



**STANDARD
FOR PORCINE SEMEN QUALITY
IN
AI CENTRES**



1st November 2011

BPEX 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 manufacturers 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 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 **2** boars, with recommendations to test from 6 boars

	Control sample	Test sample
% motile cells day 0		
% abnormal cells day 0		
% motile cells day 2 (48 ± 2 hours)		
% motile cells day 4 (96 ± 2 hours)		
% motile cells day 6 (144 ± 2 hours)		
% abnormal cells day 6 (144 ± 2 hours)		

- 2.11 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)
- 2.15 Details of checks on the temperature gradient from collection to despatch be recorded. (cf. 2.5)
- 2.16 Even if a Certificate of Conformity is supplied each batch or a representative sample of materials and equipment must be tested for toxicity. (cf. 2.10)

3 Semen Transport and Processing Laboratory (External / Internal)

- 3.1 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.
- 3.2 Containers used to transport semen from the barn / collection to the laboratory must be disposable or easy to clean / disinfect to ensure proper hygiene.
- 3.3 The Company must have satisfactory hygienic precautions in place to prevent barn air entering / contaminating the laboratory.
- 3.4 There should be no direct contact between barn employees and laboratory employees. Barn employees must change into clean protective clothing if working in the laboratory on the same day.

- 3.5 Hand cleaning shall be performed at the frequency defined in the Company protocol.
- 3.6 All hair, where appropriate, on the basis of a risk assessment, shall be fully contained by the use of a hairnet.
- 3.7 Company issued protective clothing shall be worn and changed as frequently as defined in the Company protocol.
- 3.8 Transport must be designed to prevent direct sunlight (UV) radiation.
- 3.9 During transportation to the laboratory shaking of the semen should be kept to a minimum.
- 3.10 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 miscalibration, the scales must be calibrated externally or replaced.
- 4.2.3 The accuracy of scales used for weighing ejaculate or doses must be undertaken at least weekly using standard weights and details

recorded.. Calibration of the scale should be performed with standard weights in the same range for which the scale is used.

- 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 through cross centre checking using NucleoCounter or CASA in both instances.
- 4.2.6 In house checks on the concentration measuring equipment must be performed at least four times per year with recalibration if necessary.

4.3 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 The temperature of both the heated microscope stage and slide warmer (where applicable) must be checked daily and be 38°C +/- 1°C and recorded
- 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.

4.4 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 with 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 standards. Semen must be collected at the normal collection frequency with a maximum of twice a week.
- 4.4.4 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 longevity testing with a clear description of the longevity testing interval and the expiration time after the ejaculate production.
- 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.

- 4.6.6 Semen must show a minimum of 60% motility after activation and re-warming at the expiry date (i.e. the last recommended usage date).

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.

5 Extender Preparation and Controls

- 5.1 The Company must have a written protocol for extender preparation.
- 5.2 Every new batch of extender must be tested for toxicity
- 5.3 Records must be kept of all batch numbers used on each production day.
- 5.4 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 .
- 5.5 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.
- 5.6 There should be no Colony Forming Units in batches of prepared extender on a bacterial counting plate after 24hrs storage and 48 hours incubation @ 37°C. Frequency of testing on the basis of risk assessment.

- 5.7 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.
- 5.8 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.
- 5.9 The extender powder must be mixed with an appropriate volume of production water and mixed thoroughly.
- 5.10 When not for direct use prepared extender must be stored in a clean closed container under refrigeration.
- 5.11 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.
- 5.12 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 $\pm 2^{\circ}\text{C}$ of semen temperature at the time of the initial dilution. Verification checks should be undertaken.
- 6.4 The sperm number per dose must be in the range of 2.15 – 6 billion with a tolerance for the minimum of 20% from the declared breeding Company average as stated on the documentation supplied to the customer. The recommended target is 2.3 billion sperm per dose. Lower numbers of sperm may be used with records of prior consent from customers.

- 6.5 The volume of the semen dose should be minimum of 68ml.
- 6.6 The acceptable limit for ready-diluted semen doses is less or equal to 300 CFU/ml when sampled at, or near to the expiry date and incubated for 48hrs at 30⁰ C Report results in the following bands :-

Up to 10, 11-300,301-1000, 1001– 00000 and more than 100000

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°C plus or minus 2°C.
- 8.3 Temperature must be recorded at least once a month, from a random sample of at least 5 insemination doses from the last batch processed, at the time of despatch or final packaging.
- 8.4 Semen storage areas or containers should be temperature controlled.
- 8.5 A maximum-minimum thermometer or other temperature recording system should be used that shows the range over which the temperature is varying.

9 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) .

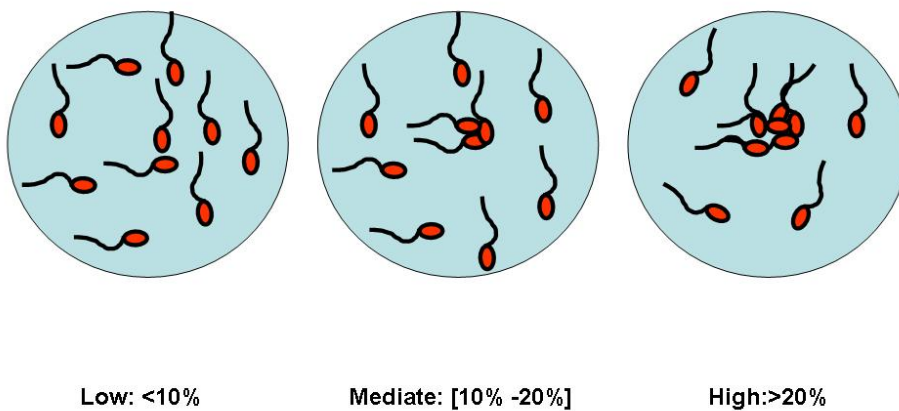
10 Independent audit and Veterinary visits

- 10.1 AI centres must receive an independent audit at intervals once every calendar year with a signed summary of the visit sent to BPEX 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		= or >	70%
	Pre / Post	= or >	4
	dilution	<	3
Agglutination (scale 0-3)			
Morphology			
Total abnormal cells		<	30%
Longevity			
% Motile cells		= or >	60%
	Expiration date	= or >	3

- Agglutination levels:
 - 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: $<20 \mu\text{S/cm}$ (0.02 mS/cm).
Extender conductivity	Manufacturer's recommendations
Temperature	Semen temperature $\pm 2^\circ\text{C}$
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: BPEX AI Standard Reference Laboratory

1. Sample testing

- 1.1. Centres should test a minimum of 4 randomly chosen semen packs from their normal production batches every calendar month and 2 for bacteriology

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 BPEX AI Standard Technical Advisory Group.

<i>Parameter</i>	<i>Limit</i>	<i>Criteria</i>
<i>Semen dose</i>		
Sperm count	<10%	Samples where more than 20% are below the declared breeding Company average.
<i>Morphology</i>		
Total abnormal cells	<7%	Samples with more than 30% abnormal sperm cells
<i>Longevity</i>		
% Motile cells at expiration date	<10%	Samples with less than 60% motility at expiration.
<i>Bacteriology</i>		
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 BPEX Programme AI Manager.
- 4.2.** Queries that are not resolved can be tabled for decision by the BPEX AI Standard Technical Advisory Group

Appendix 4: Calibration and Control of Measuring

1. Recommendations

- 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. Examples of equipment to be calibrated would include scales, colorimeters, weights, pipettes and thermometers.*
- 1.2. Calibration must be undertaken to a set schedule.*
- 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. 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.*
- 1.6. Records of all calibration testing should be maintained.*

Appendix 5: Other Recommendations

- 1 It is recommended that a separate reception room for the semen with extra ventilation is provided.)A hatch with sliding doors at each side acceptable. In standard and audited (cf. 3.3)*
- 2 It is recommended that barn employees should shower if working in the laboratory on the same day. In standard (cf. 3.4)*
- 3 Processing laboratories should ideally be air conditioned / temperature controlled as shown by temperature records (cf. 4.1)*
- 4 The use of a heated microscope stage and slide r is recommended. (cf. 4.3)*
- 5 It is recommended that the microscope be maintained professionally at least once a year. (cf. 4.3)*
- 6 It is recommended that training should be provided in the care of the microscope. A protective cover is recommended when not in use. (cf. 4.3).in the standard*
- 7 A phase contrast microscope with a heated stage is recommended.*
- 8 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)*
- 9 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)*
- 10 It is recommended to microbiologically test every batch of extender used in the production of semen doses. (cf.5.9)*
- 11 Extenders should only be used if there is adequate scientific data to demonstrate fitness for purpose*
- 12 It is recommended that a system should be in place for evaluating on farm performance on customers' or owned farms.*
- 13 It is recommended that semen should be transported to the farm at a temperature of $+17^{\circ}\text{C}$ plus or minus 2°C .*

14 It is recommended that for production units the cooling equipment should be linked to an automatic alarm system.