. Observe the [animals] at least daily for x days
36 (21 days for birds and generally 14 days for other animals).

37 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
38 causes attributable to the vaccine.

39
40 3-6. **Virus titre.** Titrate the vaccine virus by inoculation *into embryonated hens' eggs from an*
41 *SPF flock (5.2.2) or into suitable cell cultures (5.2.4)*. The vaccine complies with the test if
42 one dose contains not less than the minimum virus titre stated on the label.

43
44 3-7. **Potency.** The vaccine complies with the requirements of the test prescribed under
45 Immunogenicity (section 2-4-x) when administered by a recommended route and method. It is
46 not necessary to carry out the potency test for each batch of the vaccine if it has been carried
47 out on a representative batch using a vaccinating dose containing not more than the minimum
48 virus titre stated on the label.

3.2. INACTIVATED VIRAL VACCINES – TYPICAL PARAGRAPHS

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

1. DEFINITION

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

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

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

2-2. SUBSTRATE FOR VIRUS PROPAGATION

2-2-1. **Embryonated hens' eggs.** If the vaccine virus is grown in embryonated hens' eggs, they are obtained from a healthy flock.

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

2-3. SEED LOTS (AVIAN VACCINES)

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

2-4. CHOICE OF VACCINE COMPOSITION

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

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

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

2-4-1-1. *General safety.* For each test, use not fewer than x [animals] *that do not have antibodies against [virus]*. Administer to each [animal] a quantity of the vaccine virus equivalent to not less than x times the maximum virus titre likely to be contained in one dose of the vaccine. Observe the [animals] at least daily for x days (after the last administration).

1 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
2 causes attributable to the vaccine.

3 2-4-1-2. *Safety in pregnant [animals]*. If the vaccine *is intended for use/may be used/is not to*
4 *be contraindicated for use in pregnant [animals]*, use for the test not fewer than *x pregnant*
5 *[animals] at the stage or at different stages of pregnancy according to the schedule to be*
6 *recommended*. Administer to each [animal] a quantity of the vaccine virus equivalent to not
7 less than *x* times the maximum virus titre likely to be contained in one dose of the vaccine.
8 Observe the [animals] at least daily until *1 day after* [whelping/farrowing/calving... if a
9 specific term exists, otherwise use parturition].

10 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
11 causes attributable to the vaccine and if no adverse effects on the pregnancy or the offspring
12 are noted.

13

14 2-4-2. Immunogenicity

15 2-4-2-1. *Active immunisation*. For vaccines with claims for active immunisation against
16 [disease], a test is carried out for each route and method of administration to be recommended
17 using in each case [animals] *of the minimum age to be recommended for vaccination /*
18 *weighing...* The vaccine to be administered to each [animal] is of minimum potency.

19

20 Use for the test not fewer than *x* [animals] of the same origin and *from an SPF flock*
21 *(5.2.2)/that do not have antibodies against [virus]*. Vaccinate not fewer than *x* [animals]
22 *according to the schedule to be recommended*. Maintain not fewer than *x* [animals] as
23 controls. Challenge each [animal] after *x* days by [route] with a sufficient quantity of a
24 [virulent virus]. Observe the [animals] at least daily for *x* days after challenge. ...

25

26 The test is invalid if ... The vaccine complies with the test if ...

27

28 2-4-2-2. *Passive protection*. For vaccines with claims for passive protection against
29 [disease], a test is carried out for each route and method of administration to be
30 recommended using in each case [animals] *of the minimum age to be recommended for*
31 *vaccination ... The vaccine to be administered to each [animal] is of minimum potency.*

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

38 *The test is invalid if ... The vaccine complies with the test if ...*

39

40 2-5. MANUFACTURER'S TESTS

41 2-5-1. **Residual live virus**. The test for residual live virus is carried out... [Description of the
42 substrate, of the test procedure]. The quantity of inactivated virus harvest used is equivalent to
43 not less than *x* doses of the vaccine. The inactivated virus harvest complies with the test if no
44 live virus is detected.

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

47

1 2-5-2. **Batch potency.** It is not necessary to carry out the Potency test (section 3-x) for each
2 batch of the vaccine if it has been carried out using a batch of vaccine with a minimum
3 potency. Where the test is not carried out, an alternative validated method is used, the criteria
4 for acceptance being set with reference to a batch of vaccine that has given satisfactory results
5 in the test described under Potency. *The following test may be used.*

6
7 *[Description of the test]*
8

9 3. BATCH TESTS

10
11 3-1. **Identification.** When *injected into/administered to* [animals] that do not have antibodies
12 against [virus], the vaccine stimulates the production of such antibodies.

13 3-2. **Bacteria and fungi.** The vaccine and where applicable, the liquid supplied with it,
14 comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use*
15 *(0062)*.

16
17 3-4. **Residual live virus.** A test for residual live virus is carried out to confirm inactivation of
18 [virus].

19 *[Description of the test/tests]*

20 3-5. **Extraneous agents.** On the [animals] used for the safety test, carry out tests for
21 antibodies. The vaccine complies with the test if it does not stimulate the formation of
22 antibody against the following agents (a list is given).

23
24 3-6. **Safety.** Use *not fewer than 2* [mammals]/*10* [SPF birds] *not older than the minimum age*
25 *recommended for vaccination and preferably, that do not have antibodies against [virus]/that*
26 *do not have antibodies against [pathogen] or, where justified, use [animals] with a low level*
27 *of such antibodies as long as they have not been vaccinated against [disease] and*
28 *administration of the vaccine does not cause an anamnestic response.* Administer to each
29 [animal] by a recommended route a double dose of the vaccine. Observe the [animals] at least
30 daily for *x* days (21 days for birds and 14 days for other animals).

31
32 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
33 causes attributable to the vaccine.

34
35 3-7. **Potency.** The vaccine complies with the requirements of the test mentioned under
36 Immunogenicity (section 2-4-x) when administered by a recommended route and method.
37

3.3. LIVE BACTERIAL VACCINES – TYPICAL PARAGRAPHS

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

1. DEFINITION

... vaccine (live) is a preparation of a / one or more suitable strain of [bacteria]. This monograph applies to vaccines intended for the *active immunisation* of [animals] against [disease] *caused by [bacteria]*.

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

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

2-2. CHOICE OF VACCINE STRAIN

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

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

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

2-2-1-1. *General safety.* For each test, use not fewer than x [animals] *not older than the minimum age to be recommended for vaccination and that do not have antibodies against [bacteria]*. Administer to each [animal] a quantity of the vaccine strain equivalent to not less than 10 times the maximum number of live bacteria likely to be contained in one dose of the vaccine. Observe the [animals] at least daily for x days.

The test is invalid if more than x per cent of the [animals] show abnormal signs or die from causes not attributable to the vaccine strain. The vaccine strain complies with the test if no [animal] shows notable signs of disease or dies from causes attributable to the vaccine strain.

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

The vaccine complies with the test if no [animal] shows notable signs of disease or dies from causes attributable to the vaccine and if no adverse effects on the pregnancy or the offspring are noted.

1 2-2-2. **Increase in virulence.** The test for increase in virulence consists of the administration
2 of the vaccine strain at the least attenuated passage level that will be present between the
3 master seed lot and a batch of the vaccine to [animals: number, age, free from pathogen and
4 that do not have antibodies against pathogen] sequential passages, 5 times where possible, to
5 further similar groups and testing of the final recovered bacteria for increase in virulence. If
6 the properties of the vaccine strain allow sequential passage to 5 groups via natural spreading,
7 this method may be used, otherwise passage as described below is carried out and the
8 maximally passaged bacteria that has been recovered is tested for increase in virulence. Care
9 must be taken to avoid contamination by bacteria from previous passages.

10
11 Administer to each [animal] by [route] *a quantity of the vaccine strain that will allow*
12 *recovery of bacteria for the passages described below. Administer the strain by the route to*
13 *be recommended for vaccination most likely to lead to reversion to virulence. ...* [Description
14 of the preparation of the suspension]. Administer x ml of the [suspension] by the [route] to
15 each of x other [animals] of the same age. Carry out this passage operation not fewer than 5
16 times; verify the presence of the bacteria at each passage. If the bacteria is not found at a
17 passage level, carry out a 2nd series of passages.

18
19 *Carry out the test for ... (safety) (section 2-4-1) using unpassaged vaccine strain and the*
20 *maximally passaged bacteria that has been recovered.*

21
22 The vaccine strain complies with the test if no indication of increased virulence of the
23 maximally passaged bacteria compared with the unpassaged bacteria is observed. If bacteria is
24 not recovered at any passage level in the 1st and 2nd series of passages, the vaccine strain also
25 complies with the test.

26
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*
29 *recommended for vaccination / weighing...* The quantity of the vaccine strain to be
30 administered to each [animal] is not greater than the minimum number of live bacteria to be
31 stated on the label and the strain is at the most attenuated passage level that will be present in
32 a batch of vaccine.

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

40
41 The test is invalid if ... The vaccine complies with the test if ...

42 43 3. BATCH TESTS

44
45 3-1. **Identification.** *Each strain present in the vaccine is identified by suitable morphological,*
46 *serological and biochemical methods and by culture on selective medium.*

47
48 3-2. **Safety.** Use 2 [animals] not older than the minimum age recommended for vaccination
49 and *that do not have antibodies against [bacteria]/preferably,* that do not have antibodies
50 against [bacteria] *or, where justified, use [animals] with a low level of such antibodies as*
51 *long as they have not been vaccinated against [disease] and administration of the vaccine*

1 *does not cause an anamnestic response.* Administer to each [animal] by a recommended route
2 10 doses of the vaccine. Observe the [animals] at least daily for 14 days.

3 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
4 causes attributable to the vaccine.
5

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

10 The vaccine complies with the test if it does not contain extraneous micro-organisms.
11

12 **3-4. Live bacteria.** Make a count of live bacteria on a solid medium suitable for the culture of
13 [bacteria + strain].

14 The vaccine complies with the test if one dose contains not less than the minimum number of
15 live [bacteria] stated on the label.
16

17 **3-5. Potency.** The vaccine complies with the requirements of the test prescribed under
18 Immunogenicity (section 2-2-x) when administered by a recommended route and method. It is
19 not necessary to carry out the potency test for each batch of the vaccine if it has been carried
20 out on a representative batch using a vaccinating dose containing not more than the minimum
21 number of live [bacteria] stated on the label.
22

3.4. INACTIVATED BACTERIAL VACCINES – TYPICAL PARAGRAPHS

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

1. DEFINITION

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

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

Production of the vaccine is based on a seed-lot system. The seed material is cultured in a suitable medium to ensure optimal growth under the chosen incubation conditions ; *each strain is cultivated separately and identity is verified using a suitable method*. 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 of the harvest is verified using suitable methods.

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

2-2. CHOICE OF VACCINE COMPOSITION

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

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

2-2-1. Safety

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

2-2-1-1-1. *General safety*. For each test, use not fewer than *x* [*animals*] *that do not have antibodies against [bacteria]*. Administer to each [*animal*] a quantity a double dose of the vaccine, (then one dose after the interval to be recommended, depending on the recommended vaccination schedule). Observe the [*animals*] at least daily for *x* days after the last administration. Record body temperature the day before vaccination, at vaccination, 4 h (2 h, 4 h and 6 h in specific cases) later and then daily for 4 days ; note the maximum temperature increase for each [*animal*].

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

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

11
12 The vaccine complies with the test if:
13 —no [animal] shows notable signs of disease or dies from causes attributable to the vaccine;
14 —in specific cases, the average body temperature increase for all [animal] does not exceed
15 1.5°C and no [animal] shows a rise greater than 2.0°C ;
16 —and if no adverse effects on the pregnancy and offspring are noted.

17
18 2-2-1-2. *Field studies*. The [animal] used for field trials are also used to evaluate safety. Carry
19 out a test in each category of [animal] for which the vaccine is intended. Record body
20 temperature the day before vaccination, at vaccination and during the 2 days following
21 vaccination.

22 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
23 causes attributable to the vaccine and if the average temperature increase for all [animals]
24 does not exceed 1.5 °C and no [animal] shows a rise greater than 2.0°C.

25 26 2-2-2 **Immunogenicity**

27
28 2-2-2-1. *Active immunisation*. For vaccines with claims for active immunisation against
29 [disease], a test is carried out for each route and method of administration to be recommended
30 using in each case [animals] *of the minimum age to be recommended for vaccination /*
31 *weighing...* The vaccine to be administered to each [animal] is of minimum potency.

32
33 Use for the test not fewer than *x* [animals] *that do not have antibodies against [bacteria]*.
34 Vaccinate not fewer than *x* [animals] according to the schedule to be recommended. Maintain
35 not fewer than *x* [animals] as controls. Challenge each [animal] after *x* days by [route] with a
36 sufficient quantity of a [virulent strain]. Observe the [animals] at least daily for *x* days after
37 challenge. ...

38
39 The test is invalid if ... The vaccine complies with the test if ...

40
41 2-2-2-2. *Passive protection*. For vaccines with claims for passive protection against
42 [disease], a test is carried out for each route and method of administration to be
43 recommended using in each case [animals] *of the minimum age to be recommended for*
44 *vaccination / weighing...* The vaccine to be administered to each [animal] is of minimum
45 potency.

46
47 Use for the test not fewer than *x* [animals] *that do not have antibodies against [bacteria]*.
48 Vaccinate not fewer than *x* [animals] according to the schedule to be recommended.
49 Maintain not fewer than *x* [animals] as controls. Challenge each [animal] after *x* days by
50 [route] with a sufficient quantity of a [virulent strain]. Observe the [animals] at least daily

1 *for x days after challenge. ...*

2

3 *The test is invalid if ... The vaccine complies with the test if ...*

4

5 2-3. MANUFACTURER'S TESTS

6

7 2-3-1. **Batch potency test.** It is not necessary to carry out the relevant Potency test *or tests*
8 (section 3-x) for each batch of the vaccine *if it has/they have been carried out* using a batch of
9 vaccine with a minimum potency. Where the relevant test *or tests* is/are not carried out, an
10 alternative validated method is used, the criteria for acceptance being set with reference to a
11 batch of vaccine that has given satisfactory results in the test(s) described under Potency.

12

13 2-3-2. **Bacterial endotoxins.** *A test for bacterial endotoxins (2.6.14) is carried out on the final*
14 *lot or, where the nature of the adjuvant prevents performance of a satisfactory test, on the*
15 *bulk antigen or the mixture of bulk antigens immediately before addition of the adjuvant. The*
16 *maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that*
17 *has been shown satisfactory in safety tests 2-x given under Choice of vaccine composition or*
18 *in safety test 3-x described under Batch tests, carried out using x [animals]. Where the latter*
19 *test is used, note the maximum temperature increase for each [animal] ; the vaccine complies*
20 *with the test if the average body temperature increase for all [animal] does not exceed 1.5°C.*
21 *The method chosen for determining the amount of bacterial endotoxin present in the vaccine*
22 *batch used in the safety test for determining the maximum acceptable level of endotoxin is*
23 *used subsequently for testing of each batch.*

24

25 3. BATCH TESTS

26

27 3-1. **Identification.** When *injected into/administered to* [animals] that do not have antibodies
28 against [bacteria], the vaccine stimulates the production of such antibodies.

29

30 3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it
31 comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use*
32 *(0062).*

33

34 3-3. **Safety.** Use 2 [animals] not older than the minimum age recommended for vaccination
35 and *that do not have antibodies against [bacteria] / preferably, that do not have antibodies*
36 *against [bacteria] or, where justified, use [animals] with a low level of such antibodies as*
37 *long as they have not been vaccinated against [disease] and administration of the vaccine*
38 *does not cause an anamnestic response/10 fish of one of the species for which the vaccine is*
39 *intended, having, where possible, the minimum body mass recommended for vaccination.*
40 Administer to each [animal] by a recommended route a double dose of the vaccine. Observe
the [animals] at least daily for x days.

41

42 The vaccine complies with the test if no [animal] shows notable signs of disease or dies from
43 causes attributable to the vaccine; *a transient temperature increase not exceeding 2.0°C may*
occur.

44

45 3-4. **Residual live bacteria.** Describe a test to detect residual live bacteria when the test 3-2
Bacteria and fungi above is not able to detect them.

46

47 3-5. **Potency.** The vaccine complies with the requirements of the test or test(s) mentioned
48 under Immunogenicity (section 2-2-x) when administered by a recommended route and
method.