

September 2009

CHAPTER 4.5-6.

**COLLECTION AND PROCESSING OF  
BOVINE, SMALL RUMINANT AND PORCINE SEMEN**

Article 4.56.1.

**General considerations**

The purposes of official sanitary control of semen production are to:

1. maintain the health of *animals* on an *artificial insemination centre* at a level which permits the international distribution of semen with a negligible *risk* of infecting other *animals* or humans with pathogens transmissible by semen;
2. ensure that semen is hygienically collected, processed and stored.

*Artificial insemination centres* should comply with recommendations in Chapter 4.65.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 4.56.2.

**Conditions applicable to testing of bulls and teaser animals**

Bulls and teaser animals should enter an *artificial insemination centre* only if they fulfil the following requirements.

1. Pre-quarantine Prior to entering pre-entry isolation facility

The *animals* should comply with the following requirements prior to entry into isolation at the ~~quarantine station~~ pre-entry isolation facility where the country of origin is not free.

- a. Bovine brucellosis=~~The animals should comply with~~ point 3 or 4 of Article 11.3.5.
- b. Bovine tuberculosis=~~The animals should comply with~~ point 3 or 4 of Article 11.7.5.
- c. Bovine viral diarrhoea-mucosal disease (BVD-MD)

The *animals* should be subjected to ~~the following tests~~:

- i. a virus isolation test or a test for virus antigen, with negative results; and
- ii. a serological test to determine the serological status of every *animal*.

d. Infectious bovine rhinotracheitis-infectious pustular vulvovaginitis

If the *artificial insemination centre* is to be considered as infectious bovine rhinotracheitis-infectious pustular vulvovaginitis free (IBR/IPV), the *animals* should either:

- i. come from an IBR/IPV free *herd* as defined in Article 11.13.3.; or
- ii. be subjected, with negative results, to a serological test for IBR/IPV on a blood sample.

e. Bluetongue

The *animals* should comply with Articles 8.3.6., 8.3.7. or 8.3.8., depending on the bluetongue status of the country of origin of the *animals*.

2. Testing in the ~~quarantine station~~ pre-entry isolation facility prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, bulls and teaser animals should be kept in a quarantine station pre-entry isolation facility for at least 28 days. The *animals* should be ~~subjected to diagnostic tests~~ tested as described below a minimum of 21 days after entering the ~~quarantine station pre-entry isolation facility~~, except for *Campylobacter fetus* subsp. *venerealis* and *Tritrichomonas foetus*, for which testing may commence after 7 days in quarantine pre-entry isolation. All the results should be negative except in the case of BVD-MD antibody serological testing (see point 2b)i) below).

a. Bovine brucellosis

The *animals* should be subjected to a serological test with negative results.

b. BVD-MD

- i. All *animals* should be tested for viraemia as described in point 1c) above.

Only when all the *animals* in quarantine pre-entry isolation test negative for viraemia, may the *animals* enter the semen collection facilities upon completion of the 28-day quarantine pre-entry isolation period.

- ii. After 21 days in quarantine pre-entry isolation, all *animals* should be subjected to a serological test to determine the presence or absence of BVD-MD antibodies.
- iii. Only if no sero-conversion occurs in the *animals* which tested seronegative before entry into the ~~quarantine station~~ pre-entry isolation facility, may any *animal* (seronegative or seropositive) be allowed entry into the semen collection facilities.
- iv. If sero-conversion occurs, all the *animals* that remain seronegative should be kept in quarantine pre-entry isolation ~~over a prolonged time~~ until there is no more seroconversion in the group for a period of 3 weeks. Serologically positive *animals* may be allowed entry into the semen collection facilities.

c. *Campylobacter fetus* subsp. *venerealis*

- i. *Animals* less than 6 months old or kept since that age only in a single sex group prior to ~~quarantine~~ pre-entry isolation should be tested once on a preputial specimen, with a negative result.
- ii. *Animals* aged 6 months or older that could have had contact with females prior to ~~quarantine~~ pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

d. *Tritrichomonas foetus*

- i. *Animals* less than 6 months old or kept since that age only in a single sex group prior to ~~quarantine~~ pre-entry isolation, should be tested once on a preputial specimen, with a negative result.
- ii. *Animals* aged 6 months or older that could have had contact with females prior to ~~quarantine~~ pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

e. IBR-IPV

If the *artificial insemination centre* is to be considered as IBR/IPV free, the *animals* should be subjected, with negative results, to a diagnostic test for IBR/IPV on a blood sample. If any *animal* tests positive, the *animal* should be removed immediately from the ~~quarantine station~~ pre-entry isolation facility and the other *animals* of the same group should remain in ~~quarantine~~ pre-entry isolation and be retested, with negative results, not less than 21 days after removal of the positive *animal*.

f. Bluetongue

The *animals* should comply with the provisions referred to in Articles 8.3.6~~9~~, 8.3.7~~10~~. or 8.3.8~~11~~, depending on the bluetongue status of the country or zone where the semen collection centre is located ~~of origin of the animals~~.

3. ~~Testing for BVD-MD prior to the initial dispatch of semen from each serologically positive bull~~

~~Prior to the initial dispatch of semen from BVD MD serologically positive bulls, a semen sample from each animal should be subjected to a virus isolation or virus antigen test for BVD MD. In the event of a positive result, the bull should be removed from the centre and all of its semen destroyed.~~

4. ~~Testing of frozen semen for IBR/IPV in artificial insemination centres not considered as IBR/IPV free~~

~~Each aliquot of frozen semen should be tested as per Article 11.13.7.~~

53. Testing programme for bulls and teasers resident in the semen collection facilities

All bulls and teasers resident in the semen collection facilities should be tested at least annually for the following *diseases*, with negative results, where the country or zone where the semen collection centre is located ~~of origin~~ is not free:

- a. Bovine brucellosis
- b. Bovine tuberculosis
- c. BVD-MD

*Animals* negative to previous serological tests should be retested to confirm absence of antibodies.

Should an *animal* become serologically positive, every ejaculate of that *animal* collected since the last negative test should be either discarded or tested for virus with negative results.

- d. *Campylobacter fetus* subsp. *venerealis*
  - i. A preputial specimen should be cultured.
  - ii. Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.
- e. Bluetongue

The *animals* should comply with the provisions referred to in Articles ~~8.3.6., 8.3.7. or 8.3.8~~11., depending on the bluetongue status of the country of origin of the *animals*.

- f. *Tritrichomonas foetus*
  - i. A preputial specimen should be cultured.
  - ii. Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.
- g. IBR-IPV
 

If the *artificial insemination centre* is to be considered as IBR/IPV free, the *animals* should comply with the provisions in point 2)c) of Article 11.13.3.

4. Testing for BVD-MD prior to the initial dispatch of semen from each serologically positive bull

Prior to the initial dispatch of semen from BVD-MD serologically positive bulls, a semen sample from each *animal* should be subjected to a virus isolation or virus antigen test for BVD-MD. In the event of a positive result, the bull should be removed from the centre and all of its semen destroyed.

5. Testing of frozen semen for IBR/IPV in artificial insemination centres not considered as IBR/IPV free

Each aliquot of frozen semen should be tested as per Article 11.13.7.

## Article 4.56.3.

**Conditions applicable to testing of rams/bucks and teaser animals**

Rams/bucks and teaser animals should only enter an *artificial insemination centre* if they fulfil the following requirements.

1. Pre-quarantine Prior to entering pre-entry isolation facility

The *animals* should comply with the following requirements prior to entry into isolation at the ~~quarantine station~~ pre-entry isolation facility.

- a. Caprine and ovine brucellosis ~~≡ The *animals* should comply with Article 14.1.6.~~
- b. Ovine epididymitis ~~≡ The *animals* should comply with Article 14.7.3.~~
- c. Contagious agalactia ~~≡ The *animals* should comply with points 1 and 2 of Article 14.3.1.~~
- d. Peste des petits ruminants ~~≡ The *animals* should comply with points 1, 2, and 4 or 5 of Article 14.8.7.~~
- e. Contagious caprine pleuropneumonia ~~≡ The *animals* should comply with Article 14.4.5. or Article 14.4.7., depending on the CCPP status of the country of origin of the *animals*.~~
- f. Paratuberculosis ~~≡ The *animals* should be free from clinical signs for the past 2 years.~~

## g. Scrapie

~~If the *animals* do not originate from a scrapie free country or zone as defined in Article 14.9.3., the *animals* should comply with Article 14.9.6.~~

hg. Maedi-visna ~~≡ The *animals* should comply with Article 14.6.2.~~ih. Caprine arthritis/encephalitis ~~≡ In the case of goats, the *animals* should comply with Article 14.2.2.~~

## ji. Bluetongue

The *animals* should comply with Articles 8.3.6., 8.3.7. or 8.3.8., depending on the bluetongue status of the country of origin of the *animals*.

kj. Tuberculosis ~~≡ In the case of goats, the *animals* should be subject to a single or comparative tuberculin test, with negative results.~~

## l. Border disease

~~The *animals* should be subject to a viral agent isolation test with negative results.~~

2. Testing in the quarantine station pre-entry isolation facility station prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, rams/bucks and teasers should be kept in a ~~quarantine station~~ pre-entry isolation facility for at least 28 days. The *animals* should be subjected to diagnostic tests tested as described below a minimum of 21 days after entering the ~~quarantine station~~ pre-entry isolation facility, with negative results.

- a. Caprine and ovine brucellosis ~~≡ The *animals* should be subject to testing as described in point 1c) of Article 14.1.8.~~
- b. Ovine epididymitis ~~≡ The *animals* and semen should be subject to testing as described in points 1d) and 2 of Article 14.7.4.~~
- c. Maedi-visna and caprine arthritis/encephalitis ~~≡ The *animals* and semen should be subjected to a serological test for antibodies on *animals* and semen.~~
- d. Bluetongue

The *animals* should comply with the provisions referred to in Articles 8.3.69., 8.3.710. or 8.3.811., depending on the bluetongue status of the country or *zone* where the semen collection centre is located ~~of origin of the *animals*.~~

### 3. Testing programme for rams/bucks and teasers resident in the semen collection facilities

All rams/bucks and teasers resident in the semen collection facilities should be tested at least annually for the following *diseases*, with negative results, where the country or *zone* where the semen collection centre is located ~~of origin~~ is not free:

- a. caprine and ovine brucellosis;
- b. ovine epididymitis;
- c. Maedi-visna and caprine arthritis/encephalitis;
- d. tuberculosis (for goats only);
- e. bluetongue

The *animals* should comply with the provisions referred to in Article 8.3.11.

Article 4.56.4.

### Conditions applicable to testing of boars

Boars should only enter an *artificial insemination centre* if they fulfil the following requirements.

#### 1. Pre-quarantine Prior to entering pre-entry isolation facility

The *animals* should be clinically healthy, physiologically normal and comply with the following requirements within 30 days prior to entry into isolation at the ~~quarantine station~~ pre-entry isolation facility.

- a. Porcine brucellosis ~~≡ The *animals* should comply with Article 15.4.3.~~
- b. Foot and mouth disease ~~≡ The *animals* should comply with Articles 8.5.10., 8.5.11. or 8.5.12.~~
- c. Aujeszky's disease ~~≡ The *animals* should comply with Article 8.2.8. or Article 8.2.9.~~
- d. Teschovirus encephalomyelitis

~~The *animals* should comply with Article 15.6.4. or Article 15.6.6.~~

- ed. Transmissible gastroenteritis ~~≡ The animals should comply with Article 15.7.2.~~
- fe. Swine vesicular disease ~~≡ The animals should comply with Article 15.5.5. or Article 15.5.7.~~
- gf. African swine fever ~~≡ The animals should comply with Article 15.1.5. or Article 15.1.6.~~
- hg. Classical swine fever ~~≡ The animals should comply with Articles 15.3.5. or 15.3.6.~~
- ih. Porcine reproductive and respiratory syndrome ~~≡ The animals should be subject to the test complying with the standards in the *Terrestrial Manual*.~~

2. Testing in the ~~quarantine station~~ pre-entry isolation facility prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, boars should be kept in a ~~quarantine station~~ pre-entry isolation facility for at least 28 days. The *animals* should be subjected to diagnostic tests as described below a minimum of 21 days after entering the ~~quarantine station~~ pre-entry isolation facility, with negative results.

- a. Porcine brucellosis ~~≡ The animals should comply with Article 15.4.5.~~
- b. Foot and mouth disease ~~≡ The animals should comply with Articles 8.5.13., 8.5.14., 8.5.15. or 8.5.16.~~
- c. Aujeszky's disease ~~≡ The animals should comply with Articles 8.2.12., 8.2.13. or 8.2.14.~~
- d. ~~Teschovirus encephalomyelitis~~  
~~The animals should comply with Article 15.6.8. or Article 15.6.9.~~
- ed. Transmissible gastroenteritis ~~≡ The animals should comply with Article 15.7.4.~~
- fe. Swine vesicular disease ~~≡ The animals should comply with Article 15.5.9. or Article 15.5.10.~~
- gf. African swine fever ~~≡ The animals should comply with Article 15.1.8. or Article 15.1.9.~~
- hg. Classical swine fever ~~≡ The animals should comply with Articles 15.3.8. or 15.3.9.~~
- ih. Porcine reproductive and respiratory syndrome ~~≡ The animals should be subject to the test complying with the standards in the *Terrestrial Manual*.~~

3. Testing programme for boars resident in the semen collection facilities

All boars resident in the semen collection facilities should be tested at least annually for the following *diseases*, with negative results, where the *compartment/zone* or country is not free:

- a. Porcine brucellosis ~~≡ The animals should comply with Article 15.4.5.~~
- b. Foot and mouth disease ~~≡ The animals should comply with Articles 8.5.13., 8.5.14., 8.5.15. or 8.5.16.~~
- c. Aujeszky's disease ~~≡ The animals should comply with Articles 8.2.12., 8.2.13. or 8.2.14. regarding testing every four months.~~
- d. ~~Teschovirus encephalomyelitis~~

~~The *animals* should comply with Article 15.6.8. or Article 15.6.9.~~

~~ed. Transmissible gastroenteritis = The *animals* should comply with Article 15.7.4.~~

~~fe. Swine vesicular disease = The *animals* should comply with Article 15.5.9. or Article 15.5.10.~~

~~gf. African swine fever = The *animals* should comply with Article 15.1.8. or Article 15.1.9. Routine test to be applied at least every six months.~~

~~hg. Classical swine fever = The *animals* should comply with Articles 15.3.8. or 15.3.9.~~

~~ih. Porcine reproductive and respiratory syndrome = The *animals* should be subject to the test complying with the standards in the *Terrestrial Manual*.~~

Article 4.56.5.

### General considerations for hygienic collection and handling of semen

Observation of the recommendations described in the Articles below will very significantly reduce the likelihood of the semen being contaminated with common bacteria which are potentially pathogenic.

Article 4.56.6.

### Conditions applicable to the collection of semen

1. The floor of the mounting area should be easy to clean and to disinfect. A dusty floor should be avoided.
2. The hindquarters of the teaser, whether a dummy or a live teaser animal, should be kept clean. A dummy should be cleaned completely after each period of collection. A teaser animal should have its hindquarters cleaned carefully before each collecting session. The dummy or hindquarters of the teaser animal should be sanitized after the collection of each ejaculate. Disposable plastic covers may be used.
3. The hand of the person collecting the semen should not come into contact with the *animal's* penis. Disposable gloves should be worn by the collector and changed for each collection.
4. The artificial vagina should be cleaned completely after each collection where relevant. It should be dismantled, its various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be disinfected before re-assembly using approved *disinfection* techniques such as those involving the use of alcohol, ethylene oxide or steam. Once re-assembled, it should be kept in a cupboard which is regularly cleaned and disinfected.
5. The lubricant used should be clean. The rod used to spread the lubricant should be clean and should not be exposed to dust between successive collections.
6. The artificial vagina should not be shaken after ejaculation, otherwise lubricant and debris may pass down the cone to join the contents of the collecting tube.
7. When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The vagina should also be changed when the *animal* has inserted its penis without ejaculating.
8. The collecting tubes should be sterile, and either disposable or sterilised by autoclaving or heating in an oven at 180°C for at least 30 minutes. They should be kept sealed to prevent exposure to the environment while awaiting use.

9. After semen collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory.

Article 4.56.7.

### Conditions applicable to the handling of semen and preparation of semen samples in the laboratory

#### 1. Diluents

- a. All receptacles used should have been sterilised.
- b. Buffer solutions employed in diluents prepared on the premises should be sterilized by filtration (0.22  $\mu\text{m}$ ) or by autoclaving (121°C for 30 minutes) or be prepared using sterile water before adding egg yolk (if applicable) or equivalent additive and antibiotics.
- c. If the constituents of a diluent are supplied in commercially available powder form, the water used must have been distilled or demineralised, sterilized (121°C for 30 minutes or equivalent), stored correctly and allowed to cool before use.
- d. Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluent, the product must be free of pathogens or sterilised; milk heat-treated at 92°C for 3-5 minutes, eggs from SPF flocks when available. When egg yolk is used, it should be separated from eggs using aseptic techniques. Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by, for example, pasteurisation or irradiation to reduce bacterial contamination, may be used. Other additives must also be sterilized before use.
- e. Diluent should not be stored for more than 72 hours at +5°C before use. A longer storage period is permissible for storage at -20°C. Storage vessels should be stoppered.
- f. A mixture of antibiotics should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen: gentamicin (250  $\mu\text{g}$ ), tylosin (50  $\mu\text{g}$ ), lincomycin-spectinomycin (150/300  $\mu\text{g}$ ); penicillin (500 IU), streptomycin (500  $\mu\text{g}$ ), lincomycin-spectinomycin (150/300  $\mu\text{g}$ ); or amikacin (75 $\mu\text{g}$ ), divekacin (25 $\mu\text{g}$ ).

The names of the antibiotics added and their concentration should be stated in the *international veterinary certificate*.

#### 2. Procedure for dilution and packing

- a. The tube containing freshly collected semen should be sealed as soon as possible after collection, and kept sealed until processed.
- b. After dilution and during refrigeration, the semen should also be kept in a stoppered container.
- c. During the course of filling receptacles for dispatch (such as insemination straws), the receptacles and other disposable items should be used immediately after being unpacked. Materials for repeated use should be disinfected with alcohol, ethylene oxide, steam or other approved *disinfection* techniques.
- d. If sealing powder is used, care should be taken to avoid its being contaminated.

### 3. Conditions applicable to the storage of semen

Semen for export should be stored separately from other genetic material not meeting these ~~recommendations~~ requirements of this chapter ~~in~~ with fresh liquid nitrogen in sterilised/sanitised flasks before being exported.

Semen straws should be sealed and code marked in line with the international standards of the International Committee for Animal Recording (ICAR)<sup>1</sup>.

Prior to export, semen straws or pellets should clearly and permanently be identified and placed into new liquid nitrogen in a new or sterilised flask or container under the supervision of an *Official Veterinarian*. The contents of the container or flask should be verified by the *Official Veterinarian* prior to sealing with an official numbered seal before export and accompanied by an *international veterinary certificate* listing the contents and the number of the official seal.

### 4. Sperm sorting

Equipment used for sex-sorting sperm should be clean and disinfected between *animals* according to the ~~manufacturer's recommendations~~ of the licensor of the system.

Where seminal plasma, or components thereof, is added to sorted semen prior to cryopreservation and storage, it should be derived from *animals* of same or better health status.