STANDARDS OF LABORATORY FOR COLLECTION AND PROCESSING OF SEMEN

General consideration

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

1. Maintain the health of animals on semen production center or unit at a level which permits the international distribution of semen with a negligible risk of infecting other animals or humans with specific pathogenic organisms that can be transmitted through semen;
2. Ensure that semen is hygienically collected, processed and stored.

Part A . Bovine semen

1. Conditions applicable to semen production centre or unit

1.1. The semen production centre or unit shall comprise of:
   a) Accommodation areas for bulls used for semen production (including one isolation facility for sick animals), bull waiting yard and a semen collection room. These two premises are hereon designated pre-requisite for semen production.
   b) Semen processing laboratory and semen storage and dispensing room.
   c) Administration offices.

A *quarantine station* may also be attached to the centre provided that it is on a different location from that of part (a) and (b) of section 1.1.

2. Conditions applicable to semen processing area

2.1. The semen production unit should include separate and distinct areas for accommodating complete health tested resident bulls, bull waiting yard, semen collection yard, feed store, silage pit and isolation of disease suspected bulls.

2.2. Only animals associated with semen production should be permitted to enter the semen production area. All animals meant for semen production must meet the minimum health requirements for donor animals as prescribed by the Ministry.

2.3. Animals should be adequately isolated to prevent the transmission of disease from farm livestock and other animals. Measures should be in place to prevent the entry of wild animals.

2.4. Personnel at the unit/ centre should wear special protective clothing and footwear at all times while in the semen processing unit to preclude introduction of pathogenic organisms.

2.5. Visitors to the semen collection facilities should be kept to a minimum and visits should be subject to formal authorization and control. Equipment for use with the livestock in the semen processing area should be disinfected prior to entry.

2.6. Vehicles used for transport of animals to and from the semen production unit should not be allowed to enter the premises.

2.7. The housing for animals and semen collection areas should be cleaned and disinfected at regular interval.

2.8. Fodder introduction and manure removal should be done in a manner which posses not significant animal health risks.

3. Conditions applicable to semen processing laboratories

3.1. A proper bio-security must be maintained at the semen processing laboratory for semen evaluation and processing, semen pre-storage and storage with separate areas for artificial vagina cleaning and preparation. Entry to the laboratory should be prohibited to unauthorized personnel.

3.2. The laboratory personnel should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms during semen evaluation, processing and storage.

3.3. Visitors to the laboratory should be kept to a minimum and visits should be subject to formal authorization and control.

3.4. A laboratory should be constructed with materials that permit effective cleaning and disinfection.

3.5. A laboratory should be regularly cleaned. Work surfaces for semen evaluation and processing should be cleaned and disinfected at the end of each working day.

3.6. The laboratory should be rodents and insects proof as needed to control pests.

3.7. A storage rooms and individual semen containers should be easy to clean and disinfect.

3.8. Only semen collected from donors bulls having a certified pedigree and health status should be processed in the laboratory.

4. Conditions applicable to donor bulls and teasers animals

4.1. Bulls and teaser animals can enter semen production center only if they fulfill following requirements:

4.1.1 Pre-quarantine testing

Bovine must appear healthy and normal and must comply with the following requirements prior to entry into isolation at the quarantine station or isolation unit prior to entering the semen collection areas.

a) Bovine brucellosis

Bovine kept in a country or zone free from bovine brucellosis, or from a herd officially free from bovine brucellosis and subjected to a serological test for bovine brucellosis with negative results during the 30 days prior to shipment; or

Bovine kept in a herd free from bovine brucellosis and subjected to buffered brucella antigen and complement fixation tests with negative results during the 30 days prior to shipment.

b) Bovine tuberculosis

Bovine subjected to the tuberculin test for bovine tuberculosis with negative results during the 30 days prior to shipment and come from a herd officially free from bovine tuberculosis; or

Bovine subjected to the tuberculin test for bovine tuberculosis with negative results during the 30 days prior to shipment and come from a country or zone officially free from Bovine Tuberlcosis

c) Bovine viral diarrhoea-mucosal disease (BVD-MD)

The animal should be subjected to the following test:

A virus isolation test or a test for virus antigen, with negative results;

A serological test to determine the serological status of every animal.

d) Infectious bovine rhinotracheitis-infectious pastular vulvovaginitis(IBR-IPV)

If the artificial insemination center is to be considered as IBR/IPV free, the animals should either:

- Come from an IBR/IPV free herd, or
- Be subjected to a serological test for IBR/IPV on a blood sample with negative results,

4.1.2. Test in the quarantine station prior to entering the semen processing unit

Prior to entering the semen processing unit, bovines must be kept in a quarantine station 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, except for Campylobacter fetus and Trichomonas foetus, for which testing may commence after at least 7 days in quarantine station, and the result should be negative except in the case of BVD-MD antibody serological testing.

a) Bovine brucellosis

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

b) BVD-MD

1. All animals should be tested for viraemia as described in point 4.1.1 c) above.
2. After 21 days in quarantine, all animals should be subjected to a serological test to determine the presence or absence of BVD-MD antibodies.
3. Only if no sero-conversion occurs in the animals that tested sero-negative before entry into the quarantine station, may any animal (seronegative or seropositive) be allowed entry into the semen collection unit.
4. If sero-conversion occurs, all the animals that remain sero-negative should be kept in quarantine station over a prolonged time until there is no more sero-conversion in the group for a period of 3 weeks.

c) *Compylobacter fetus* subsp. venerealis

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

d) Trichomonas foetus

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

e) IBR- IPV

If the Semen collection center or unit is to be considered as IBR/IPV free, the animals should be subjected to a diagnostic test with negative result on a blood sample. If any animal test positive, these animals should be removed immediately from the quarantine station and other animals of the same group should remain in quarantine and be tested with negative result, not less than 21 days after removal of the positive animals.

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

Prior to dispatch of semen from BVD-MD serologically positive bulls, semen sample from each animal should be subject to a virus isolation or virus antigen ELISA 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.

4.1.4. Testing of frozen semen for IBR/IPV in Semen collection center or unit not considered as IBR/IPV free.

Each aliquot of frozen semen should be subjected to a virus isolation test, with negative result.

4.1.5. Testing program for bovines resident in the semen collection facilities

All bovines resident in the semen collection facilities should be tested at least annually for the following diseases, with negative results:

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) *Camplylobacter fetus* **(**subsp. *Venerealis)*

1. A preputial specimen should be cultured.
2. 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) Trichomonas foetus:

1. A preputial specimen should be cultured.
2. 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.

f) IBR/IPV

If the Semen collection center is to be considered as IBR/IPV free, diagnostic tests on blood samples for IBR/IPV of all breeding bulls repeated at maximum intervals of 12 months, with a negative result.

5. Conditions applicable to the management of bulls

The objective is to keep bulls in a satisfactory state of cleanliness, particularly of the lower thorax & abdomen.

5.1. Whether on pasture or housed, the bull should be kept under hygienic conditions. If housed, the litter must be kept clean and renewed as often as necessary.

5.2. The Coat of the bull should be kept clean and generally short.

5.3. The Length of the tuft of hairs at the preputial orifice, which is invariably soiled, should be cut to about 2 cm. The hair should not be removed altogether because of its protective role. If cut too short, irritation of the preputial mucus may result.

5.4. The animal should be brushed regularly and where necessary on the day before semen collection paying special attention to the underside of the abdomen.

5.5. In the event of obvious soiling, there should be careful cleaning with soap or a detergent of the preputial orifice and the adjoining areas followed by thorough rinsing and drying.

5.6. When the bull is brought to the collection area, the technician must make sure that the bull is clean and that it is not carrying any excessive litter or particles of feed on its body or its hooves for such materials are always heavily contaminated.

6. Conditions applicable to the collection of semen

6.1. The floor of the mounting area should be easy to clean and to disinfect. A dusty floor should be avoided.

6.2. The hindquarters of the teaser, whether a dummy or a live teaser animal must be kept clean.

6.3. Before actual collection, bull should be allowed one or two false mount for sexual stimulation.

6.4. The hand of the person collecting the semen must not come in contact with the bull's penis. Disposable gloves should be worn by the collector and changed for each collection.

6.5. The Artificial Vagina must be cleaned completely after each collection; 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 reassembly using approved chemical or method such as the use of 70% ethyl /or 98-99% isopropyl, ethylene oxide or steam. Once reassembled, it should be kept in a cupboard, which is regularly cleaned and disinfected.

6.6. The lubricant used should be clean. The rod used to spread the lubricant must be clean and should not be exposed to dust between successive collections.

6.7. 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.

6.8. When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The A.V. should also be changed when the bull has inserted its penis without ejaculation.

6.9. The Collecting tubes should be sterile and either disposable or sterilized by autoclaving or heating in an oven at 180o c for at least 30 minutes. They should be kept sealed to prevent exposure to the environment while awaiting use.

6.10. 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.

7. Conditions applicable to frozen semen production in the laboratory

7.1. Diluents preparation

7.1.1. All receptacles used should be sterilized.

7.1.2. Buffer solution employed in diluents preparation on the premises should be sterilized by filtration or by autoclaving (121^{o}c for 30 minutes) or be prepared using sterile water before adding egg yolk and antibiotics.

7.1.3. If the constituents of diluents are supplied in commercially available powder form, the water used must be distilled or dimeneralised, sterilized (121^{o} c for 30 minutes or equivalent) stored correctly and allowed to cool before use.

7.1.4. When egg yolk is used, it should be separated from egg albumen using aseptic techniques. Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by for example pasteurization or irradiation to reduce bacterial contamination may be used.

7.1.5. Diluents 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.

7.1.6. A mixture of antibiotic should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen produced: either gentamicin (250 μg), tylosin (50 μg), lincomycin-spectinomycin (150/300 μg), or penicillin (500 IU), streptomycin (500 IU). The names of the antibiotic added and their concentration should be stated in the international veterinary certificate.

7.2. Semen evaluation, processing and packaging

7.2.1. The tube containing freshly collected semen should be sealed and kept in the water bath of temperature ranging from 37 + 1°C until evaluation, dilution and processed. The evaluation of semen (visual & microscopical) should not take more than 5 minutes;

a) Visual examination; reading the volume, colour and presence of foreign bodies is done immediately after the collection.

b) Microscopical examination; mass activity, initial motility, concentration.

7.2.2 Dead percentage of freshly collected semen during evaluation should not exceed 30%. Similarly major and minor abnormalities should not exceed more than 20 and 30 % respectively.

7.2.3. The mass activity should be minimum scale of 2 (1-good, 2 – very good, 3- excellent) and initial motility of minimum 60% shall be accepted for processing.

7.2.4. After the evaluation add equal volume of extender to the fresh semen until the final concentration is worked out using standard procedure. The final dilution should be worked out at minimum of 20 x 106 spermatozoa per straw.

7.2.5. After dilution and during refrigeration the semen should also be kept in a stoppered container.

7.2.6. During the course of filling, receptacles such as semen straws and other dispatch items should be used immediately after being unpacked. Materials for repeated use should be sterilized with alcohol, ethylene oxide, steam or other approved sterilization techniques.

7.2.7. If sealing powder (pvc) is used care should be taken to avoid its contamination.

7.2.8. The filled and sealed straws, after equilibration of the straws at 4°C, should be LN2 vapour freezed at -80 to -90 °C for 10 minutes before plunging in liquid nitrogen.

7.2.9. The minimum post thaw motility (PTM) fit for storage and distribution should be 40%.

7.3. Storage, quarantine and distribution of frozen semen

7.3.1. Only semen having minimum of 40% PTM should be stored.

7.3.2. Before distribution the frozen semen should undergo a quarantine period of 1 month during which microbial load is studied in the batch of semen using standard procedure. During the quarantine period all semen which do not fulfill the required standard should be discarded.

7.3.3. Semen for distribution should be stored in liquid nitrogen in LN2 containers. Semen straws in the goblet should be code marked for records and identification.

Part B. Small ruminant semen

1. Conditions applicable to semen processing centre

1.1. The centre should be under the direct supervision of a veterinarian approved by the Department of Livestock.

1.2. The centre should be built so as to ensure total isolation, thus preventing any contact with animals on the outside, and so as to facilitate cleaning and disinfection of the various installations. Each centre should comprise at least the following distinct installations;

a) Housing and isolation of animals

b) Semen collection room or mounting area
c) Room for cleaning, disinfection and storage of artificial vaginas
d) Laboratory where the semen is prepared, which should contain separate facilities for cleaning and sterilization (glassware, etc);
e) Storage room
f) Room for the distribution of semen.

1.3. Only personnel at the centre and the veterinary authority are permitted to enter installations were animals are housed or the laboratory area; no other person may do so unless authorized and supervised by the centre veterinarian. The personnel shall not enter the animal section or the laboratory section without first removing outdoor clothing and putting on clothing which is specific to each section. Only animals having attained a health status equivalent to that of the population of the centre (see below) shall be allowed to enter the centre. Any vehicles used for their transport must remain outside. No vehicle or material from outside the centre shall be allowed within the closed area of the animal section, other then new materials to be used exclusively within the centre.

1.4. Semen processing centre situated in areas where viral vector-borne diseases occur should be constructed and managed in such a way that the animals are protected from vectors, or semen collection should take place only during seasons when the vectors are at low level of activity.

1.5. The semen processing centre must be subjected to regular inspection at least once a year by the official BAFRA.

1.6. They must be supervised so as to ensure that only sheep and goat with the required health status are housed therein and that only authorized personnel may enter, after passing through a suitable cloakroom area.

1.7. A register shall be maintained containing the information of each animal's identification, its date of birth, date of entry, date of departure or death, breed and all the diagnostic test results, vaccination and treatment carried out.

1.8. The good condition of the animals and the mounting area including hygiene of the premises must be maintained well.

2. Conditions applicable to the introduction of donor animals.

2.1. Only duly identified animals from an approved quarantine station are authorized to enter the centre. These animals should have spent a minimum of 30 days in the quarantine station where they should not be in contact with any animal.

2.2. The animals should originate from flocks which are not subjected to any movement restrictions on health grounds. The flocks must have been free from disease that are notifiable, free or officially free from caprine and ovine brucellosis and free from clinical signs of the following diseases:
a) Contagious agalactia (Mycoplasma agalactiae), for at least 6 months;

b) Peste des petits ruminants, Contagious caprine pleuropneumonia, Caseous lymphadenitis and Ovine epididymitis, for at least 12 months;
c) Paratuberculosis, for at least 2 years;
d) Scrapie, Pulmonary adenomatosis and Maedi-visna or Caprine arthritis/encephalitis (CAE), for at least 3 years.

2.3. During quarantine, the animals must be subjected to clinical examination, in particular to check the integrity of their reproductive organs and their good health status. The results of the microscopic examination of their semen must be compatible with artificial insemination usage. Authorization should not be given for the dissemination of semen collected in the quarantine station.

2.4. The animals must be subjected to following diagnostic tests with negative results in countries considered affected;
a) for tuberculosis (for goat only), single for comparative tuberculin test;
b) for caprine and ovine brucellosis, buffered antigen test coupled with complement fixation test;
c) for ovine epididymitis, a complement fixation test coupled with culture of a semen sample;
d) for maedi-visna or CAE, a serological test;
e) for Border disease, a virus isolation test;
f) for contagious caprine pleuropneumonia (for goats only), a serological test;
g) for blue tongue, a serological test.

2.5. Animals leaving a quarantine station for a semen processing centre must be free from any clinical signs of disease and must originate from quarantine station which meets the following condition on the day of departure:
a) be situated in the centre of a 10 km zone in which there has been no case of foot and mouth disease and Peste des petits ruminants for at least 30 days;
b) be free form notifiable disease of the species concerned.

3. Testing programme for donor animals

All animals housed in Semen processing centre shall undergo, with favorable results, the following examinations and controls at least twice a year:

3.1. Clinical examination, with particular attention given to the reproductive organs (testicular and epididymal palpation);

3.2. Microscopic examination of semen and examination of any somatic cells present; in the event of any anomalies, a microbe count with qualitative detection of specific micro-organisms should be carried out;

3.3. Diagnostic tests in countries infected:
a) for caprine and ovine brucellosis;
b) for ovine epididymitis, serological tests coupled with culture of semen sample;
c) for maedi-visna or CAE;

d) for tuberculosis (for goat only), single or comparative tuberculin test;
e) for blue tongue.

Should any of the above tests or examinations give unfavorable results, the accreditation of the centre should be suspended and the animal concerned must be isolated and eliminated as quickly as possible and its semen collected since the date of last negative examination discarded. This semen may be sent to the official veterinary laboratories for further investigation if necessary.

The health status of the remaining animals in the centre should be reviewed and, if relevant, appropriate procedures for restoration of its accreditation should be carried out.

4. Conditions applicable to the collection, processing, packing and storage of semen

4.1. Only semen collected in the centre may be processed therein.

4.2. The collection, handling, packing and storage of semen should be carried out exclusively in the areas set aside for this purpose.

4.3. Any equipment coming into contact with semen or the donor animal during collection, processing or packing must be suitably cleansed, disinfected and sterilized immediately after use.

4.4. Any products of animal origin used in the treatment of semen, including diluents and additives, shall originate from a source free from any health risks, or be treated prior to use to render the products safe.

4.5. Receptacles used for storage and transport must be suitably disinfected and sterilized before the start of any filling operation.

4.6. Where a cryogenic agent is used, this should not have been used previously for other products of animal origin.

4.7. Each individual dose of semen should be clearly marked to enable the date of collection of semen, the identity of the donor animal, and the name of the centre to be easily ascertained, if necessary by means of code.

5. Conditions applicable to trading of semen

Semen intended for trade must fulfill following conditions:

5.1. Semen straws shall be code marked in line with national standards.

5.2. Containers must be sealed before export and accompanied by an international veterinary certificate listing the following contents.

5.2.1. Semen collected in an accredited centre duly supervised as described above, and in which there were no case of foot and mouth disease, peste des petits ruminants within the radius of 10 km during the three months prior to and 30 days following collection;

5.2.2. Semen come from animals which:

a. Spent an uninterrupted period of at least 30 days prior to and 30 days after collection in the centre.

b. Showed no clinical signs of disease during that period;

c. Were not used for natural service during the 30 days prior to collection;

5.3. Have been securely stored, for at least 30 days before shipment, in receptacles which were cleansed, disinfected and sterilized before use and which left the storage place duly sealed.

An international veterinary certificate attesting compliance with the above conditions must accompany the semen, suitably identified, during transport. The names and concentration of antibiotics included in the semen diluent should be stated in the certificate.

Part C: Porcine semen

1. Conditions applicable to semen processing centre

1.1. The centre should be officially approved by the Department of Livestock.

1.2. The centre should be under the sanitary control of BAFRA including the checking of health and welfare of animals at the centre at least every 6 months.

1.3. The centre should be under the overall supervision of the Department of Livestock

1.4. Only swine associated with semen production should be permitted to enter the centre. Other species of livestock may exceptionally be resident on the centre, provided that they are kept physically apart from the swine.

1.5. Swine on the centre should be adequately isolated from farm livestock on adjacent land or buildings for instance by natural or artificial means.

1.6. The entry of visitors should be strictly controlled. Personnel at a centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only on the centre should be provided.

1.7. Individual semen containers and storage rooms should be capable of being disinfected.

2. Conditions applicable to the introduction of boars

2.1. Boars should enter a semen processing centre if they fulfill the requirements laid down by the BAFRA.

2.2. The semen from boars with genetic defects or associated with genetic defects in near relatives may not be eligible for export.

2.3. Boars must be clinically healthy and physiologically normal and must pass pre-entry tests within the 30 days prior to entry into isolation at a semen processing centre. The prescribed diseases and tests are listed under point 3-testing programme for boars.

2.4. Boars must remain in isolation at the semen processing centre for a period of at least 30 days before being retested to meet the standards listed under the point 3-testing programme for Boars. Boars may enter the stud on the successful completion of these tests and must be clinically healthy.

3. Testing programme for boars

3.1. Definitions

Prescribed tests cover a minimal range of diseases from which all boars on semen processing centre must be free.

Routine tests are tests applied at regular intervals to confirm the continued freedom from disease of the stud.

3.2. Prescribed tests

a) Brucellosis (B. abortus, B. suis)

Boars to give negative results to serological tests.

3.3. Routine tests

Routine tests to be applied at least every 6 months for a & b and every 12 months for c & d.

a) Swine vesicular disease
Boars to give negative results to a serum-neutralization test.

b) Swine fever
Boars to give negative results to enzyme-linked immunosorbent assay and indirect immunofluorescent tests.

c). Enterovirus encephalomyelitis (ex Teschen disease)
Boars to meet certification standards.

d) Vesicular stomatitis

Boars to give negative results to a complement fixation test.

Claims of country freedom from some viral and bacterial infections of swine may be given consideration providing such claims are backed by serological survey data and epidemiological investigation.

4. Optional tests and requirements

Semen processing centre may be required by the BAFRA to include in their veterinary prophylactic programme a number of other diseases, either through vaccination or by requiring negative results to serological tests.

Additionally, some importing countries may require assurances of freedom from a disease (for example: classical swine fever, Aujeszky's disease) based on negative serology or other biological tests. The range of infections to be covered is extensive and beyond the capacity of semen processing centres to support totally. Thus, only optional tests remain to be applied and interpreted by bilateral agreement when importation of semen is being considered.

Records of the progeny of a donor boar should be maintained as far as possible to determine that he is not associated with any genetic defect. The records of the boar should indicate his fertility. The semen must be obtained from a boar with a normal libido.

5. Conditions applicable to diluents

Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluents, the product must be free of pathogens or sterilized; milk heat-treated at 92°C for 3-5 minutes, eggs from disease free flocks when available. The inclusion of penicillin, streptomycin, polymixin etc. is permitted, provided that this is declared in the international veterinary certificate.

6. Conditions applicable to the packing of semen

6.1. The examination of ejaculates, and the dilution and freezing of semen must be carried out in a laboratory maintaining the hygienic standards set by the BAFRA.

6.2. Only semen of a health standard equivalent to that produced in a semen processing centre should be handled.

6.3. The tube containing freshly collected semen should be sealed and kept in the water bath of temperature ranging from 37 $\pm$ 1°C until evaluation, dilution and processed. The evaluation of semen (visual & microscopical) should not take more than 5 minutes;

a) Visual examination; reading the volume, colour and presence of foreign bodies is done immediately after the collection.