# STANDARD

# FOR PORCINE SEMEN QUALITY

# IN

# AI CENTRES

**January 2017**

# AHDB PORK STANDARD FOR PORCINE SEMEN QUALITY IN AI CENTRES

## 1. Legal Requirements

1.1 The collection, processing and distribution of semen must be carried out in premises, which comply with The Artificial Insemination of Pig (Animal Health) (England and Wales) Regulations 1964, (domestic market only) The Artificial Insemination of Pigs (EEC) Regulations 1992 and the Animal Product (Import and Export) Regulations 2004.

## 2 Semen Collection

2.1 The Company must have a documented protocol for semen collection.

2.2 The Company must have documented cleaning schedules in place for the cleaning and disinfection of the semen collection area. These must include responsibility for cleaning, frequency of cleaning, methodology, cleaning chemicals to use, dilution rates, contact time and reference to the manufacturer's instructions in their use.

2.3 The effectiveness of the cleaning and disinfection must be verified.

2.4 Semen should be diluted with a volume of extender that is no less than 75% of the ejaculate volume within 15 minutes after collection.

2.5 The temperature gradient from collection to despatch must be checked and recorded as a minimum, once per month.

2.6 All materials used in the collection of raw semen and its transportation to the laboratory must be kept in a warming cabinet at the temperature range of + 33°C to + 40°C prior to collection.

2.7 Equipment used to heat materials must be visually clean and temperature controlled.

2.8 The temperature settings of the pre collection warming cabinets and or boxes in the collection area must be checked on collection days. A maximum-minimum thermometer must be used that shows the range over which the temperature is varying.

2.9 The warming cabinet must have the capacity to achieve a set temperature of between +33°C and +40°C.

2.10 All materials and equipment used for the collection of semen must be non-spermicidal and should either be tested or be supplied with a Certificate of Conformity. Semen tested from a minimum of **6** boars, with recommendations to test from **10** boars.

| | Control sample | Test sample |
|---|---|---|
| % motile cells day 0<br>% progressive motile cells day 0<br>% abnormal cells day 0 | | |
| % motile cells day 3 (72 ± 2 hours) *<br>% progressive motile cells day 3 (72 ± 2 hours)* | | |
| % motile cells day 6 (144 ± 2 hours)**<br>% progressive motile cells day 6 (144 ± 2 hours)**<br>% abnormal cells day 6 (144 ± 2 hours)** | | |

** If day 3 is on a day that the center is closed for production, you may choose either to perform this
test on day 2 or day 4. Preference is for day 4.

** If day 6 is on a day that the center is closed for production, you may choose to perform this test
on day 7

2.11 The Company Protocol must state what level of deterioration in the test sample relative to the control sample is acceptable, and how the results from each ejaculate should be considered (e.g. averaged or each result considered individually) before a particular product will be deemed to have failed the toxicity test. Records must be maintained of all testing undertaken.

2.12 Records must be maintained of supplies received.

2.13 Appropriate action should be taken to address any equipment failures and temporary arrangements organised to cover any deficiency.

2.14 Details of the cleaning and disinfection of the semen collection area must be recorded ( cf 2.3 )

## 3 Semen Transport and Processing Laboratory (External / Internal)

3.1 A separate reception room for semen or a hatch with sliding doors at each side must be provided

3.2 The Company must have a documented protocol, which describes the hygiene and temperature control procedures in place between collection of the semen and delivery to the laboratory.

3.3 Employees who have worked with pigs must shower and change clothes if they are to work in the laboratory on the same day.

3.4 Containers used to transport semen from the barn / collection to the laboratory must be disposable or easy to clean / disinfect to ensure proper hygiene.

3.5 The Company must have satisfactory hygienic precautions in place to prevent barn air entering / contaminating the laboratory.

3.6 Hand cleaning shall be performed at the frequency defined in the Company protocol.

3.7 All hair, where appropriate, on the basis of a risk assessment, shall be fully contained by the use of a hairnet.

3.8 Company issued protective clothing shall be worn and changed as frequently as defined in the Company protocol.

3.9 Transport must be designed to prevent direct sunlight (UV) radiation.

3.10 During transportation to the laboratory shaking of the semen should be kept to a minimum.

3.11 The transport protocol must detail precautions to prevent large temperature fluctuations.

## 4 Semen Quality Assessment

4.1 General

4.1.1 On arrival at the laboratory all ejaculates should be assessed for quality.

4.1.2 There must be a written protocol for recording temperature and control in the processing area.

4.1.3 The Company must have documented cleaning schedules in place for the cleaning and disinfection of the Laboratory. These should include responsibility for cleaning, frequency of cleaning, methodology, cleaning chemicals to use, dilution rates, contact time and reference to the manufactures instructions in their use.

4.1.4 The temperature in the warming cabinets must be monitored. The collection / transfer hatch does not need to be temperature controlled unless it is being used as a warming cabinet as well, in which the semen is stored between 30 to 34 degrees C, until processed.

## 4.2 Macroscopic Fresh Semen Assessment

4.2.1 The Company must have a written protocol for macroscopic semen assessment and rejection of ejaculates that do not meet standards.

4.2.2 When checking with standard weights indicates a mis-calibration, the scales must be calibrated externally or replaced.

4.2.3 Weighing scales used for ejaculate or doses must be calibrated at least weekly using standard weights and details recorded. Calibration of the scales should be performed with standard weights in the same range for which the scale is used. The Company Protocol must state the calibration points and acceptable deviation at each calibration point for each weighing scale. Calibration record sheets must show the calibration points and acceptable ranges. Actions taken in the event of a calibration reading outside of the acceptable range must be recorded.

4.2.4 External calibration of weights should be undertaken on the basis of risk assessment and may not be necessary where commercially available standard weights are used.

4.2.5 The accuracy of concentration measuring equipment must be tested using an external reference laboratory annually, or by using a Nucleo-Counter or CASA. (Discussed at length 2016)

4.2.6 In house checks on the concentration measuring equipment must be performed at least four times per year with recalibration if necessary.

4.2.7 In the case of equipment failure, procedures must be in place to ensure a thorough review is undertaken of the likely effect on product quality since the last test.

## 4.3 Microscopic Fresh Semen Assessment

4.3.1 The Company must have a written protocol for microscopic semen assessment, which must describe equipment used, requirements of equipment, control of proper functioning of equipment and operational guidelines.

4.3.2 A heated microscope stage and / or slide warmer must be used. The temperature of both the heated microscope stage and slide warmer

(where applicable) must be checked daily and be 38ºC +/- 1ºC or within the range defined in the Company protocol. Records of checking must be kept.

4.3.3 Pipettes must be cleaned and calibrated – internally every month and externally once a year or as per manufacturer's instructions if more frequent checking is recommended. Records of pipette cleaning and calibration should be maintained.

4.3.4 Amounts being weighed must be within normal range of scales.

4.3.5 Microscope objectives and oculars must be cleaned on a regular basis but at least once a month

4.3.6 The microscope must be maintained / serviced on a regular basis.

4.3.7 Records must be kept of maintenance and servicing undertaken.

4.3.8 The microscope must be maintained professionally at least once a year.

4.3.9 Training must be provided in the care of the microscope and a protective cover must be used when not in use.

## 4.4 Morphological semen assessment

4.4.1 The Company must have a written protocol for morphological semen assessment including quality control checking.

4.4.2 Every boar in use must be fully evaluated for semen morphology at least once every 4 weeks. This evaluation must be carried out using stain and at least 100 cells must be counted. There must be at least one interim rapid check between full counts. A full check must be carried out where the rapid check indicates that morphological defects could be over 30%.

4.4.3 Boars which have morphological defects in excess of 30% in their ejaculates, must be evaluated for morphology every week – until at least two ejaculates meet the standards. Semen must be collected at the normal collection frequency with a maximum of twice a week.

4.4.4 Where full (stained) line-speed morphology tests are carried out, the Company has the discretion to sell the next good ejaculate from a boar which had previously failed a morphology test. Where full line-speed morphology tests are not carried out, semen must not be sold from a boar which has failed a morphology test until after two successive collections have been shown to have normal morphology.

4.4.5 The maximum percentage of morphologically abnormal cells permitted is 30%. Ejaculates with more than 30% abnormal cells must be rejected with the exception of pooled semen where semen from boars with up to

35% morphological defects may be used but the mixed pool must have less than 30% morphological defects and the use must be declared to customers.

## 4.5 Post Dilution Motility

4.5.1 The Company must have a written protocol for post-dilution semen motility assessment.

4.5.2 Post dilution motility assessment must be performed on a minimum of 6 different batches of semen on each production day or on all batches if less than 6 being produced.

## 4.6 Longevity Test

4.6.1 The Company must have a written protocol for a longevity testing regime with a clear description of which ejaculates will be tested and the testing interval to be applied for each ejaculate. The regime must be sufficiently robust to identify any boars whose ejaculates deteriorate below 60% motility by the relevant expiry date. The protocol must take into account the days of the week on which samples are typically collected and tested.

4.6.2 The Company must have a written protocol for the re-activation of stored / cooled semen

4.6.3 The longevity of semen from all boars in use must be tested as a minimum of once every two weeks unless ejaculates are pooled. Ejaculates which are pooled must be longevity tested once every two weeks and in batches which fail all boars within pools that fail must be individually tested.

4.6.4 Every ejaculate from boars with abnormal longevity must not be used in production and the boars must be tested and ejaculates not used in production until 2 ejaculates in a row have normal longevity again.

4.6.5 Every ejaculate from boars with potentially affected longevity (on the basis of within Company risk assessments of e.g. health, vaccination, etc) should be tested until 2 ejaculates in a row have normal longevity.

## 4.7 Standard for Semen Quality

4.7.1 Operational procedures must be designed and followed so as to ensure that all ejaculates processed for sale meet the minimum requirements for semen quality, which are listed in the Table of Minimum Semen Quality requirements (Appendix 1).

4.7.2 Where scoring scales are used the Company must describe in detail how the scale intervals are defined and related to objective measurements

4.8 Data Recording

4.8.1 All data related to semen quality assessment must be recorded in such a way that one can report the history on a per boar and / or per day basis. If an electronic data sheet is set up to record these data, it should be backed up regularly.

4.9 A system must be in place to monitor the performance of semen on customers farms or farms owned by the Breeding Company.

## 5 Extender Preparation and Controls

5.1 Extenders should only be used if there is adequate scientific data to demonstrate fitness for purpose

5.2 The Company must have a written protocol for extender preparation.

5.3 Every new batch of extender must be tested for toxicity

5.4 Records must be kept of all batch numbers used on each production day.

5.5 The Company must have a protocol in place for the measurement of the pH of each batch of extender used in production or a representative sample as determined on the basis of a risk assessment. Records must be kept of number of batches tested and readings for exceptions. The pH (fresh extender) should be the extender pH recommended by the supplier ± 0.3.

5.6 The Company must have a protocol in place for the measurement of conductivity of water. Checks must be undertaken on the conductivity of water following de-ionisation or distilling for every batch used.

5.7 There should be no Colony Forming Units in batches of prepared extender on a bacterial counting plate after 24hrs storage and 48 hours incubation @ 30$^{o}$C. Frequency of testing on the basis of risk assessment, but as a minimum new batches of extender must be tested when first used.

5.8 In instances of >0 – 50 Colony Forming Units, the affected diluent may be regarded as acceptable if the AI made from this diluent is <300cfu/ml after culture on the day of expiry. A risk assessment of this practice must occur every quarter, using all of the 4 monthly QC samples being produced from this stored diluent. Full traceability of AI doses produced from this stored diluent must be documented.

5.8.1 5.8 is only applicable for AI centres that can demonstrate a consistent 90% pass rate for the rolling previous 12 months, via their monthly QC checks and random sampling by AHDB PORK. This would be ongoing and the count set to “0 months passed’’ if a failure level above 10% occurs *for any subsequent batch*.

5.9 Records must be maintained of mineral tests, change of cartridges and servicing. Frequency of testing and servicing must be on the basis of risk assessment.

5.10 The Company must have a protocol for cleaning and disinfection procedures in place for the purifier and the storage tanks. These must include responsibility for cleaning, frequency of cleaning, methodology, cleaning chemicals to use, dilution rates, contact time and reference to the manufactures instructions in their use. The interval between cleaning and disinfection procedures can be determined on the basis of a risk assessment protocol, which describes bacteriological testing, cleaning and disinfection. This protocol must be available for inspection. Alternatively cleaning and disinfection must be carried out at a frequency of not less than once a month.

5.11 The extender powder must be mixed with an appropriate volume of production water and mixed thoroughly.

5.12 When not for direct use prepared extender must be stored in a clean closed container under refrigeration.

5.13 Bacteriological quality of stored extender must be checked by culturing samples for microbiological contamination. Centres must establish that bacteriological quality can be maintained up to the maximum potential storage time for extenders under their own conditions, which requires testing at intervals for bacterial growth up to the maximum storage time used. This should be repeated on several batches and repeated at yearly intervals to establish if extenders can be kept for the specified maximum storage time.

5.14 Extender must be stored according to the Manufacturer’s recommendations

## 6 Semen Dilution

6.1 The Company must have a written protocol for semen dilution.

6.2 The addition of the extender must be carried out within 15 minutes of collection.

6.3 The temperature of the extender should be within ± 2°C of semen temperature at the time of the initial dilution. Verification checks should be undertaken.

6.4 The sperm number per dose must not be less than 1.8 billion. There is also a maximum tolerance of 20% below the declared breeding Company average dose as stated on the documentation supplied to the customer. The declared average dose will depend upon the processing method and expected level of variation. Sperm number supplied for low dose post-cervical AI will be as per customer requirements.

6.5 Bacteriology testing

The Company must have a written protocol for bacteriology testing which defines when samples are to be taken, when and where they are to be tested and what action should be taken in the event of a result which is out of tolerance. The testing protocol must include incubation at $30^0$C for 48 hours. A minimum of 2 diluted semen doses and 1 sample of prepared extender must be tested per month. The semen does must be tested within 24 hours of the expiry date. The acceptable limit for semen doses is less or equal to 300 CFU/ml; the acceptable limit for prepared extender is less than 10 CFU/ml. The lab results must be reported in the following bands :- <10, 11-300, 301-1000, 1001– 100000 and >100000. Records must be kept of any actions taken in response to out of tolerance results.

## 7 Production Data Recording

7.1 The Company must retain records, which demonstrate effective control of semen quality and cover the scope of this standard.

7.2 All records must be genuinely produced and be legible.

7.3 Records must be retained for a minimum of 2 years.

7.4 All records must be stored in such a manner as to be readily retrievable.

## 8 Semen Storage/Despatch

8.1 The Company must have a documented protocol for semen storage.

8.2 Semen must be cooled and stored at a temperature of +17$^o$C plus or minus 2$^o$C.

8.3 Once a month, a sample of at least 5 insemination doses must have their temperature checked and recorded. This check should be carried out on days when the interval from semen dilution to collection from the stud is at its shortest. Records must be kept of the batch number of the packs checked, the date and time of dilution, the date and time of the temperature

check and the temperature obtained. If out of tolerance results are obtained, corrective action must be taken and recorded.

8.4 Semen storage areas or containers must be temperature controlled.

8.5 A maximum-minimum thermometer or other temperature recording system must be used that shows the range over which the temperature is varying. If the cool-room is deliberately set to a lower temperature for the first few hours after collection, the temperature monitoring regime should be adjusted accordingly.

**Product quality assurance**

9.1 AI centres have to demonstrate how they assure product quality standards are maintained for sperm count, longevity and morphology (from a minimum average of 4 samples per month) and bacteriology (from a minimum average of 2 samples per month) . In addition 1 sample of prepared extender should be submitted for bacteriology and have <10 cfu / ml @$30^0$ c and 48hrs incubation. See Appendix 3.

## 10 Independent and Internal audits

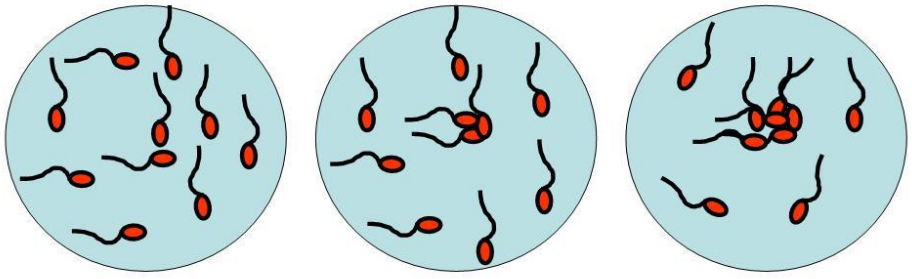
10.1 AI centres must receive an independent audit at intervals once every calendar year with a signed summary of the visit sent to AHDB Pork following each visit.

10.2 AI centres must undertake internal assessments at least once per year at regular interval(s) between external audits to review policy, procedures and performance

**Appendix 1:** Minimum Semen Quality requirements.

| | | | |
|---|---|---|---|
| ***Macroscopic*** | | | |
| Colour | | | Grey / white |
| Odour | | | normal |
| Contamination | | | none visible |
| ***Microscopic*** | | | |
| Fresh semen quality | | | |
| % Motile cells | Pre / Post dilution | = or > | 70% |
| | | = or > | 4 |
| Agglutination (scale 0-3) | | < | 3 |
| ***Morphology*** | | | |
| Total abnormal cells | | < | 30% |
| ***Longevity*** | | | |
| % Motile cells | Expiration date | = or > | 60% |
| | | = or > | 3 |

- Agglutination levels:
  - Low: <10%
  - Mediate: [10% -20%]
  - High:>20%



Low: <10% Mediate: [10% -20%] High:>20%

**Appendix 2:** Extender requirements

| | |
|---|---|
| Osmolarity | Depends on extender, see technical information from provider ± 15 mOsm |
| Water quality | Deionised water (water quality 1) |
| Water conductivity maximum | At 25°C: <20 µS/cm (0.02 mS/cm). |
| Extender conductivity | Manufacturer's recommendations |
| Temperature | Semen temperature ± 2°C |
| pH (fresh extender) | Manufacturer's recommendations ± 0.3 |
| Bacterial contamination | 0 CFU* after 48 hours incubation at 37°C |

*CFU = Colony Forming Units

## Appendix 3: Random Monthly Testing

### 1. Sample testing

1.1. Centres must test a minimum of 4 randomly chosen semen packs from their normal production batches every calendar month for sperm count, morphology and longevity and 2 for bacteriology. The sperm count test can either be carried out in-house using CASA or SP100 equipment, or by an independent accredited laboratory. Morphology, longevity and bacteriology can be carried out at an independent lab or at a lab owned by the breeding company other than the lab at the specific stud (i.e. a stud cannot carry out tests for this purpose in its own lab), unless morphology and longevity screening is undertaken by a named trained technician not normally involved with semen analysis. If the doses collected during the AHDB Pork random sampling process do not achieve the acceptable pass rate, then the 4 monthly AI samples have to be sent to an external lab for independent screening until the pass rate has been achieved.

1.2. A record must be kept of the results supplied by AHDB Pork from the random samples, to show the pass level achieved.

### 2. Benchmarks

2.1. It is the responsibility of individual Centres to ensure that in every rolling period of 13 calendar months with effect from October 1, 2007 the results from samples tested meet the benchmarks for parameters agreed by the AHDB PORK AI Standard Technical Advisory Group.

| ***Parameter*** | ***Limit*** | ***Criteria*** |
|---|---|---|
| ***Semen dose*** | | |
| Sperm count | <10% | Samples where more than 20% below the declared breeding Company average. |
| ***Morphology*** | | |
| Total abnormal cells | <7% | Samples with more than 30% abnormal sperm cells |
| ***Longevity*** | | |
| % Motile cells at expiration date | <10% | Samples with less than 60% motility at expiration. |
| ***Bacteriology*** | | |
| CFU/ml at expiration date | <10% | Samples with more than 300 CFU/ml at expiration. |

### 3. Compliance

**3.1.** Centres which do not meet one or more of the agreed benchmarks in any rolling period of 13 calendar months may test additional samples at their own expense to demonstrate that they are in compliance with the Standard.

**3.2.** Centres must not fail to meet the agreed benchmarks for the rolling periods of 13 calendar months for more than 6 consecutive months.

## 4. Appeals procedure

**4.1.** Any queries relating to sample testing should be raised, in writing, with the AHDB Pork AI Programme Manager.

**4.2.** Queries that are not resolved can be tabled for decision by the AI Standard Technical Advisory Group

## Appendix 4: Calibration and Control of Measuring

1.1. Appropriate equipment for inspecting, measuring, weighing and testing must be available that is, where necessary, regularly calibrated against nationally recognised standards. Where a traceable calibration is not possible, the Company shall demonstrate the basis by which the standardisation is carried out. Equipment to be calibrated would include scales, colorimeters, weights, pipettes, pH meters, conductivity meters and thermometers.

1.2. Calibration must be undertaken to a set schedule and where standard solutions are involved, these must be within their use-by date.

1.3. The calibration status of equipment must be identified.

1.4. The accuracy required for each piece of equipment must be appropriate to its function.

1.5. Records of all calibration testing should be maintained.

1.6. The company protocol must define the acceptable range for each piece of equipment to be calibrated. This information must be incorporated into relevant record sheets. Records must be kept of actions taken when out of tolerance results are obtained.

## Appendix 5: Other Recommendations

***As from October 2015, the ability to opt out of being audited against the recommendations is withdrawn, but non-compliances will not require rectification.***

1. *Processing laboratories should ideally be air conditioned / temperature controlled as shown by temperature records (cf. 4.1)*

2. *A phase contrast microscope is recommended.*

3. *It is recommended that quality control checks should be undertaken on all new batches of extender pre-mix, which should include physical appearance, bacteriology, pH, conductivity, and toxicity e.g. split ejaculate for both existing and new batch. (cf. 5.5)*

4. *The Company should have a protocol in place for the measurement the conductivity or osmolarity of each batch of extender used in production or a representative sample as determined on the basis of a risk assessment. Records must be kept of number of batches tested and readings for exceptions. For a 12-month period there should be random testing of osmolarity of extender batches. Osmolarity should be within a range of ± 15 mOsm of that indicated in the technical information supplied by the provider. (cf.5.7)*

5. *It is recommended that semen should be transported to the farm at a temperature of +17°C plus or minus 2°C.*

6. *It is recommended that the cooling equipment should be linked to an automatic alarm system.*

7. *It is recommended that toxicity testing is carried out on a minimum of 10 boars' ejaculates per sample.*

8. *It is recommended that during internal audits lab staff are calibrated for their accuracy of motility and morphology assessments with records of these calibrations retained to show what was done and the results achieved.*

9. *It is recommended that all batches of diluted semen are temperature checked using an infra-red thermometer prior to despatch.*