STANDARD

FOR PORCINE SEMEN QUALITY

IN

AI CENTRES

September 2024

AHDB PORK STANDARD FOR PORCINE SEMEN QUALITY IN AI CENTRES

Operational procedures must be designed and followed so as to ensure that all ejaculates processed for sale meet the minimum requirements for semen quality.

Standards are marked as C=Critical, S=Significant, LS= Less Significant, R=Recommendation

Actions must be taken towards removing non-conformances of Standards marked as C in 72hours, marked as S in 1 week and LS in 2 weeks, respectively, with a timeline to completion. Failure will lead to suspension.

1. Protocols

1.1 Semen Collection

1.1.1 The Company must have a documented protocol for semen collection including good hygiene technique when using hand collection method. S

1.1.2 The Company must have documented cleaning schedules in place for the cleaning and disinfection of the semen collection area and equipment used for in pen collection in own pens. These must include the 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. S

1.2 Semen Transport and Processing Laboratory (External / Internal)

1.2.1 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. LS

1.2.2 There must be a protocol for transport of semen from the moment of collection to the time the semen is received at the lab. Staff must follow the protocol and take appropriate precautions to prevent large temperature fluctuations during transport. S

1.3 Semen Quality Assessment - General

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

1.3.2 The Company must have documented cleaning schedules in place for the cleaning and disinfection of the Laboratory. These include but are not limited to the responsibility for cleaning, frequency of cleaning, methodology, cleaning chemicals to use, dilution rates, contact time and reference to the manufactures instructions in their use. S

1.4 Semen Quality Assessment -Macroscopic Fresh Semen Assessment

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

1.4.2 Weighing scales used for ejaculate or doses must be calibrated at least weekly using standard weights and the details recorded. Calibration of the scales must 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. S

1.5 Semen Quality Assessment -Microscopic Fresh Semen Assessment

1.5.1 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. If CASA equipment is used, it must be done according to manufacturer's guide. C

1.6 Semen Quality Assessment - Post Dilution Motility

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

1.7 Semen Quality Assessment - Longevity Test

1.7.1 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. C

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

1.8 Semen Quality Assessment -Standard for Semen Quality

1.8.1 Where scoring scales are used for semen quality assessment the Company must describe in detail how the scale intervals are defined and related to objective measurements S

1.9 Extender Preparation and Controls

1.9.1 The Company must have a written protocol for extender preparation. C

1.9.2 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 and also trade name, vat identification, water volume, extender powder weight, water conductivity (or resistivity), extender conductivity (or osmolarity or refractive index), pH (section 1.9.2), extender temperature, date, and technician initials. The pH (fresh extender) must be the extender pH recommended by the supplier ± 0.3.An additional internal QC step to confirm proper extender preparation , With the options of conductivity, refractometry, osmolarity be within the reference range supplied by the extender supplier S

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

1.9.4 The Company must have a protocol for cleaning and disinfection procedures in place for the purifier and the storage tanks. These must include the 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 no less than once a month. S

1.10 Semen Dilution

1.10.1 The Company must have a written protocol for semen dilution. S

1.10.2 The Company must have a written protocol for bacteriology testing of diluted semen. It defines, when samples are to be taken, when and where they are to be tested, and what action shall be taken in the event of a result which is out of tolerance. The testing

protocol must include incubation at 30°C for 48 hours. 22-44 diluted semen doses must be tested per quarter, and at least 1 sample of prepared extender and 1 pure water sample must be tested per month.

The semen doses must be tested within 24 hours of the expiry date. The acceptable limit for semen doses is less or equal to ≤100 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. It is not necessary to test new batches of semen extender for bacteriology prior to use. S

1.11 Semen Storage/Despatch

1.11.1 The Company must have a documented protocol for semen storage. C

2. Collection Barn

2.1 Semen Collection

2.1.1 Details of the cleaning and disinfection of the semen collection area must be recorded. LS

2.1.2 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. LS

2.1.3 Equipment used to heat materials for semen collection must be visually clean and temperature controlled. LS

2.1.4 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. LS

2.1.5 The warming cabinet used during semen collection must have the capacity to achieve a set temperature of between +33°C and +40°C. LS

2.2 Semen Transport and Processing Laboratory (External / Internal)

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

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

2.2.3 Transport of the semen must be designed to prevent direct sunlight (UV) radiation. LS

2.2.4 During transportation from the collection area to the laboratory shaking of the semen must be kept to a minimum. S

3. Lab processing

3.1 Semen Collection

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

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

3.2 Semen Transport and Processing Laboratory (External / Internal)

3.2.1 The lab for semen processing must have a separate reception room or a hatch with sliding doors at each side. S

3.3 Semen Quality Assessment - General

3.3.1 On arrival at the laboratory all ejaculates must be assessed for quality. C

3.4 Semen Dilution

3.4.1 The addition of the extender to the semen must be carried out within 15 minutes of collection. S

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

4. Semen quality assessment: Post dilution

4.1 Semen Quality Assessment -Morphological semen assessment

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

4.1.2 Every boar in use must be fully evaluated for semen morphology at least once every 4 weeks. This evaluation must be carried out using the stain method or the CASA system

with auto-morphology feature. Using the stain or fixed mount method 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%. Where a CASA system with an auto-morphology feature is in place, every boar in production is analysed at time of collection using the CASA system. A full stain, fixed mount check is only required for new boars entering the stud. Each company to record internal competency testing of staff . C

4.1.3 New boars which have morphological defects in excess of 30% in their ejaculates, must be evaluated prior to or at the next collection using stain, fixed mount method . Boars which pass morphology analysis following a failed test can only be used in production after a manual count . C

4.1.4 The maximum percentage of morphologically abnormal cells permitted is 35%. Ejaculates with more than 35% abnormal cells must be rejected with the exception of pooled semen where the mixed pool must have less than 30% morphological defects. For mixed pools semen, boars with the maximum of 30% morphological defects may be used. Morphologically abnormal cells can be compensated for by increasing the number of cells. Every 5% of extra abnormalities needs 10% extra cells. The minimum number of cells added needs to be 1.23 billion cells. S

4.2 Semen Quality Assessment - Post Dilution Motility

4.2.1 Post dilution motility assessment must be performed on all batches of semen on each production day . S

4.3 Semen Quality Assessment - Longevity Test

4.3.1 The longevity of semen from all individual boars in use must be tested at every collection unless ejaculates are pooled. Ejaculates which are pooled must be longevity tested every pool and in batches which fail all boars within pools that fail must be individually tested. C

4.3.2 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 consecutive ejaculates have normal longevity again. S

4.3.3 Every ejaculate from boars with potentially affected longevity (on the basis of Company risk assessments of e.g. health, vaccination, etc) must be tested until 2 consecutive ejaculates have normal longevity. S

5. Semen Storage & dispatch

5.1 Semen Storage/Dispatch

5.1.1 Semen must be cooled and stored as per extender manufacturer's recommendation. S

5.1.2 Once a month, a sample of at least 5 stored insemination doses must have their temperature checked and recorded. This check shall 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. S

5.1.3 Semen storage areas or containers must be temperature controlled. S

5.1.4 For semen storage 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 must be adjusted accordingly. S

6. Staff

6.1 Semen Transport and Processing Laboratory (External / Internal)

6.1.1 Hand cleaning during all processes must be performed at the frequency defined in the Company protocol. LS

6.1.2 Company issued protective clothing must be worn and changed as frequently as defined in the Company protocol. S

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

7. Maintenance & monitoring of lab & kit

7.1 Semen Collection

7.1.1 Appropriate action must be taken to address any equipment failures for the semen collection and temporary arrangements organised to cover any deficiency. S

7.2 Semen Quality Assessment - General

7.2.1 The temperature in the warming cabinets in the laboratory 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˚C, until processed. LS

7.3 Semen Quality Assessment -Macroscopic Fresh Semen Assessment

7.3.1 Weighing scales used for ejaculate or doses must be calibrated externally or replaced, when checking semen with standard weights indicates a mis-calibration. S

7.3.2 External calibration of weights for weighing scales used for ejaculate or doses must be undertaken on the basis of risk assessment and may not be necessary where commercially available standard weights are used. LS

7.3.3 The accuracy of concentration measuring equipment must be tested using an external reference laboratory annually, or by using a Nucleo Counter that is tested against an external reference laboratory at least annually S

7.3.4 In house checks on the concentration measuring equipment must be performed at least four times per year with recalibration if necessary. This applies to CASA and Nucleo-Counter if used for measuring concentration. The ability of individual technicians to reliably maximize the potential of the instrument should also be demonstrated . S

7.3.5 In the case of quality assessment 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. S

7.3.6 In case of quality assessment equipment failure there must be a documented contingency plan in place to ensure production can be continued fulfilling the Standard. C

7.4 Semen Quality Assessment -Microscopic Fresh Semen Assessment

7.4.1 For microscopic assessment of fresh semen, CASA equipment or a phase contrast microscope with a heated 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 38˚C +/- 1˚C or within the range defined in the Company protocol. Records of checking must be kept. LS

7.4.2 Pipettes used in quality assessment 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 must

be maintained. S

7.4.3 Amounts being weighed during quality assessment must be within normal range of scales. LS

7.4.4 Microscope objectives and oculars for quality assessment must be cleaned on a regular basis of at least once a month. Where CASA is used, the CASA camera must be checked and cleaned at least twice per year or on the basis of risk assessment. The camera focus must be checked at the start of processing and checked periodically during processing. All Cleaning must be recorded. LS

7.4.5 The microscope used for quality assessment must be maintained / serviced internally on a regular basis. The microscope must be maintained professionally at least once a year. Both applies if the CASA instrument is used for assessment. Records must be kept for all maintenance and services. S

7.4.6 Records of undertaken maintenance and servicing of all microscopy equipment must be kept. LS

7.4.7 Training must be provided in the care of the microscope and, if used, the CASA equipment. A protective cover for the microscope must be used when not in use. Records must be kept for the trainings. S

7.5 Extender Preparation and Controls

7.5.1 Frequency of testing and servicing of the water purification kit must be on the basis of risk assessment. Records must be maintained of mineral tests, change of cartridges and servicing. LS

8. Extender preparation

8.1 Extender Preparation and Controls

8.1.1 Extenders must only be used if there is adequate scientific data to demonstrate fitness for purpose. S

8.1.2 Records must be kept of all extender batch numbers used on each production day. trade name, vat identification, water volume, extender powder weight, water conductivity (or resistivity), extender conductivity (or osmolarity or refractive index), pH (section 1.9.2), extender temperature, date, and technician initials. S

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

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

8.1.5 Extender powder must be stored according to the Manufacturer's recommendations.

9. Toxicity testing

9.1 Semen Collection

9.1.1 Records must be kept of all incoming consumables which come into contact with semen and / or extender. These records must include the batch number. Where the manufacturer does not give a batch number, the lab must assign one itself to each separate delivery such that traceability can be maintained. LS

9.1.2 All materials and equipment used for the collection of semen must be non- spermicidal and must either be tested or be supplied with a Certificate of Conformity. Semen tested from a minimum of 6 boars. S

9.2 Extender Preparation and Controls

9.2.1 Every new batch of extender powder must be tested for toxicity. S

9.2.2 Every batch of prepared extender must be checked for physical appearance and tested for pH and conductivity prior to use. If pH and / or conductivity are outside of manufacturer's recommendations, the batch must not be used. S

9.3 Semen Collection

9.3.1 The Company protocol for semen collection must state what level of deterioration in the test sample relative to the control sample is acceptable, and how the results from each ejaculate shall 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. S

10. Bacteriology

10.1 Extender Preparation and Controls

10.1.1 Made up extender which has been stored for 24 hrs or longer should have no Colony Forming Units on a bacterial counting plate after incubation for 48hrs at 30°C. Frequency of testing on the basis of risk assessment, but as a minimum new batches of extender must be tested when first used. LS

11. General

11.1 Legal Requirements

11.1.1 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 Animal Insemination of Pigs (EEC) Regulations 1992 and the Animal Product (Import and Export) Regulations 2004. C

11.2 Semen Quality Assessment

11.2.1 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 must be backed up regularly. C

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

11.3 Semen Dilution

11.3.1 When Diluted, There will be 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 AI (for example post-cervical) will be in accordance to client requirements subject to their approval S

11.4 Production Data Recording

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

11.4.2 All production data records must be genuinely produced and be legible. S

11.4.3 Production data records must be retained for a minimum of 2 years. S

11.4.4 All production data records must be stored in such a manner as to be readily retrievable. S

11.5 Product quality assurance

11.5.1 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 must be submitted for bacteriology and have <10 cfu / ml at 30˚C and 48hrs incubation. See Appendix 3. C

11.6 Independent and Internal audits

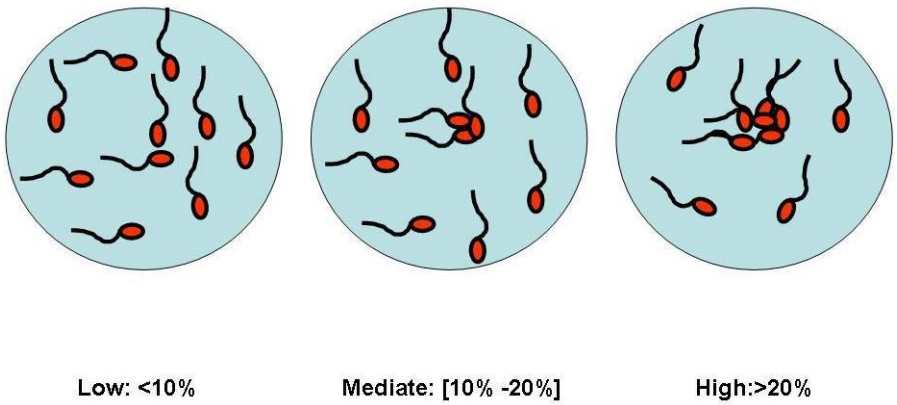
11.6.1 AI centres must receive an independent audit at intervals once every calendar year with a signed summary of the visit sent to AHDB Pork following each visit. C

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

Appendix 1 Minimum Semen Quality requirements. C

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

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



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

Appendix 2 Extender requirements C

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. Morphology testing can be done using slides or using the stud's own CASA or another CASA machine owned by the same company. 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. S

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

2. Benchmarks

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

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 100 CFU/ml at expiration.
Water resistivity		>10 MOhm

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 the problem has been rectified. S

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. C

4. Appeals procedure

4.1 Any queries relating to sample testing must be raised, in writing, with the AHDB Pork AI Programme Manager. Queries that are not resolved can be tabled for decision by the AI Standard Technical Advisory Group. S

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 must 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. If a CASA machine is used, density must be calibrated against an SP100 or another CASA. CASA morphology must be tested against stained slides or Fixed mount. " S

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

1.3 The calibration status of equipment must be identified. S

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

1.5 Records of all calibration testing must be maintained. LS

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. S

Appendix 5 - Other Recommendations

As from October 2015, the ability to opt out of being audited against the recommendations is withdrawn. As from October 2020, non-compliances will require rectification.

1. *The Company should have a protocol in place for the measurement and the 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. Osmolarity, conductivity or refractometry methods can be used. For a 12-month period there should be random testing of extender batches. Osmolarity should be within a range of ± 15 mOsm of that indicated in the technical information supplied by the provider. R*

2. *It is recommended that semen should be transported to the farm at a temperature within the appropriate recommended range for the extender in use, unless alternative delivery has been approved by the client. R*

3. *It is recommended that the cooling equipment and cooling room should be linked to an automatic alarm system. R*

4. *It is required that during internal audits lab staff are assessed and deemed competent for their accuracy of motility and morphology assessments including operating the CASA equipment when available, with records of these competencies retained to show what was done and the results achieved . R*

5. *It is recommended that there will be random sampling of diluted semen every production day where temperature is checked using an infra-red thermometer prior to despatch. R*

6. *All hair, where appropriate, on the basis of a risk assessment and following the company's protocol, should be fully contained by the use of a hairnet. R*