

**Effective October 1, 2014**, the European Union has amended its requirements for export of equine semen and embryos as stated in Annex D of Directive 92/65/EEC, as follows:

**Equine semen:**

1. Chapter I(I)(1.1): Removed reference to “permanent” supervision of the center by the center veterinarian, acknowledging that collection of equine semen is seasonal. The center must be under the supervision of the center veterinarian during the collection period.
2. Chapter II(I)(1.5): Regarding laboratories approved to conduct required testing for EIA, EVA and CEM, in addition to a “laboratory recognized by the competent authority,” added a requirement that the laboratories are assessed and accredited in accordance with ISO 17025.
3. Chapter II(I)(1.5)(b): In addition to the virus isolation test, added the option for a PCR or real-time PCR test for EVA:  
“a test for the isolation of the equine arteritis virus or the detection of its genome by polymerase chain reaction (PCR) or real-time PCR carried out with negative result on an aliquot of the entire semen of the donor stallion, unless the donor stallion has reacted with negative result at a serum dilution of one in four in a serum neutralization test for EVA.”
4. Chapter II(I)(1.5)(c): Changed CEM testing requirements, as follows:  
“an agent identification test for contagious equine metritis, carried out with negative result in each case on three specimens (swabs) taken from the donor stallion on two occasions with an interval of not less than seven days, and in no case earlier than seven days (systemic treatment) or 21 days (local treatment) after possible antimicrobial treatment of the donor stallion, from at least the following sites:
  - the penile sheath (prepuce),
  - the urethra,
  - the fossa glandis.

The specimens shall be subjected to at least one of the following tests:

- (i) culture under microaerophilic conditions for at least 7 days for the isolation of *Taylorella equigenitalis*, set up within 24 hours after taking the specimens from the donor animal, or 48 hours where the specimens are kept cool during transport; or
- (ii) polymerase chain reaction (PCR) or real-time PCR for the detection of genome of *Taylorella equigenitalis*, carried out within 48 hours after taking the specimens from the donor animal.”

5. Chapter II(I)(1.5)(a-c). Changes to the 3 testing options (resident, temporary, walk-on) are **underlined**:

- (a) If the donor stallion is continuously resident on the semen collection centre for at least 30 days prior to the date of the first semen collection and during the collection period, and no equidae on the semen collection centre come into direct contact with equidae of lower health status than the donor stallion, the tests required in point 1.5 shall be carried out on samples taken from the donor stallion at least once a year at the beginning of the breeding season or prior to the first collection of semen intended for trade in fresh, chilled or frozen semen and not less than 14 days following the date of the commencement of the residence period of at least 30 days prior to the date of first semen collection;
  
- (b) if the donor stallion is resident on the semen collection centre for at least 30 days prior to the date of the first semen collection and during the collection period, but may leave the centre occasionally under the responsibility of the centre veterinarian for a continuous period of less than 14 days, and/or other equidae on the collection centre come into direct contact with equidae of lower health status, the tests required in point 1.5 shall be carried out as follows:
  - i. at least once a year on samples taken from the donor stallion at the beginning of the breeding season or prior to the first collection of semen intended for trade in fresh, chilled or frozen semen and not less than 14 days following the date of the commencement of the residence period of at least 30 days prior to the date of first semen collection; and
  - ii. during the period of collection of semen intended for trade in fresh, chilled or frozen semen as follows:
    - the test required in point 1.5(a) on samples taken not more than 90 days prior to the collection of semen for trade,
    - the test required in point 1.5(b) on samples taken not more than 30 days prior to the collection of semen for trade, unless the non-shedder state of a donor stallion is confirmed by virus isolation test, PCR or real-time PCR carried out on samples of an aliquot of the entire semen taken not more than 6 months prior to the collection of semen for trade and the donor stallion has reacted with positive result at a serum dilution of at least one in four in a serum neutralisation test for equine viral arteritis,
    - the test required in point 1.5(c) on samples taken not more than 60 days prior to the collection of semen for trade, which in the case of PCR or real-time PCR may be carried out on three specimens (swabs) taken on a single occasion.

- (c) if the donor stallion does not meet the conditions in points (a) and (b) and the semen is collected for trade in frozen semen, the tests required in point 1.5 shall be carried out on samples collected from the donor stallion as follows:
- i. at least once a year at the beginning of the breeding season;
  - ii. during the storage period provided for in point 1.3(b) of Section I of Chapter III and before the semen is removed from the centre or used, on samples taken not earlier than 14 days and not later than 90 days following the date of collection of the semen.

By way of derogation from point (ii) of the first subparagraph, post-collection sampling and testing for equine viral arteritis as described in 1.5(b) is not required in case the non-shedder state of a seropositive donor stallion is confirmed by virus isolation test, PCR or real-time PCR carried out with negative result on samples of an aliquot of the entire semen of the donor stallion taken twice a year at an interval of at least four months and the donor stallion has reacted with positive result at a serum dilution of at least one in four in a serum neutralisation test for equine viral arteritis.

#### **Equine embryos:**

1. Chapter III(II)(1.8) has been reworded:  
The embryos shall be washed and have an intact *zona pellucida*, or the embryonic capsule in case of equine embryos, before and immediately after washing. In accordance with the IETS Manual, the standard washing procedure shall be modified to include additional washes with the enzyme trypsin where recommended for the inactivation or removal of certain pathogens;
2. Chapter III(II)(1.10) has been reworded and magnification has increased from 40x to 50x. The *zona pellucida* of each embryo, or the embryonic capsule in case of equine embryos, shall be examined over its entire surface area at not less than 50 × magnification and certified to be intact and free of adherent material.

#### **Chapter IV. Requirements for equine embryo donor females:**

4. In addition to the requirements laid down in Directive 90/426/EEC, donor mares shall:
  - 4.1 not be used for natural breeding during at least 30 days prior to the date of collection of ova or embryos and between the date of the first sample referred to in points 4.2 and 4.3 and the date of the collection of ova and embryos;
  - 4.2 be subjected with negative result to an agar-gel immuno-diffusion test (Coggins test) or an ELISA for equine infectious anaemia carried out on a blood sample taken not less than 14 days following the date of the commencement of the period

of at least 30 days referred to in point 4.1 and not more than 90 days prior to the collection of ova or embryos for trade;

4.3 be subjected to an agent identification test for contagious equine metritis, carried out with negative result in each case in a laboratory referred to in point 1.5 of Chapter (II)(I) on at least two specimens (swabs) taken from the donor mare in no case earlier than seven days (systemic treatment) or 21 days (local treatment) after possible antimicrobial treatment of the donor mare, from at least the following sites:

- the mucosal surfaces of the clitoral fossa,
- the clitoral sinuses.

The specimens shall be taken during the period referred to in point 4.1 on two occasions with an interval of not less than seven days in the case of the test referred to in point (i), or on one occasion in the case of the test referred to in point (ii).

The specimens shall be placed in transport medium with activated charcoal, such as Amies medium, before dispatch to the laboratory.

The specimens shall be subjected to at least one of the following tests:

- (i) culture under microaerophilic conditions for at least seven days for the isolation of *Taylorella equigenitalis*, set up within 24 hours after taking the specimens from the donor animal, or 48 hours where the specimens are kept cool during transport; or
- (ii) polymerase chain reaction (PCR) or real-time PCR for the detection of genome of *Taylorella equigenitalis*, carried out within 48 hours after taking the specimens from the donor animal.