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BOAR STUD MANAGEMENT GUIDELINES

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WELCOME TO THE 2017 EDITION OF THE PIC BOAR STUD MANAGEMENT GUIDELINES

We are pleased to present the 2017 PIC Boar Stud Management Guidelines. These guidelines provide recommendations for staff working at boar studs. The 2017 edition replaces the 2014 edition and includes the latest available knowledge and technology.

The goal of this manual is to share practical recommendations in an easy-to-digest format. We have structured this manual into four main sections. Each section contains information about expectations or targets, best management practices and detailed instructions about important working steps.

Compared to the 2014 edition we have added more information about quality assurance/control (QA-QC). We believe QA-QC processes are essential for every boar stud since the quality of an artificial insemination (AI) dose can have significant influence on the performance of an entire production system.

This manual is meant to be applicable to boar studs across the world and country-specific regulations and practices have been excluded. The intent is to provide useful information regardless of your geographical location, operation size, facilities or technical equipment. We recognize that there are different ways to achieve the same results so these guidelines do not reject other management strategies. At all times, please follow the best practices and appropriate standards with respect to animal health and welfare as outlined by the local governing body within the customer's country of operation.

We hope these guidelines help you further improve the performance of your boar stud operations. In case you have questions, please reach out to us.

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GLOSSARY OF TERMS & ACRONYMS

SECTION 1

Intervention level
The actual performance value that should trigger defined actions to break a performance trend and improve.

PRRS
PRRS stands for Porcine Reproductive and Respiratory Syndrome. It is a disease that can be spread via semen and cause reproductive failure in sows besides other symptoms.

ppm
ppm stands for parts per million. It is the concentration of a solute in a liquid or gas expressed as a unit of solute dissolved in one million units of solution.

cfm
cfm stands for cubic feet per minute. It is an expression of the volume of air moving through a ventilation system or other space.

fpm
fpm stands for feet per minute. It is an expression of the speed of air moving through a ventilation system or other space.

micron (micrometer)
Micron is a length unit equal to one millionth of a meter. Symbol µm.

Mcal/ME
Mcal/ME stands for Megacalories Metabolisable Energy. It is a measure of energy value (of food).

PCR
PCR stands for Polymerase Chain Reaction. It is a test method that allows for rapid and highly specific diagnosis of infectious diseases.

AI
AI stands for Artificial Insemination.

Libido
Libido refers to sexual desire. In the context of AI boars it means the willingness to jump a dummy for semen collection.

Warm-up-pen
The warm-up-pen is a pen a boar is transferred to prior semen collection. By exposure to another boar being collected in the next pen, the boar in the warm up pen will be sexually stimulated which will speed up the time until he jumps the dummy when entering the collection pen.

Prepuce
Prepuce is the skin surrounding and protecting the head of the penis, also known as penile sheath.

Persistent frenulum
Persistent frenulum is a thin membrane of tissue between the tip and the corpus of the penis. Normally it forms back prior birth.

AC
AC stands for Artificial Cervix. In automatic semen collection systems it fixes the penis and applies pressure for stimulation.

Prepucial fluid
Prepucial fluid is an accumulation of bacterial contaminated fluid in the prepuce that contains urine and other secrets.

Epididymis
Epididymis is an elongated organ on the posterior surface of a testis that stores sperm while they mature.

Shelf-life
In the context of AI shelf-life refers to the time until semen doses should be used for insemination.

Sterile
Sterile means free from bacteria or other living microorganism.

Contamination (microbiological)
Contamination refers to the non-intended or accidental introduction of infectious material like bacteria or their toxins and by-products.

Disinfection
Disinfection refers to the act of disinfecting, using specialized cleansing techniques that destroy or prevent growth of organisms capable of infection.

SECTION 2

Motility (of sperm)
Motility is the general ability of sperm to move, normally expressed as a % of total sperm.

Progressive Motility (of sperm)
Progressive motility is the ability of sperm to move in a forward direction, normally expressed as % of total sperm.

Primary sperm defects
Primary sperm defects are sperm defects that originate on dysfunctions of the spermatogenesis (sperm production) such as head deformations.

Secondary sperm defects
Secondary sperm defects are sperm defects that occur during the passage of the epidymidis such as cytoplasmic droplets.

CASA
CASA stands for Computer Assisted Sperm Analysis. It is a system that uses special software and hardware to automatically estimate different semen parameters like sperm motility and morphology.

(Spectro-)Photometer
A Spectro-Photometer is a device that measures the intensity of light. The concentration of ejaculates can be measured by such devices by measuring the light intensity prior and after passing a semen sample.

NaCl
NaCl stands for Sodium Chloride.

Andrology
Andrology is the medical specialty that deals with male health, particularly relating to the problems of the male reproductive system.

Accuracy
Measurement accuracy is identified as the difference between the measurement of a factor and the accepted value for that factor from a trusted external source, or the percentage by which the two values differ.

Acrosome
Acrosome is an organelle covering the head of animal sperm and containing enzymes that digest the egg cell coating, thus permitting the sperm to enter the egg.

Formaldehyde
Formaldehyde is a chemical that can be used for preservation of sperm cells for evaluation.

Oil immersion (microscopy)
In light microscopy, oil immersion is a technique used to increase the resolving power of a microscope. This is achieved by immersing both the objective lens and the cover slip in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.

TDS
TDS is the abbreviation for Total Dissolved Solids and is a measure of the combined content of all inorganic and organic substances contained in a liquid.

Isothermic
Isothermic means something occurring at same temperature. In the context of semen preservation it means mixing a semen sample with extender of same temperature.

SECTION 3

Calibration
Calibration is the comparison of measurement values delivered by a device under test with those of a calibration standard of known accuracy.

QA – Quality Assurance
QA is a way of preventing mistakes or defects in manufactured products and focused on providing confidence that quality requirements will be fulfilled.

QC – Quality Control
QC is a procedure or set of procedures intended to control that a manufactured product adheres to a defined set of quality criteria.

Precision
Precision is the extent to which a given set of measurements of the same sample agree with their mean.

Sensitivity
Sensitivity is the smallest absolute amount of change that can be detected by a measurement.

Accuracy
Measurement accuracy is identified as the difference between the measurement of a factor and the accepted value for that factor from a trusted external source, or the percentage by which the two values differ.

Tolerance
Tolerance refers to the total allowable error within a measurement.

Hemocytometer
Hemocytometer is a device used in manual blood and sperm cell counts consisting of a counting chamber of uniform depth that is covered by a ruled cover glass so that the region under each ruled square contains a known volume of the diluted sample.

Flow cytometry
Flow cytometry is a laser- or impedance-based, technology employed in areas like cell counting or cell sorting by suspending cells in a stream of fluid and passing them through an electronic detection apparatus. A flow cytometer allows simultaneous multi-parametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

Biofilm
Biofilm is a thin usually resistant layer of microorganisms (such as bacteria) that form on and coat various surfaces like water pipes.

UV-sterilization
UV-sterilization is the process of sterilizing material by exposure to ultraviolet light.

RO-Reverse Osmosis
RO is a water purification technology that uses a semi-permeable membrane to remove ions, molecules, and larger particles from water.

Coliform bacteria
Coliform bacteria is a certain kind of bacteria commonly used as indicator of sanitary quality of food or water. Fecal coliform are such bacteria that generally originate in the intestine of warm-blooded animals.

EPA
EPA stands for United States Environmental Protection Agency.

Confidence Level
Confidence level is a measure of the reliability of a result. A confidence level of 95 percent or 0.95 means that there is a probability of at least 95 percent that the result is reliable.

Deviation level
Deviation level is a measure that is used to quantify the amount of variation or dispersion of a set of data values.

HACCP
HACCP stands for Hazard Analysis and Critical Control Point. It is a food industry originated monitoring system for identification and control of associated health hazards. It is aimed at prevention of contamination, instead of end-product evaluation.

CFU
CFU stands for colony forming units. It is a measure for bacterial contamination normally expressed per ml or cm^2.

SECTION 4

Terminal sire
Terminal sire refers to a boar that is used to produce commercial or slaughter generation pigs for meat production.

GP sire
GP sire refers to a boar that is used to produce daughters to be used as parent females.

GGP sire
GGP sire refers to a boar that is used to produce pureline progeny .

Camborough®
Camborough refers to the PIC parent female which is a cross between PIC L02 (Landrace) and PIC L03 (Large White).

OBL
OBL stands for Optimum Boar Life. It is a tool used by PIC Genetic Services for boar stud culling recommendations.

SECTION 1:

BARN AND BOAR MANAGEMENT FOR BEST PERFORMANCE

This section starts with the characteristics and targets of an excellent boar stud operation. In addition, it describes practices to help achieve these targets including general management recommendations, bio-security considerations and advice on how to train and collect boars.

BOAR PERFORMANCE REVIEW

Good AI boar performance is the result of good boar management that includes proper boar quarantine and training, high standards in nutrition, and excellent hygiene and semen collection practices. Table 1.1 defines the targets for an excellent boar stud management program.

TABLE 1.1: BOAR STUD MANAGEMENT PROGRAM TARGETS

TRAIT	TARGET	INTERVENTION LEVEL
Boars trained after 4 weeks	>90 %	<80%
Un-trainable boars	≤3%	>10%
Age at which first doses for use are produced	≥220 days - <300 days	<200 days - >300 days
Boars collected per collection technician per hour[1]	≥5	≤3
Avg. semen output per boar per week[2]	≥90B cells	<75B cells
Non productive boars (lameness, sick, semen quality, ...)[3]	≤5%	>10%
Ejaculates with poor quality[4]	6-10%	>12%
Annual boar mortality	<5%	>5%

1 Depending on barn setup: Walking distances collection area-pen/stall, Proximity pass through window-collection area etc. Semi-Automatic collection systems might raise number up to 10 boars per collection technician and hour.

2 Depending on breed and boar age. The displayed numbers consider a stud with terminal boars only and 70% replacement rate.

3 Numbers are annual averages and dependent on geographical locations as well as barn temperatures. During summer the number of non productive boars could raise to >10%.

4 Poor quality defined as ejaculates with <70% motility and/or >30% abnormal cells. The given value is considered as a whole year average. Especially during summertime the monthly trash rates can be higher.

BIO-SECURITY

Most boar studs serve a large amount of sows/farms and disease that can spread via semen (e.g. PRRS, Classical Swine Fever) can cause tremendous economic damage. Therefore, protection of the health status of the boar herd is crucial. Proper location, quarantine and barn management can help to reduce the risk of introducing pathogens to the boar stud and customer sow farms. Table 1.2 summarizes biosecurity best practices. Additional guidelines and practices should be documented and implemented with support of your local veterinarian.

TABLE 1.2: BIO-SECURITY RECOMMENDATIONS

AREA	CHARACTERISTICS OF A BIO-SECURE BOAR STUD
Facility location	• Establish stud as far away as possible from other pig farms, major roads, etc.; • Keep stud protected from access by unintended visitors (lockers, fence, ...)
Staff	• Ensure min. 48 hrs. downtime from other pig contact; • Use shoe disinfection/cover; • Shower in and complete change of clothes and shoes; • Ensure one night downtime if changing from quarantine to production barn
Visitors	• Only allow essential visitors (maintenance personal etc.); • Apply the same rules as for barn staff; • Sign in to visitor book (name, reason for visit, when/where last pig contact, confirm bio-security rules); • Keep frequently used (maintenance) tools in stock at the stud; • Clean and disinfect all tools / supplies that need to enter the stud
Supplies	• Clean and disinfect supplies prior to introduction; fog with disinfectant; • Consider an offsite delivery drop-off location to reduce the amount of outside traffic entering the boar stud; • Remove shipment packaging (i.e. outside cardboard boxes) to enhance the disinfection process
Vehicles/trailers	• Clean, disinfect, and dry prior to approaching the production barn (animal transports); • Set appropriate trailer downtime standards between animal transports
Other	• Establish rodent and other pest control procedures; • Install positive pressure air filtration of the barn; • Establish independent quarantine of all incoming replacement boars for a set period of time prior to introduction into the herd

For further information on biosecurity strategies consult PIC Health Assurance.

GENERAL MANAGEMENT RECOMMENDATIONS

In this section we provide an overview of general management strategies that should work equally well in the quarantine and the production barn. Key information is listed in Table 1.3.

Disclaimer: Under all circumstances, producers should adhere to the locally applicable laws that regulate management and housing practices, even if they differ from the recommendations presented in these guidelines.

TABLE 1.3: GENERAL RECOMMENDATIONS FOR BOAR STUD MANAGEMENT

FACTOR	QUARANTINE	PRODUCTION BARN
HOUSING		
Barn Temperature[1]	• <20°C (<68°F)	
Humidity	• >40% - <70%	
Gasses (max. ppm per m³ air)	• Ammonia: 20 ppm; • Carbone Dioxide: 3,000 ppm; • Hydrogen Sulfide: 5 ppm	
Ventilation (in Cubic Feet per Minute (CFM) per head)	• Min (cold): 14 CFM; • Max: 150 CFM	
Airspeed (in Feet per Minute (FPM)	• 400-800 FPM	
Housing[2]	• House boars individually; • Maintain visual and/or direct contact with other animals; • House boars at same age/breed close to each other to make visual comparison easier	
Flooring[2]	• Solid floor: Sloped to avoid manure and liquid build up; • Insulated lying area; • Slatted floor: Keep dry and clean; • Straw: Exchange at least once a week. Regular mycotoxin tests recommended. Ensure safe source (pig manure free); • Sawdust: Exchange 1-3 times/year (depending on humidity); • Keep lying area dry and free of feces as much as possibl	
FEEDING MANAGEMENT		
Water	• Have clean and fresh water always available; • Use nipple drinkers (80-90 cm above floor) or troughs; • Daily demand approx. 17 L (4.5 Gallons) per boar/day; • Minimum flow rate 1 L/min (0.26 Gallons). Test monthly; • Test nipple drinker when cleaning troughs; • Water quality according to Appendix A. (check twice a year)	
Diet	• Specific for age/weight to maintain required body condition. See information on specifics and energy demand in Appendix B. For more information contact PIC Nutrition Services • Average grain particle size 750-900 micron; • Avoid the use of by-products or co-products where mycotoxins may be concentrated. Boar feed should be produced out of tested raw material; • Use of mycotoxin binders may be beneficial, • Check for mycotoxin contamination regularly; • Adding anti-oxidants may be beneficial; • Contact PIC Nutrition Services for additional information or support	
Feeding strategy	• Feed 1-2 times per day; • Clean troughs prior to feeding; • If automatic feeders are used, check accuracy by weighing and adjusting every 2 weeks; • To adapt to new feed and environment provide a reduced ration (2/3 of the regular amount) for 2-3 days; After the first 2-3 days feed boars in order to maintain a body condition of 2; • At entry weights of approx. 160 kg, it is normal to provide about 2.5 kg feed with 7.9 Mcal/ME feed per day	• Adjust individual feeding based on visual body conditioning score; • Target is >95% of boars in normal body condition[3].

FACTOR	QUARANTINE	PRODUCTION BARN
HEALTH		
Treatments	• Herd veterinarian should follow local regulations; • Minimize treatments as much as possible as negative effects on semen quality can occur; • Oral application is preferred over injection if available. Drugs are dissolved in solvents or sterile water. Solvent ingredients may have an adverse effect on semen production and/or quality. Typically drugs are not tested for these effects; • Injections should never be done in the collection pen; • Keep records for all treatments given.	
	• Vaccinations should not start within the first 3 days after arrival; • Some vaccines have a negative effect on semen quality. Apply those vaccines as early as possible to give the boar time to recover prior to semen collection starting. Negative effects might be seen up to 8 weeks after treatment.	• Split herd vaccinations over several groups with time to mitigate potential negative effects on semen quality; • Reduce no. of vaccinations to an absolute minimum.
Diagnostic testing[4]	• Conduct diagnostic testing on diseases and with test methods according to herd veterinarian and local regulations; • Sample as gentle as possible. Consider saliva testing, blood sampling from leg (tarsal vein) instead of jugular vein etc.; • Contact your herd veterinarian for detailed instructions.	
	• Conduct initial test on the boars within seven days of arrival; • Test 100% of the population at the end of the isolation period.	• Conduct regular tests on common diseases transmittable by semen and mandatory ones.
Clinical monitoring	• Walk barns daily to check boars and to identify those that did not clean up their feed or show signs of sickness; • Measure and record temperature for every suspicious boar; • In case of fever (≥39°C or ≥102°F) contact your herd veterinarian immediately and skip collection of the boar that day. A diagnostic work-up on blood samples including PRRSV PCR is recommended; • Get boars up every day at feeding to observe for lameness; • Consult your herd veterinarian, in case of doubts; • Keep records for any boars that are treated or off-feed.	
Semen collection management of sick animals	• Don't collect lame animals until full recovery and following the conclusion of painkiller treatments; • Don't collect animals that display blood in the semen for 2 weeks. If blood continues to appear after the rest period, medical investigation and proper treatment according to your herd veterinarian should be done; • Don't collect animals with depressed general condition until recovery.	

FACTOR	QUARANTINE	PRODUCTION BARN
SEMEN COLLECTION		
Start collection[5]	• Don't start collection training until 180 days of age or after 270 days of age; • Don't start collection training until at least 5 days after arrival.	• Don't start semen collection for use in AI prior to 220 days of age; • 2 consecutive ejaculates that pass the minimum thresholds should be collected prior to using the doses for AI (2nd good can be used).
Collection frequency	• Conduct collection training 2 consecutive days following 1 rest day and repeat until the boar has successfully been collected 2 consecutive training days; • After successful training, collect boars once every 7 days.	• Collect boars below 12 months of age: 1/week; • Collect boars above 12 months of age: • Min.: 1/week • Max.: every 3 days; • Response to collection frequency may vary by boar.
Non collects	• Don't collect lame or sick boars until recovery; Boars with blood in the semen should rest 2 weeks.	
Collection schedule	• During training, first collect the boars with high libido, followed by the more challenging animals.	• Manage collection schedule via proactive planning and scheduling; • Prioritize by index and eligibility (collect highest indexed boars with adequate rest days and good semen quality first).

1 We are aware that barn temperatures below 20˚C/68°F are difficult to realize in some regions or during summer. However it has been shown that this is the critical temperature threshold prior to negative impacts on semen production.
2 Always follow local legislation concerning housing and bedding requirements for AI boars.
3 Examples for body condition categories to be found in the chapter below.
4 Should be defined together with herd veterinarian.
5 Differences might appear depending on whether boars are trained on quarantine or in the stud.

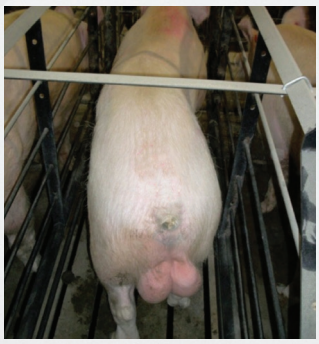
BODY CONDITION

The nutritional strategies in Table 1.3 and Appendix A, B and C will help to keep boars in proper body condition. The PIC score of boar body condition is shown in Graphic 1. However there are differences between genetic lines that cannot be considered in this illustration. At normal body condition (score 2) it is possible to feel the backbone with firm palm pressure but not to see it (especially near the tail).

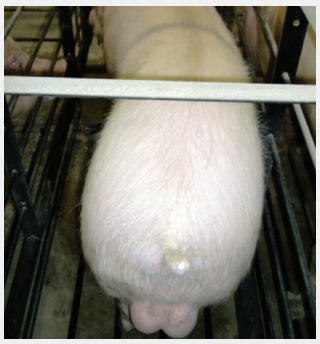
Keep in mind that the amount of feed has to be adjusted for every individual boar and the specific nutritional characterization of the local diet. Automatic feeding systems help to execute a more consistent feeding regime. The process of body condition scoring should ideally be done by the same person every time for consistency purposes.

GRAPHIC 1.1: BOAR BODY CONDITION

Thin Body Condition



Good Body Condition



Fat Body Condition

SUCCESSFUL BOAR TRAINING

A solid preparation of boars for AI is the foundation of a high productive boar inventory. Mistakes during collection training might have a persistent negative effect on the "mounting quality" of boars or lead to boars that are not able to be collected at all. The key elements of successful boar training are listed below.

PREPARATION

- Use a record system to keep track of individual boars training and success.
- Remove any source of distraction in the collection area.
- Ensure the collection pen doesn't allow the boar to wander too much.
- Position a warm-up pen next to or behind the collection area.
- Ensure staff safety. Make sure the boar is comfortable with human contact.
- Have experienced and patient staff conduct training.
- Adjust dummy height to the boar's size (~120° angle rear legs-belly).

COLLECTION

- Squeeze the prepuce to stimulate the boar and make every effort to get the boar to pay attention to the dummy.
- Once the boar mounts the dummy, lock the penis and collect the ejaculate.
- Observe any possible anatomical problems with the boar (i.e., limp penis, persistent frenulum) during this process and report back to your boar supplier.

WORK FLOW

- While staff are collecting the first boar, the next boar should be placed in the warm-up area to prepare for training.
- If the boar does not jump the dummy after ~5 minutes, stop the training and continue the next day.
- Alternatively, consider the application of a natural prostaglandin (if permitted in your country; consult herd veterinarian).
- Avoid any type of manipulation (vaccination, cutting teeth, etc.) in the collection area.
- Once the boar is trained, repeat the process for 2 days in a row to reinforce the learning experience.
- After training is successfully completed, collect the boar once every 7 days until he reaches one year of age.

TRAINING USING AN AUTOMATIC COLLECTION SYSTEM

This requires a slightly different approach. Details are outlined below.

- An automatic collection system includes an artificial cervix (AC), slide arm, AC holder and dummy.
- The AC mimics a sow's cervix and provides pressure to stimulate the boar.
- The slide arm allows free back and forth movement during collection.
- Follow the manual collection steps (outlined above) for the first day of collection.
- On day 2, collect the first portion of the ejaculate manually for approximately 1 minute with the left hand.
- After 1 minute, attach the penis to the automatic collection system and allow the boar to finish the collection.
- Repeat the process after the rest day on day 3 of training.
- Each boar will acclimate to the system at their own pace. Not every boar accepts automatic collection. If he does not acclimate to the system after 4 weeks you should consider collecting him by hand.

For more details on training boars for successful collection check the QR code below (Leads to Successful boar training presentation)

ROUTINE SEMEN COLLECTION

Next all aspects of an efficient semen collection process are described.

COLLECTION AREA DESIGN

A proper collection area can help to make semen extraction quicker, safer, more hygienic, and productive. It provides a significant advantage in terms of hygiene compared to collection in the housing area. The key elements of an effective collection area are listed below:

- Separated from animal housing area.
- As small as possible.
- Easy to clean (surfaces/walls/floor/dummy).
- Flooring with good traction.
- Boar collection from outside or with a staff escape route.
- Collecting the boar from a collection pit to reduce strain injuries along with knee and back problems for the technician.
- Easy to clean dummy (easy to flip up) with adjustable height.
- (Semi) automatic systems to relieve technician from repetitive strain injuries.
- Operated in combination with a "warm-up area"
    - While current boar is collected, the next one is stimulated by watching/hearing/smelling his predecessor.

SEMEN COLLECTION PROCEDURE

The semen collection procedure is one of the key components for the production of high quality semen. Ejaculate bacterial contamination can be greatly reduced if this process is done correct.
Best semen collection practices can be found in Table 1.4.

TABLE 1.4: RECOMMENDATIONS AROUND SEMEN COLLECTION

AREA	RECOMMENDATION
Prior to collection	• Refer to the management table (Table 3) for information on collection frequencies/schedules; • Store all required supplies like gloves, collection cups, bags, etc. in a clean, closed cabinet; • Use an insulated (pre-warmed to 37°C/100°F) collection cup to house a plastic bag in which the ejaculate gets collected; • Cover the cup with a filter (gauze, milk-filter, commercially available product) to separate the bulbourethral secretion (gel phase) from the ejaculate; • Do not place the collection cup on the floor or other contaminated surfaces for hygiene reasons; • For better stimulation it is recommended to work with a warm-up area. Boars entering the warm-up pen will be stimulated by smelling, hearing, and watching another boar being collected; • Have boars mount the dummy within <5 minutes after entering the collection area.
During collection	• The double glove method helps increase hygiene of the collection: ○ The outer glove is removed after evacuating the prepuce and stimulating the boar until the penis is exposed; ○ Prior to fixing the penis the outer glove is removed. The penis is only allowed to be touched with the inner clean glove. • Fix the penis with one hand, leaving the tip uncovered; • Keep penis with tip above boars belly level (higher) to avoid preputial fluid from running down into the collection cup; • Do not touch the filter nor the tip of the penis with your hand; • Do not collect the first, clear fraction of the ejaculate but dump this on the floor. This removes most of the potential contaminants from the ejaculate; • Continue semen collection until the boar finishes ejaculation and pulls back his penis.
After collection	• After collection, the first priority is to prepare the ejaculate for delivery to the lab; • Do NOT squeeze the filter in an attempt to remove liquid from the gel fraction. The filter may be contaminated and can be damaged so squeezing will result in gel particles ending up in the ejaculate with negative effects on semen quality.

HANDLING BOARS WITH POOR SEMEN QUALITY

Proper handling of boars with poor semen quality can help increase the likelihood and timeliness of returning them to production. Note that boars with persistent damage in the testicles will not recover.

- If two consecutive ejaculates below the minimum quality criteria are collected, flag the boar. This is also known as putting the boar "on-hold" pending further testing;
- Have flagged boars follow a separate program as outlined in the list below;
- Cull boars with a very low index or those slated for replacement display poor semen quality;
- Inspect the animal for signs that could help explain poor semen quality like:
  - Lameness/pain
  - Poor body condition
  - Abnormal testis/epididymis (large asymmetry, inflammation, lesions, atrophic, etc.)
- Apply treatment if appropriate;
- Factors such as heat stress, vaccinations, mycotoxins etc. can have negative effects on semen quality.

PROGRAM FOR FLAGGED BOARS:

1. Take the body temperature of the boar, prior to collection. Elevated body temperatures could be a reason for poor semen quality.
2. Review the health and treatment records of the boar. Is there a probable cause listed? Pay particular attention to the records from 3-4 weeks earlier;
3. Remove boar from the regular collection schedule, but keep collecting the boar:
   - < 12 months: strictly 1x per week
   - >12 months: min 1x per week

   Make exceptions for boars with health issues/lameness/blood in ejaculate (see Table 1.3). Schedule the collection of flagged boars on low production days so there is sufficient time to review the ejaculate;
4. Evaluate every ejaculate for motility and morphology to track recovery. Conduct a detailed morphology assessment at high magnification (refer to Section II, Chapter "Semen Evaluation") to confirm the percentages of each type of cell defect. Also check for cell density if low ejaculate concentration is the boar's problem;
5. If necessary, send a sample of the ejaculate to a 3rd party laboratory for microbiological testing;
6. If two consecutive ejaculates with good semen quality are obtained, place the boar back into the normal collection schedule;
7. Monitor to see if semen quality returns to normal after approximately 6-8 weeks after the cause for quality drop is solved.

HYGIENE

Bacterial contamination of ejaculates has a negative effect on the semen quality and shelf-life of the produced semen doses. Although it is hard to collect sterile ejaculates under commercial conditions, it is a priority during collection to reduce the bacterial contamination of raw ejaculates as much as possible. Key areas of focus to maximize hygiene are highlighted in Table 1.5.

TABLE 1.5: GENERAL RECOMMENDATIONS FOR HYGIENIC COLLECTION

AREA	RECOMMENDATION
Boar housing	• Ensure dry and clean lying area for boars; • Exchange bedding material regularly; • Regularly execute complete cleaning and disinfection of the whole barn. Schedules differ depending on factors like bedding material and humidity. Minimum 1x/year
Boar	• Have the boar enter collection area free from feces, bedding material etc.; • Clean, and dry his belly/underside if needed; • Trim preputial hair on regular basis.
Collection area/ Dummy	• Keep separated from housing area, if possible; • Clean disinfect and dry after every production day (power wash, detergent, disinfectant); • Emphasize cleaning the underside of the dummy (flip dummy up for cleaning, if possible); • Replace dummies with deep scratches on surface
Utensils (Cups, gloves, ...)	• Store all material nearby the collection area in a closed cabinet to minimize contamination; • Prepare collection cups (styrofoam and gauze/filter) in a clean environment; • If collection bags with integrated filters are used, consider preparation in the barn; • Don't touch collection filters and inside of the cup/bag with your hands; • Use a warm, insulated flask to hold the collection cup/bag
Collection technique	• Refer to the paragraph "Semen Collection" earlier in this section
Warming cabinet	• Clean, disinfect, and dry after every production day (detergent-dry-alcohol).
Insulated flasks	• Clean, disinfect, and dry after every production day (detergent-dry-alcohol).

TROUBLE SHOOTING

In this section we present the most common challenges in studs along with some intervention strategies. Please note that this is a non-exclusive list. For recommendations on other problems or more detailed advice please contact your PIC Account Manager or PIC Technical Services.

PROBLEM: HIGH NUMBERS OF UN-TRAINABLE BOARS

Table 1.6 includes intervention strategies if you have less than 80% boars trained after 4 weeks or a total of >5 % un-trainable boars. Note that there can be a difference between genetic lines.

TABLE 1.6: POINTS TO REVIEW AND INTERVENTIONS WHEN HAVING HIGH AMOUNTS OF UN-TRAINABLE BOARS.

POTENTIAL CAUSE	INTERVENTION
Lack of stimulation	• Let boar with high libido mount dummy first to leave odor on the dummy; • Let boar observe other boar being collected prior to his turn; • A portable dummy can help to "mimic the sow" better and interact with the boar
Lack of comfort	• Adjust dummy to proper height (approx. 120° angle leg-belly); • Ground the dummy with good traction – no slipping; • Create a safe position on dummy (no leg pattering)
Training schedule	• Conduct multiple training sessions over the first week. Try 2 consecutive days of training followed by 1 day rest and another 2 days of training.
Distraction	• Don't conduct other activities (like feeding, power washing etc.) during training.
Negative priming	• Do not treat boars in the collection pen and avoid slick floors/dummy or other stressors.
Age at collection	• Boars show best responsiveness if older than 6.5 month of age at start of training; • After approx. 9 month of age training might get more difficult.

PROBLEM: INCREASED AMOUNT OF ABNORMAL CELLS

There are a number of reasons why boars produce ejaculates with increased numbers of abnormal cells. A selection of potential causes and intervention strategies is listed in Table 1.7. In a lot of cases this could be due to multiple, overlapping reasons, so no single cause can be identified. Especially during summer, overall semen quality can decrease due to seasonal and temperature effects. Note that the intensity of response to such stressors can differ between genetic lines and individuals.

TABLE 1.7: POINTS TO REVIEW AND INTERVENTIONS WHEN HAVING EJACULATES WITH AN INCREASED NUMBER OF ABNORMAL CELLS.

POTENTIAL CAUSE	INTERVENTION
Ambient barn temperatures above 22°C/72°C	• Equip barn with cool cells or other devices to lower temperature; • Increase ventilation rate for better heat convection; • Place more sensitive boars/breeds closer to cool cells; • Conduct boar collection, training, feeding during cooler morning hours; • Feed smaller portions more frequent; • Check water supply; • Don't vaccinate during high summer; • If possible, avoid boar transport during high summer (or do early morning/at night).
Elevated body temperature (fever)	• Split boars in groups for vaccination and apply treatment of groups 4-6 weeks apart; • Apply antipyretics if core temperature rises over 39°C/102°F (take diagnostic sample to rule out PRRS virus infection); • If together with sickness/lameness, apply treatment based on herd veterinarian's recommendation.
Treatments/Vaccination	• Refer to "Elevated body temperature".
Boar age	• Boars below 220 days of age are still developing sperm cell production and therefore may have a lower percentage of normal cells. They can be trained/collected, however it is advised not to use their semen until boar is approximately 220 days old; • Boars above 3 years of age typically display a slight increase in abnormal cells.
Mycotoxins	• Check feed for mycotoxins; • Clean troughs prior feeding (avoid mold); • Use mycotoxin binders in feed; • Consult your nutritionist for additional intervention options.
Wrong collection frequency	• Avoid schedules of <1 and >2 collections/week in adult boars; • Avoid schedules of >1 collection/week in boars <12 month of age; • Collect boars at least every 7 days.
Lameness/sickness	• Apply treatment according to herd veterinarian and do not collect boars until recovery.
Pathological testis (injuries, lesions, strong asymmetry, inflammation, ...)	• Consult your herd veterinarian for proper treatment. If damage is persistent with no recovery of semen quality, cull the boar.

PROBLEM: INCREASED AMOUNT OF ANIMALS WITH LAMENESS OR CRACKED HOOFS

Lameness or cracked hoofs can have multiple reasons. The most common reasons along with intervention strategies are outlined in Table 1.8.

TABLE 1.8: CAUSES AND INTERVENTION STRATEGIES FOR LAMENESS/CRACKED HOOFS

POTENTIAL CAUSE	INTERVENTION
Infection of joints, physical damage	• Apply (antibiotic and anti-inflammatory) treatment based on herd veterinarians´ recommendation; • Do not collect boar until recovery.
High humidity/ softened hoof	• Pay attention on dry flooring. Increase ventilation to improve drying.

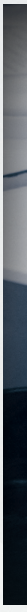
Wrong flooring/sharp edges	• Check housing area for critical points:
	  • Check flooring is not too rough/with sharp edges (especially new facilities);
	  • Check metal troughs might have sharp edges;
	  • Check crate/fence for sharp edges/protruding parts;
	  • Check attachment of dummy to floor (screws)

PROBLEM: HIGH LEVELS OF BACTERIAL CONTAMINATION IN EJACULATES

If microbiological testing of semen doses indicates the barn as potential place where contamination occurs, conduct a thorough review based on Table 1.9.

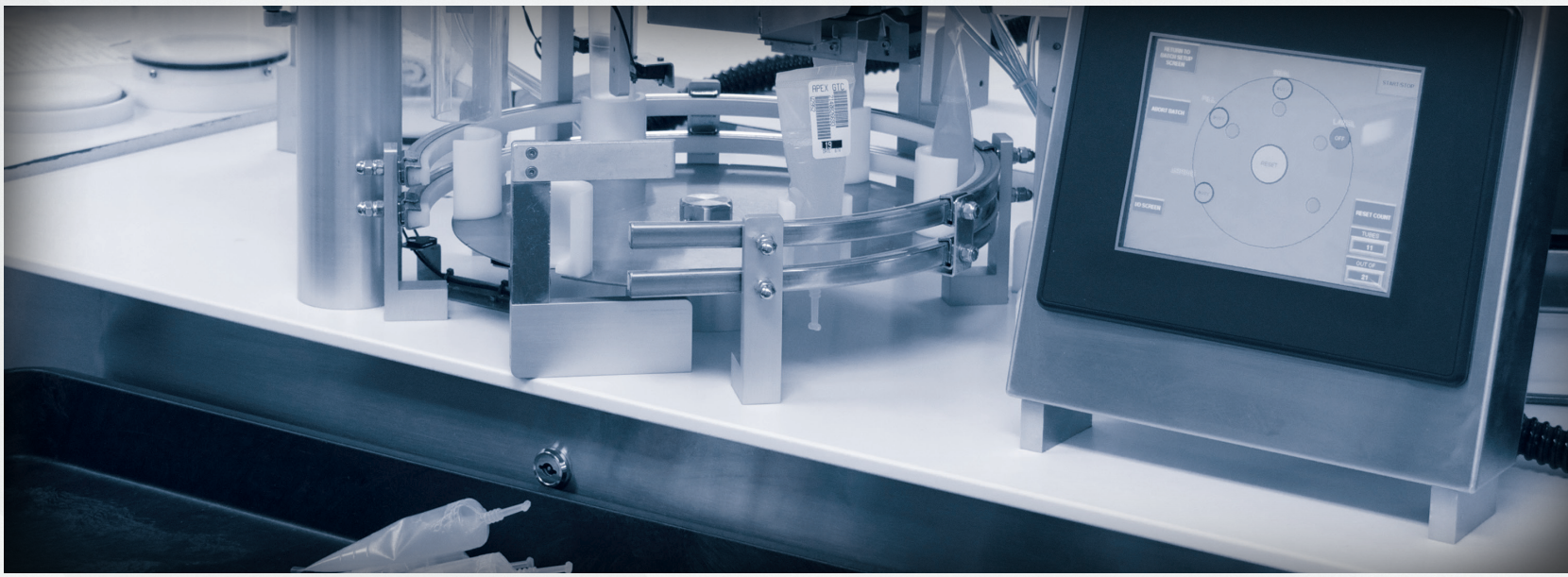
TABLE 1.9: CAUSES AND INTERVENTION OF BACTERIAL EJACULATE CONTAMINATION

POTENTIAL CAUSE	INTERVENTION
High bacterial load on boar surface due to dirty housing area	• Keep boar housing area as dry and clean as possible by removing bedding material more often, increase air flow on ground level, adjust nipple drinkers to not sprinkle floor; • Clean housing area more frequent; • Clean boars belly/prepucial area with dry paper-towel prior collection
High bacterial contamination in collection area	• Clean and disinfect dummy and collection area after every production day with emphasis on underside of dummy; • Work with warm-up pens for boars to pee and defecate prior entering collection area; • Evacuate prepuce in warm-up area; • Remove feces from collection area with shovel in between collections
Contaminated supplies	• Store collection gloves, cups and filters clean and dry until usage; • Do not place collection cup on floor; • Do not touch collection filter/inside of collection bag with hands
Contamination caused by wrong collection technique	• Refer to instructions made in this section, chapter “semen collection” and “hygiene”
Wrong choice of detergents/disinfectants or wrong application	• Ensure that agents are effective against suspected bacterial flora; • Use agents according to manufacturer’s instructions.



SECTION 2:

LABORATORY QUALITY MANAGEMENT



This section provides an overview of management strategies to ensure that only semen with acceptable quality gets processed while also providing details about best processing strategies and hygiene in the lab.

EXPECTATIONS

The primary target of every AI laboratory is to produce consistent semen doses of good quality which will fertilize a sow. The goal of the laboratory process is the efficient evaluation of ejaculates to determine whether they meet the established quality standards as described in Table 2.1 and the production of doses that satisfy both in-house and customer requirements.

TABLE 2.1: EXPECTED RESULTS FROM A LABORATORY MANAGEMENT PROGRAM

TRAIT	TARGET	INTERVENTION LEVEL
Consistent amount of cells per AI dose	≤+/- 5% off target	> +/- 10% off target
Consistent AI dose volume	≤+/- 1 ml off target	> +/- 2 ml off target
AI dose minimum total motility at expiration date	≥70%	<60%
AI dose minimum progressive motility at expiration date	≥60%	<50%
AI dose maximum amount of abnormal cells (primary and secondary defects)	≤30%	>30%
AI dose maximum amount of cytoplasmic droplets only	≤20%	>20%
AI dose bacterial cell count	<1 cfu/ml	>1 cfu/ml

LABORATORY SETUP

A proper lab design as described in Table 2.2 sets the stage for high quality, efficient semen dose production. Lab design affects hygienic production and efficient work flows. Lab design details often have to be decided on a case by case basis depending on the unique situation encountered at each stud (space, staffing, etc.).

TABLE 2.2: MAJOR PRINCIPLES AND TARGETS OF AI LABORATORY DESIGNS

PRINCIPLE	EXPLANATION
Separation between production lab and: • Cleaning area (wet lab) • Supply storage room • Semen storage (cool) room • Office • Break room • Sanitary rooms • Facility rooms (water, power, compressor, etc.)	The production lab is uniquely used for the evaluation and processing of semen. The layout is set up in a way that this room is easy to clean and disinfect. To prevent contamination of the processed semen, all other activities require rooms separate from the production area.
Place only items/machines essential for production in lab.	The less items or equipment the easier the cleaning of the lab.
Have short distances between working stations.	Allows for faster processing
In-line-workflow	No crossing of working ways from "dirty" (ejaculate arrival, evaluation) to "clean" (extension, filling) areas to avoid cross contamination.
Replace air condition filters regularly.	Less distribution of dirt/bacteria via air.
Lab furniture movable (wheels)/open space to floor	Allows to clean area beneath
Avoid storage cabinets in the production lab. Work with mobile carts containing daily supplies.	Cabinets need to be cleaned as well and this is often not done on a regular basis.
All surfaces (ceiling, walls, floor, countertops, furniture) composed of the appropriate materials and constructed for ease of cleaning and compatibility with disinfection.	Facilitates proper cleaning and disinfection
Allow distance between wires/pipes and the wall/floor.	Facilitates easy access to clean
No 90° angles between floor and wall	Facilitates cleaning
Floor coating without seams and drains	Facilitates easy cleaning and no gathering of dirt and bacteria
Power sockets above equipment area	Facilitates cleaning with no wires on the floor or against the wall or on the horizontal surfaces
Separate rooms for storage of filled semen doses and lab supplies.	Facilitates cleaning

LABORATORY HYGIENE

Besides the aforementioned rules to setup a proper lab, a variety of practices help to reduce bacterial load and cross contamination during semen processing:

- Enter the lab only after changing clothes (lab coat, pants and shoes, hair/beard net).
- Make it mandatory to wash hands and disinfect prior entering the laboratory (Graphic 2).
- No food or beverages in the laboratory (can be consumed in the break room).
- Wash hands and disinfect after meals and after visiting the restroom.
- If semen pass-through window is used, never open both sides at the same time.
- Do not store unnecessary items in the pass-through window.
- Nothing from the barn besides the ejaculate (in a plastic bag or comparable) should enter the laboratory.
- If people have to transfer from the barn to the laboratory on the same day (not preferred), make it mandatory to shower and change clothes.
- Unpack supplies from outer packaging (if possible) prior to entering the laboratory.
- Don't touch anything that comes in direct contact with semen/extender with bare hands.
- Use disposable plastic bags to line pitchers that will contain ejaculate/semen. This will reduce the risk for cross contamination and limit cleaning.
- Remove spilled ejaculate/extender from countertops immediately with paper towel/tissue and disinfected with alcohol wipes.
- Filter pressurized air prior to use (i.e. pneumatic tube systems, filling/packing machines).
- Dispose of trashed semen and residual extender after production in the toilet or an alternative disposal place outside the production lab or in the sink that is used only for cleaning.
- Sanitize hands in between ejaculates being processed and every time there is any possibility of contamination.
- Perform regular cleaning of the laboratory as explained in Section III, chapter "Cleaning and Disinfection".

GRAPHIC 2.1: INSTRUCTIONS FOR PROPER HAND WASHING

SEMEN EVALUATION

The foundation of AI dose production is the measurement of raw ejaculate sperm cell numbers as well as the evaluation of two key quality parameters, semen motility and cell morphology. Only ejaculates that meet a set standard of minimum criteria listed in Table 2.3 should be processed. Discard ejaculates that do not meet the minimum standards.

TABLE 2.3: THRESHOLDS FOR SEMEN EVALUATION

SEMEN VARIABLE	NORMAL VALUE	MINIMUM THRESHOLD
Appearance	Milky to creamy consistency	
Color	Gray-white to white in color	
Volume	100 – 500 ml	≤ 50 ml
Total no. of sperm cells	20-120 Billion	>15 Billion
Motile sperm cells (raw ejaculates)[1]	80-95%	To meet minimum required semen motility at expiration date
Progressive motile sperm cells (raw ejaculate)[1]	60-90%	To meet minimum required semen motility at expiration date
Agglutination	0-10%	≤ 30%
Abnormal sperm cells (primary and secondary defects)	10-15%	≤ 30%
Cytoplasmic droplets (as part of abnormal cells)	5-10%	≤ 20%
Maximum motility loss/24 hours	2.5 – 3.0%	≤ 3%
Maximum progressive motility loss/ 24 hours	2.5 – 3.0%	≤ 3%
Motile sperm cells at expiration date		≥ 70%
Progressive motile sperm cells at expiration data		≥ 60%

1 Measurement results can vary depending on the used CASA system

MACROSCOPIC EVALUATION

Macroscopic evaluation is a simple but important step. The goal is to discard unsuitable ejaculates, especially contaminated ones prior to entering the analysis and processing flow. Table 2.4 lists the factors for evaluation.

TABLE 2.4: MACROSCOPIC SEMEN EVALUATION

FACTOR	NORMAL	ABNORMAL
Color	White, grey-white, yellow-white	Red or brown (all variations)
Consistency	Creamy, milky	Water-like, flaky
Impurities	None	Blood, pus, urine, feces, others
Odor	Neutral	Urine, feces, putrid

MEASUREMENT OF SPERM CONCENTRATION/TOTAL CELL COUNT

Different methods are available to measure the sperm cell concentration of the ejaculate.
The requirements for an accurate measurement of sperm concentration with different devices are listed in Table 2.5. The multiplication of the cell concentration with the ejaculate volume (measured with a scale; 000.0 g) provides the total number of cells in the ejaculate.

The most frequent devices for concentration measurement in AI studs are computer assisted semen analysis (CASA) machines and photometers (i.e. spectrophotometers).

Measurements are typically done on the raw or possibly the pre-extended ejaculate (depending on the laboratory protocol and device).

TABLE 2.5: REQUIREMENTS FOR ACCURATE MEASUREMENTS WITH CASA MACHINES AND PHOTOMETERS

PHOTOMETER	CASA
SAMPLE PREPARATION	
No abnormal color/purification of the ejaculate	
Proper and thorough mixing of the ejaculate prior to sampling	
Precise pipetting of the sample. Use of an automatic pipetting system as alternative	
Depending on the system, a dilution of the ejaculate sample prior to measurement is needed. Accuracy and precision of this step has a large influence on the measurement results	
Mix sample in cuvette by gently inverting it 5X (use cap or parafilm to cover cuvette instead of finger tip to avoid contamination)	Filling of the standard counting chamber (according to manufacture instructions). Avoid under/overfilling
MEASUREMENT	
Every measurement series to start with a 0 calibration of the device (according to manufacturer. Normally done with 0.90% NaCl solution).	• Proper software settings (species, sample dilution, type of counting chamber, cutoff values motility etc.). • Confirmation of all settings prior use
Avoid touching the measurement area/field of the cuvette/chamber	
	Check for proper light adjustment and contrast settings
Measure immediately after sample preparation	
TO CONSIDER	
Air bubbles in the sample or a dirty/scratched cuvette can jeopardize measurements.	Measurement chambers to be free of dust, debris, moisture, and mold.
Avoid obstructions to the photometer-shaft.	
Photometers usually have a sigmoid light absorption curve. Measurement is only reliable on the linear part of the curve.	A minimum of 400-600 cells to be counted (depending on chamber and CASA manufacturer).
Regular service and calibration (min. 2x/year) of the device with standard samples (according to manufacturer)	Regular (min. 1x/year.) maintenance by specialized technician.

EVALUATION OF SPERM MOTILITY

The evaluation of sperm motility is an important step in the overall quality control of raw ejaculates. All requirements for a good motility measurement are listed in Table 2.6. Only ejaculates with a minimum motility of 70% or more are recommended for further evaluation and processing. Motility evaluation is performed on a microscope with phase contrast and a heated (38°C/100°F) stage. Evaluation can be done by manual estimation or automated with help of a CASA system.

TABLE 2.6: REQUIREMENTS FOR GOOD MOTILITY MEASUREMENTS

TECHNICIAN ESTIMATION	MEASUREMENT WITH CASA MACHINE
SAMPLE PREPARATION	
• Warm all equipment (glass slides, microscope stage, etc.) to 38°C • All consumables (glass slides, coverslips, pipet tips, counting chambers, etc.) new and previously unused; No cleaning/reuse of items	
Proper mixing of the ejaculate prior to sample preparation	
Use of pre-warmed glass slides and cover slips	Use of pre-warmed counting chambers/ microscope slide
• Sample of 5-15 µl raw ejaculate • If high concentration (>500x10^6 cells/ml) one drop of extender or 0.9% NaCl can help to dilute.	• Pre-dilution of the sample with warmed extender to achieve optimum cell concentration for measurement (according to CASA manufacturer manual); • Pre-dilution will be done in a pre-warmed sample tube. Prepared sample to be mixed (Vortexed) again prior to analysis.
	Use a micro-pipette with fine tip to load the counting chamber. The loading volume should be ± 0.5 µl more than the actual chamber volume. Chamber volumes depend on manufacturer (MOFA® chamber: ≈2.2 µl; Leja® chamber ≈ 3 µl).
MEASUREMENT	
Samples measured immediately after pipetting to the glass slide or CASA chamber.	
Evaluation of a minimum of 5 different fields at 200-400x magnification and phase contrast.	Proper software settings (species, sample dilution rate, type of measurement chamber, etc.).
Evaluation at center region of cover slip.	Check for proper light adjustment and contrast.
Evaluation of a new sample if motility across view field varies considerably.	Measurement chambers free of dust, debris, moisture.
Evaluation on 0-100% scale	If measurement fields are selected manually, the chosen fields of view should be in the middle of the chamber.

EVALUATION OF AGGLUTINATION

Evaluation of agglutination can be done at the same time as motility/morphology evaluation. Most andrology laboratories use the categories as described in Table 2.7 where ejaculates with score 3 are not recommended for processing. Dilution of the evaluated sample can help to reduce agglutination.

TABLE 2.7: SCORING OF EJACULATE AGGLUTINATION

SCORE	DESCRIPTION (PERCENTAGES EXPRESS AGGLUTINATED CELLS WITHIN A VIEW FIELD)
0	None
1	Mild (<10%)
2	Moderate (10-30%)
3	Severe (>30%)

EVALUATION OF SPERM MORPHOLOGY

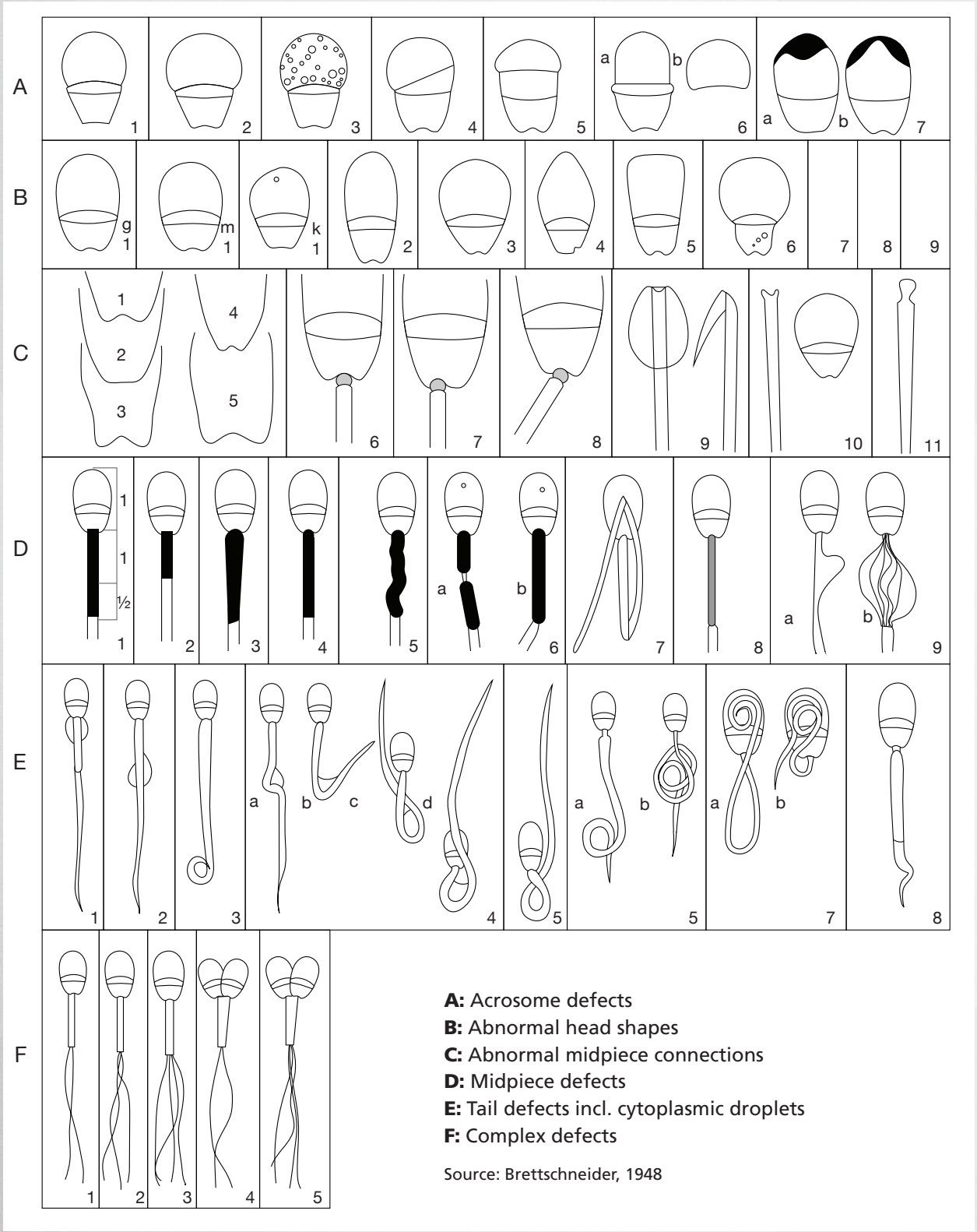
Sperm morphology can be evaluated by using a CASA system with auto-morphology or by manual microscopic methods. The accuracy of the results is dependent on the number of analyzed cells. The example shown in Table 2.8 highlights how the minimum and maximum abnormal cells counted depends on the number of total cells observed. In general the more cells can be counted, the closer the result is to the actual value. This relationship becomes crucial as ejaculate evaluation results move closer to the minimum thresholds (see framed area in table).

TABLE 2.8: ACCURACY OF SPERM CELL MORPHOLOGY EVALUATION RESULTS

CELLS COUNTED	100		200		500	
% ABNORMAL	MIN	MAX	MIN	MAX	MIN	MAX
0	0.00	3.60	0.00	1.80	0.00	0.70
1	0.00	5.40	0.10	3.60	0.30	2.30
2	0.20	7.00	0.60	5.00	1.00	3.60
3	0.60	8.50	1.10	6.40	1.70	4.90
4	1.10	9.90	1.70	7.70	2.50	6.10
5	1.60	11.30	2.40	9.00	3.30	7.30
6	2.20	12.60	3.10	10.20	4.10	8.50
7	2.90	13.90	3.90	11.50	4.90	9.60
8	3.50	15.20	4.60	12.70	5.80	10.70
9	4.20	16.40	5.40	13.90	6.60	11.90
10	4.90	17.60	6.20	15.00	7.50	13.00
15	8.60	23.50	10.40	20.70	12.00	18.40
20	12.70	29.20	14.70	26.20	16.60	23.80
25	16.90	34.70	19.20	31.60	21.30	29.00
30	21.20	40.00	23.70	36.90	26.00	34.20
35	25.70	45.20	28.40	42.00	30.80	39.40
40	30.30	50.30	33.20	47.10	35.70	44.40
45	35.00	54.30	38.00	52.20	40.60	49.50
50	39.80	60.20	42.90	57.10	45.50	54.50

It is important to mention that the automatic morphology evaluation by CASA systems is dependent on the settings and the capability of the machine to detect different anomalies. Most of the CASA systems are able to detect cytoplasmic droplets and bent/looped tails, but not acrosome defects or abnormal head shapes. Graphic 2.2 provides an overview on different sperm anomalies.

GRAPHIC 2.2: OVERVIEW ON DIFFERENT SPERM CELL ANOMALIES



In normal stud procedures it is not required to differentiate all anomalies mentioned in the table above. For simplification, Table 2.9 can be used to take notes on manually evaluated ejaculates.

TABLE 2.9: ROUTINE MORPHOLOGY TABLE

MORPHOLOGY	COUNT	% OF TOTAL
Normal cell		
Head defects (including acrosomal)		
Cytoplasmic droplets (prox. and dist.)		
Tail defects		
Other defects		

PREPARING FIXED/STAINED SAMPLES

There are two preparation methods for samples to be evaluated for abnormal cells: the unstained, wet preparation and the stained and dried preparation. Both techniques have pros and cons which are listed in Table 2.10 below.

TABLE 2.10: COMPARISON OF UNSTAINED WET PREPARATION AND STAINED SMEAR

UNSTAINED, WET PREPARATION	STAINED SMEAR
Quick/easy to do	Good contrast to detect cell anomalies
Cells float → differentiate "real" cytoplasmic droplets from free floating droplets	Slightly more complex/time consuming sample preparation
Cells move → assessment slightly tougher	Cells are easy to evaluate since fixed in position
No storage of fixed slides	Storage of fixed samples possible
Storage of fixed semen sample in sample tube possible	

UNSTAINED, WET PREPARATION

The unstained, wet preparation technique is suitable for examination by phase contrast or differential interference contrast (DIC) illumination. The sperm cells can be examined in any clear medium. Motility can be stopped by adding 10 µl of 1.5% formaldehyde[1] to 1 ml of (raw) sperm suspension (for extended ejaculates use 500 µl only). Place a small drop (e.g. 5 µl) on the slide and covered carefully with a large cover slip (e.g. 22x40 mm). The thin monolayer that is formed means that the sperm will be in the same focal plane and moving less than in a thicker layer. When oil immersion is used, some pressure is transferred through the oil to the cover slip when focusing. If the sperm suspension layer is thick, sperm will be pushed away from the field of view.

[1]Formaldehyde should only be used by veterinarians or after special training about the health and safety rules to follow when handling this chemical.

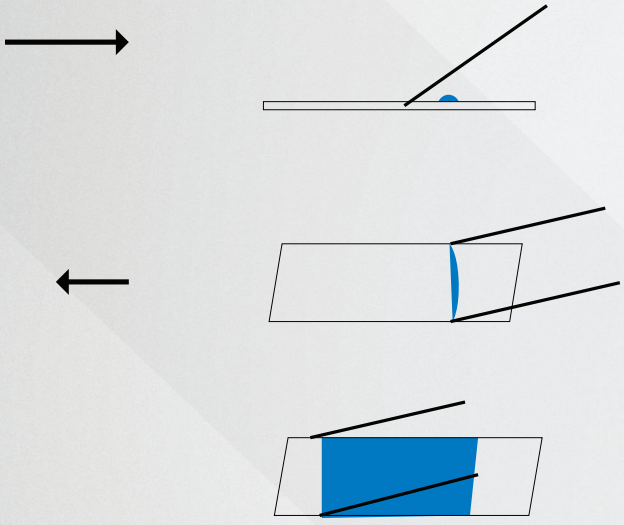
STAINED PREPARATION

The stained preparation technique only uses plain glass slides for the preparation. The glass slides and stain should be at room temperature. Put 1 droplet of raw semen and 2 droplets of stain (most common Eosin-Nigrosin) on a microscope slide. Mix the droplets smoothly with the edge of a second glass slide. By using the edge or corner of the slide, transfer a small volume of fixed and stained semen to a clean microscope slide and place it near one end of the slide, leaving space for labeling.

Using another new microscope slide (spreader) put the short edge on the slide 'downstream' from the drop. Keep the whole edge in contact with the slide and draw back the slide until it touches the drop of stained semen. Wait for the semen to spread to the full width of the slide and then push the spreader in an angle of 45° along the slide to create the smear. The smear should not be very thin (this is not a blood smear) or too thick (this may make appreciation of the differential stain difficult).

Always check the smear using a low power objective immediately after it has air dried (no additional heat). If there are too many or too few sperm or not enough or too much contrast, prepare a new slide. The smear should be even in appearance. Each low power (low magnification) field should have a number of sperm within it. Low power fields with zero or just one or two sperm mean a slide on which it will be very difficult to find sperm under oil immersion. At the other extreme, the sperm density should not be so high that sperm overlay each other. This would make it very difficult to examine individual sperm clearly.

GRAPHIC 2.3: INSTRUCTION ON HOW TO PREPARE A SMEAR



Making and evaluating smears requires practice. A good smear is characterized by:

- Enough cells per viewing field (min. 5 at 1,000x magnification)
- No overlapping cells
- Enough contrast to see all cell details
- Easy to focus (only one layer of cells)

MANUAL EVALUATION OF SAMPLES

Manual evaluation of samples should be done with a light microscope and movable stage at 1,000x magnification and oil immersion. It requires practice to "find" the cells with this magnification. Start focusing on the sperm cells with a magnification of 100x or 200x. When changing to a 1,000x from the lower magnification, only a small adjustment is needed to get the cells in focus. A minimum of 100 cells (preferable 2 x 100 cells though) should be counted and evaluated to estimate the percentage of abnormal cells in the ejaculate. Find a spot in the smear where cells are not overlaying each other as the starting point. Start at the top left of a field, follow an imaginary line to the right and assess every cell on that line. Then go 1 line lower and follow this from the right to the left going zigzag through the field. Then move to the next field and continue the process until the targeted number of cells has been evaluated. If a cell shows more than one anomaly, only count the more severe one. Head and acrosome anomalies have highest priority followed by cytoplasmic droplets and tail defects.
Time is critical when morphology evaluation is conducted during semen production. In this case an evaluation at 650x magnification without oil immersion would be sufficient.

CONSIDERATIONS FOR WORKING WITH CASA AUTO-MORPHOLOGY SYSTEMS

CASA systems differ in the types of sperm anomalies they can detect. Most identify cytoplasmic droplets (proximal and distal) and bent/looped tails. They don't identify typically defect acrosomes and abnormal head shapes. Consider this when setting thresholds for maximum allowed percentage of abnormal sperm. Contact the CASA vendor for more information about auto-morphology function. One big advantage of automated morphology is that more cells can be reviewed in a shorter time and the calculated estimates become more accurate.

SEMEN PRESERVATION

Raw ejaculates have a very limited shelf life after collection, so their capacity to fertilize oocytes needs to be preserved by adding semen extender. Usually extenders preserve semen for up to 3-7 days after collection. The dilution rate of the raw ejaculate (how many semen doses get produced) is dependent on the targeted cell count per AI dose. Semen extender can be considered the "backbone" of every AI dose and should be prepared with care.

The water used for extender preparation should be purified (ASMA type I water or equivalent) and free of bacteria. It can be either produced with an on-site water purification system or purchased from a reliable supplier. Minimum requirements for water quality can be found in Section III, Table 3.5-3.6.

Extender should be prepared for each production day in stainless steel vats which can be equipped with a disposable plastic liner for reduced hygiene risk.

The basics activities to prepare an extender are:

- Add the required amount of purified water to the vat
- As the water reaches proper temperature (according to manufacturer instruction; normally around 35°C/95°F), add the required amount of extender powder to the water and mix it well. Either manual stirring and/or recirculation of the water will aid in dissolution of the powder.
- The time until an extender preparation can be used varies between products (refer to manufacturer instruction)
- Ensure that all extender powder is dissolved in the water prior to usage
- Use a conductivity or TDS meter or refractometer to check for the correct water-extender ratio prior to using the extender (Instructions see Appendix D).
- Use a pH meter as an additional check for extender readiness. Determine the appropriate value according to the manufacturer's specifications
- After extending the first ejaculate of the day, check if the sperm motility is within the normal range. Repeat these procedures 2 hours after dilution as a further double-check of extender quality.
- Ensure that all vats, tubes, and pipelines that get exposed to extender are cleaned every time production is completed. More details to be found in Section III, Chapter "Cleaning And Disinfection".

DEDICATED CELLS PER DOSE

The number of cells per dose varies between studs and is influenced by multiple factors. No general recommendation can be given here. Most common across the globe are counts between 1.5 and 3 billion cells per dose in a 30-100 ml volume. The cell concentration per dose should not exceed about 60 million cells per milliliter as this may result in reduced shelf-life.

There are 2 different ways of expressing the dedicated cells per dose:

1. TOTAL SPERM CELLS PER DOSE

It is easy to calculate total sperm cells per dose and check days after the dose has been produced since the number of cells does not change over time. The number of doses with a dedicated total cell count that can be produced out of an ejaculate is calculated as follows:

$$\frac{\text{Ejaculate Volume (ml)} * \text{Sperm cell concentration (Million/ml)}}{\text{Targeted Cell Count per Dose (Million cells)}}$$

The amount of extender (in ml) to add to the raw ejaculate is calculated as follows:
(Doses to produce (see above) * dose volume (ml)) – ejaculate volume (ml)

2. VIABLE[1] SPERM CELLS PER DOSE

The target of viable sperm cells per dose defines a certain number of motile cells with normal morphology per dose. There is normally an overlap between non-motile cells and those with morphological defects. A calculated composite score works well to define the number of "non-viable" cells (see formula below). As we expect that only motile, normal cells are able to fertilize an oocyte this calculation makes more sense than the expression of total sperm cells per dose. However, it is tougher to control the doses for adequate viable cell count as motility drops over time. The later the sample is checked post-production, the lower the number of viable cells present. There is no way to determine if the dedicated amount of viable cells was added initially. The number of doses with a dedicated viable cell count to be produced from an ejaculate is calculated as follow:

Composite Score = Motile cells (%) * Normal morphology cells (%)
Viable cells (Million) = Composite Score * Volume (ml) * Concentration (M/ml)

The calculations for number of doses to produce and amount of extender to add are equal to what was mentioned above for the total cell calculations.

[1]Note that the original/scientific definition of "viable cells" differs from the one described above which was established in AI stud slang. Viable cells in a scientific context are ones that are still intact (intact acrosome and plasma membrane).

SEMEN EXTENSION PROCESS

The most common semen extension strategies are the one- and two-phase extension. They differ in the temperatures of the extender when added to the raw semen. The first steps are equal on both processes:

- Conduct first extension of the raw ejaculate as fast as possible but max. 15 min. after finishing the collection.
- Ensure the first extension is done isothermal; raw ejaculate and extender should have the same temperature
- Mix the extender and ejaculate in a proper, clean vessel. Special semen mixing pitchers or buckets with a disposable plastic liner work best.

1. ONE STEP DILUTION

- Complete ejaculate dilution with extender at similar temperature within 15 min. after collection.
- Alternative if 15 min. not achievable: Quickly pre-dilute the raw ejaculate with extender of same temperature (1:1 to 1:3 ejaculate-extender ratio most common) and conduct final dilution at same temperature as soon as possible.
- Package semen promptly.
- Cool to storage temperature (17 ± 2°C/63±3.6°F).

2. TWO STEP DILUTION

- Pre-dilute the raw ejaculate with extender of same temperature (1:1 to 1:3 ejaculate-extender ratio most common).
- Wait for 15-20 min to equilibrate with room temperature.
- Conduct final dilution with extender not cooler than room temperature (20°C/68°F).
- Package semen promptly.
- Cool to storage temperature (17 ± 2°C/63±3.6°F).

Recent studies (SCHULZE et al., 2013) indicate a slight quality advantage of ejaculates extended with the one step, isothermic method.

SEMEN FILLING

Most often, the extended semen gets "filled" in plastic tubes or bags with the help of packaging machines. Due to the cost of the automatic packaging machines and the lower throughput, smaller boar studs typically fill semen doses manually.

In general the dose volumes are between 30 and 90 ml, depending on the type of AI to be completed. For conventional AI, the dose volume is recommended to have a minimum of 70 ml.
Table 2.11summarizes the most important focus areas when packing semen doses.

TABLE 2.11: CRITICAL AREAS OF SEMEN PACKAGING

HYGIENE
Store hoses/ bags clean and dry until usage Ensure that everything in touch with the extended semen is: • Clean (disposable or disinfected) • Not touched by hands (unless just disinfected) • Non sperm-toxic Hoses, sinkers, needles for packing the extended semen should be changed after every ejaculate/semen batch. Establish proper disinfection and sterilization protocols for hoses, sinkers and needles.
HOMOGENEITY
• Mix the extended semen well prior to filling • If the packaging process exceeds 10 minutes, mix one more time to avoid semen settlement which equates to variance in the number of sperm cells per dose.
PACKING VOLUME
• Check the dedicated filling volume of the doses during the packaging process in order to assure the accuracy • Variations >+/- 1 ml should trigger calibration/maintenance of the filling machines
MAINTENANCE
• Clean and disinfect the filling machines after every production day. • Follow maintenance intervals based on manufacturer specifications.

SEMEN COOLING

Cool rooms are used for storing and cooling the semen prior to dispatch. Maintain temperatures at 17± 2°C/63±3.6°F and use a stir fan to ensure air circulation. Record daily high and low temperatures in the cool room through the use of an automatic recording device.

Wire shelves or trolleys are used to move, store and cool the semen. The design of these units provides optimal flow of cool air and a more uniform cooling of the doses from extension to preservation temperatures.

The combination of a cooling system along with adequate air circulation within the cool room is the key factor in reducing dose temperatures to the desired range over the appropriate time period.

SEMEN PACKAGING AND SHIPPING

The goal of semen packaging during shipment and delivery is to protect the doses against cool or warm ambient temperatures as well as physical damage including from sunlight. The goal is to maintain semen doses at a temperature of 17± 2°C/63±3.6°F with temperature fluctuations not exceeding 1°C/2°F until they are used on-farm.

If semen gets transported with the boar stud's own couriers, the best option is to equip the delivery vehicles with plug-in climate boxes using the vehicle's power supply. The units should be able to cool and warm and have an outside display for regular internal temperature checks. A stir fan helps to circulate the conditioned air and creates equal temperatures inside the whole box. When packing the boxes, there should be enough room inside for air to be circulated. Semen transported in such climate boxes does not need special insulated packaging materials such as Styrofoam boxes. A double plastic or paper bag (remove outside bag prior drop off) will be sufficient for this type of transport.

If semen gets shipped with a parcel courier service (mail, UPS®, FEDEX®, etc.) or air mail, typically no temperature controlled vehicles are available and the package can be exposed to extreme and/or fluctuating temperatures. The following factors can diminish transport temperature fluctuations to protect the semen doses:

- Ensure that semen is at the final storage temperature prior to packaging.
- Pack doses in a single thick wall or double Styrofoam box.
- Use insulating foil to cover the inner Styrofoam box (2 box system) or the single Styrofoam box in the 1 box system. The foil can also be used as an inner layer to wrap directly around the doses as well.
- Use gel packs to stabilize the transport environment around the doses as they add both volume and temperature regulation to help mitigate fluctuations. Gel packs at cool room temperature should only be placed in direct contact with the semen doses. High ambient temperature conditions can be mitigated by adding frozen gel packs to the void between the two Styrofoam boxes (i.e. 2 box systems). Conversely, warm gel packs are added to the void when there are low ambient temperatures during shipment.
- Enclose the outer Stryofoam box inside a cardboard box for added protection.
- Add a small electronic temperature recording device to track the internal temperature during shipment, which aids in troubleshooting packaging and shipment procedures.
- Keep semen transport times as short as possible.

More detailed instructions can be found in Appendix E.

SEMEN STORAGE ON FARM

Ideally semen shipments are dropped by the courier or commercial parcel service at an off-farm location to maintain high bio-security levels for both the farm and boar stud. The drop-off point can have a 17°C/63°F storage unit where the shipment box is placed by the driver. Farm staff should know when the drop-off occurs so that a timely pickup and transfer to the final storage unit can happen following the decontamination process for incoming shipments (see Section I, Table 1.2).

The key points of proper on farm semen storage are listed below:

- Store semen at 17±2°C/63±3.6°F. Avoid using semen stored outside this range.
- Use a surge-protected, fan-assisted cooler that can warm and cool.
- Space semen cooler at least 2.5 cm/1inch from the wall to work effectively.
- Open cooler shelving to allow complete air flow.
- Unpack doses before placing them in the semen cooler.
- Store doses loosely and horizontally.
- Record semen cooler temperature and who checks the inventory daily.
- Use several digital thermometers with outside readers. Place the sensors at different locations in the cooler (near cooling/heating element, near door and in the middle)
- Use semen within three days after production if possible and order new doses based on your current inventory.
- Transport semen to the breeding barn in an insulated container and use gel packs at 17°C/63°F to maintain temperature.
- Only take doses to the barn that will be used within 1 hour. Do not take doses back from the barn to the semen cooler.
- Recent research has shown that the historical practice of rotating semen daily is not needed.

SECTION 3:

QUALITY ASSURANCE AND QUALITY CONTROL

This section provides information about how to use, calibrate and maintain measurement devices as well as their targeted accuracy. Furthermore information about cleaning and disinfecting procedures as well as adequate third party monitoring programs will be provided.

DEFINITIONS

Quality assurance (QA) is a way of preventing mistakes, avoiding problems, and providing confidence that quality requirements will be fulfilled. Prevention of quality issues (i.e. proactive process) differs subtly from quality control (QC) where the objective is to detect products of substandard quality and implement the changes for improvement (i.e. reactive process). Whereas QC mainly tests finished product, QA is testing critical processes that contribute to the end product's quality. We use two guiding principles for QA: "Fit for purpose" (the product should be suitable for the intended purpose); and "right the first time" (mistakes should be eliminated). In this section these principles will be discussed in relation to the production of semen doses.

MAINTENANCE AND CALIBRATION OF EQUIPMENT AND DEVICES

In boar studs many technical devices are used. Failing equipment can delay the completion of the day's production and also may affect the quality of the product since many of these devices are important to determine the ejaculate quality.

An example is the use of pipettes or dispensers to prepare a diluted ejaculate sample in order to measure the concentration of sperm cells. If the dispensed volumes are not accurate, the dilution will be inaccurate and the concentration measurement will result in an underestimation or overestimation of the concentration. This can result in doses not containing enough sperm cells for optimal fertility.

The more we rely on equipment, the more important it is to use a daily, weekly or monthly checklist for the different equipment and to develop a calibration and maintenance plan. In addition, it is important to have an alternative protocol in place for when the equipment fails. An example of a checking, cleaning, maintenance and calibration schedule for key laboratory equipment is given in Table 3.1. For QA this schedule should be accompanied by specific instruction per equipment/device on how to perform the checks, cleaning, maintenance, and when it is necessary to calibrate and how it is to be done.

TABLE 3.1: A CHECK, CLEANING, MAINTENANCE AND CALIBRATION SCHEDULE

SERVICE/ CALIBRATION-SCHEDULE	CHECK	CLEANING[1]	INTERNAL MAINTENANCE[2]	SERVICE[2]	CALIBRATE[3]	COMMENT
Scales	Daily	Daily		1x/year	1x/year	Standard weights required
Manual pipettes and dispensers	Daily	Daily	Monthly	2x/year	2x/year	
Auto-dispenser	Daily	Daily	Weekly	1x/year	1x/year	Per manufacturer's instructions
Spectrophotometer	Daily	Daily	Quarterly	2x/year		
Heating stage microscope	Daily	Daily				Calibrated thermometer required
Heating plates	Daily	Daily	Monthly			
Heating devices extender vats	Daily	Daily	Monthly			
Heating blocks	Daily	Daily	Monthly			
Dry heat sterilizer	Daily	Daily	Weekly			Per manufacturer's instructions
Refrigerators and semen storage units	Daily	Monthly	4x/year			min-max thermometer
Thermometers	Daily	Daily	Monthly	Replace		
TDS-conductivity meter	Daily	Daily	Monthly	2x/year		
pH meter	Daily	Daily	Monthly	2x/year		
Microscopes (CASA)	Daily	Daily	Weekly	1x/year		
Air conditioners	Daily	Monthly	4x/year	2x/year		Replace filters monthly

[1] Minimum required thorough cleaning

[2] Follow instructions of manufacturer and contemplate intensity of use

[3,4] Minimum calibration rate; Additional calibration required whenever equipment is off target

SCALES

Accuracy of a scale refers to as the ability to provide a result that is as close as possible to the actual value on a consistent basis. In most boar studs there are multiple scales, all with different specifications and capabilities for ranges of weights. The specifications depend on what needs to be weighed and how precise the measurement should be. Table 3.2 lists the most common scales typically encountered in the laboratory.

TABLE 3.2: OVERVIEW OF VARIOUS SCALES

TO MEASURE	WEIGHING RANGE	SENSITIVITY
Pipette/dispenser accuracy and technician repeatability	0-50 g (analytical scale/precision scale)[1]	0.0001 g / 0.1 mg
Extender powder weight	0-5,000 g;	1 g / 1,000 mg
Ejaculate volume	0-1,000 g	1 g
Dispensing extender to ejaculate	0-5,000 g;	1 g / 1,000 mg
Tube/bag volume	0-100 g	0.1 g / 100 mg
Package weight	0-20 kg/0-20,000 g	0.1 kg / 100 g

[1]This is the scale used to check the accuracy and repeatability of the pipettes/dispensers

Check all scales before use on every production day by using standard weights. Place one or more standard weights equal to the (range of) load on the scale, take a reading and remove the weight from the scale. Check the accuracy. This operation should be repeated several times before readings are taken for production purposes. Once the scale has been turned on and "warmed-up", better repeatability will be achieved.

Scales can be very accurate even when used without being exercised first, but improved results can be obtained by going through a "warm-up" procedure. The accuracy can be calculated by taking 5-10 measurements and calculating the average. The relative (%) difference between the average and standard weight is the accuracy.

EXAMPLE ACCURACY CALCULATION FOR AN ANALYTICAL/PRECISION SCALE:
Accuracy (%) = 100 x ((average weight – targeted weight)/targeted weight))
Standard weight = 2.00 g
Average weight = 2.02 g
Accuracy (%) = 100x ((2.02-2.00)/2.00)) (%) = 100x ((0.02)/2.00)) (%) = 100x ((0.01)) (%) = 1%

Table 3.3 shows the tolerances for standard weights (maximum tolerated deviation of readout weight versus standard weight). If the average is outside the tolerated deviation, the scale needs calibration. In our example the scale is 1% off target whereas the tolerance is (maximum) 0.06% (see Table 3.3). Therefore the scale needs to be recalibrated. The decision about recalibration is dependent on how much of an affect the inaccuracy has on the final product.

For example, if you check the scale (5 kg) for dispensing extender, the tolerance is 0.01%. So if the imprecision is 0.1% (5 g), the effect on the end product is too small to be important. It would be within the acceptable volume variation of a packing machine.

TABLE 3.3: TOLERANCES FOR MEASURING VALUES OF STANDARD WEIGHTS

WEIGHT	TOLERANCE	TOLERANCE (%)	WEIGHT	TOLERANCE	TOLERANCE (%)
5kg	0.5g	0.01%	2g	1.1mg	0.06%
3kg	0.3g	0.01%	1g	0.9mg	0.09%
2kg	0.2g	0.01%	500mg	0.72mg	0.14%
1kg	0.1g	0.01%	300mg	0.61mg	0.20%
500g	70mg	0.01%	200mg	0.54mg	0.27%
300g	60mg	0.02%	100mg	0.43mg	0.43%
200g	40mg	0.02%	50mg	0.35mg	0.70%
100g	20mg	0.02%	30mg	0.3mg	1.00%
50g	10mg	0.02%	20mg	0.26mg	1.30%
30g	6mg	0.02%	10mg	0.21mg	2.10%
20g	4mg	0.02%	5mg	0.17mg	3.40%
10g	2mg	0.02%	3mg	0.14mg	4.67%
5g	1.5mg	0.03%	2mg	0.12mg	6.00%
3g	1.3mg	0.04%	1mg	0.1mg	10.00%

Source: National Institute of Standards and Technology (NIST); NIST Handbook 2003 105-8

CALIBRATION

Calibration is the comparison between the output of a scale or balance against a standard value. Calibration requires a standard weight and the balance to be set in the "calibration mode." Depending on the scale you can perform this yourself (follow instruction in the manual) or have it performed by a specialized company (which is recommended).

DISPENSING WITH PIPETTES OR (AUTO) DISPENSERS

In most cases the raw semen sample has to be diluted before measuring concentration or analyzing motility and morphology. A dilution error can have a profound effect on the number of cells per dose. Whether you use an auto-dispenser, a bottle top dispenser or pipettes, it is important to check the accuracy of the device before your daily production, to test the repeatability of the individual technician, and to test the reproducibility between technicians on a frequent basis (suggested minimum 1x/month). The bullet points below describe how to test dispensing accuracy and repeatability:

- Use a precision/analytical scale (checked with standard weights) and do 5-10 measurements before actually starting the procedure.
- Dispense the required volume (water) 10 times with a pipette tip change (if applicable) before each aspiration of liquid.
- Record the results in a spreadsheet program.
- Calculate the average and the standard deviation for the 10 measurements.
  - Accuracy (systematic error %) = 100x ((average weight – targeted weight)/targeted weight)).
  - Variation (random error) expressed as coefficient of variation (CV %) =
- 100x (Standard Deviation measurements/Average measurements).
- Keep the following in mind when interpreting the results
  - The smaller the dispensing volume, the larger the systematic and random error to be expected.
  - Accuracy is dependent on the quality, correct use, and proper maintenance of the pipettes/dispensers.
  - The repeatability is dependent on dispensing technique, quality of the pipette/dispenser, and pipette tips (if applicable). Table 3.4 illustrates the maximum permissible error, both for accuracy (systematic error) and repeatability (random error).

TABLE 3.4: PERMISSIBLE SYSTEMATIC AND RANDOM ERROR FOR DISPENSERS AND PIPETTES

MAXIMUM PERMISSIBLE		SYSTEMATIC ERROR		RANDOM ERROR	
VOLUME (µL)	VOLUME (ML)	± %	± µL	± %	± µL
1	0.001	5.0%	0.05	5.0%	0.05
2	0.002	4.0%	0.08	2.0%	0.04
5	0.005	2.5%	0.125	1.5%	0.075
10	0.01	1.2%	0.12	0.8%	0.08
20	0.02	1.0%	0.2	0.5%	0.1
50	0.05	1.0%	0.5	0.4%	0.2
100	0.1	0.8%	0.8	0.3%	0.3
200	0.2	0.8%	1.6	0.3%	0.6
500	0.5	0.8%	4.0	0.3%	1.5
1,000	1	0.8%	8.0	0.3%	3.0
2,000	2	0.8%	16	0.3%	6.0
5,000	5	0.8%	40	0.3%	15
10,000	10	0.6%	60	0.3%	30

Source: ISO 8655:2002

SPECTROPHOTOMETER

Many boar studs use a spectrophotometer to measure ejaculate sperm concentration, also known as ejaculate density. The concentration is calculated based on the amount of light transmittance through the semen versus absorbance by the semen. Since this is an indirect way of measurement, the accuracy of the equipment should be measured against reference equipment/samples where the accuracy has been tested and is within permissible limits. Typically a spectrophotometer is calibrated by using samples with different concentrations which are checked with reference equipment and methods.

A spectrophotometer typically shows a sigmoid curve and the measurements taken will only be reliable on the linear portion of the curve. It is important to know both the minimum and maximum concentrations that can be measured in a reliable way on the said spectrophotometer.

In order to check if a spectrophotometer is working correctly on a daily basis, use formalin[1] fixed samples (both low and high density) as a reference. Sperm concentrations in formalin-fixed1 semen (dilution 1:1) remain stable at concentrations from 0.1 M/ml to 100 M/ml for at least 5 weeks at 4°C/40°F (refrigerator temperature).

Take a sample from the low density and high density formalin[1] fixed semen samples, measure the concentration with the spectrophotometer, and decide if the spectrophotometer is still accurate.

[1]Formaldehyde should only be used by veterinarians or after special training about the health and safety rules to follow when handling this chemical.

Clean the spectrophotometer daily and test at least every week (but preferably every day). Maintain according to the manufacturer's recommendations and service at least 2x per year (preferably quarterly). We recommend checking multiple semen samples monthly for concentration with a reference laboratory to assure the spectrophotometer remains accurate.
Four methods are generally used by reference laboratories to check the concentration of submitted samples prepared with your boar stud spectrophotometer (or other equipment):

1. Counting cells with a hemocytometer
   A sample is loaded into a cell counting chamber. A grid in the chamber represents a certain volume. The number of cells in a standard number of grids is counted and then the cells per ml are calculated. If for example a sample is diluted 1:10 (11x; 1 part sample + 10 parts diluent) and 325 cells were counted in 5 squares the total cell count/ml is calculated as:
   Total cells/ml = (325 x 11 x 10,000)/5= 7.15 Million cells/ml

2. Using a flow cytometer
   A sample is incubated with a fluorescent mammal DNA probe that binds to pig DNA. The cells will emit a fluorescent signal once the probe bound DNA flows through the machine and is measured. Based on the total signal strength, the number of cells in the sample is calculated. Then the concentration of the original sample is calculated by using the appropriate dilution factor used to prepare the flow cytometry sample. Typically this method is very accurate since it is a direct measurement and many cells are counted (>50,000). Flow cytometers are not considered to be basic equipment for an AI laboratory but can be found in professional 3rd party andrology centers.
3. Using a Nucleocounter®
   The Nucleocounter® follows the same principle as the flow cytometer. The Nucleocounter® is not quite as accurate as a flow cytometer since less cells are counted (2,000-5,000). However, since the cost of the Nucleocounter® is significantly lower than a flow cytometer, these machines are found in many boar studs and are being used as an "in-house" reference for dose density.
4. Using CASA and Counting Chambers
   Counting chambers and CASA systems are routinely found in reference laboratories and are being used to determine the concentration of submitted boar stud doses.
   See Section II, Chapter "Semen Evaluation" for further information about CASA system and counting chamber use.

HEATING DEVICES AND THERMOMETERS

In semen production many devices are used that need to be warmed or heated. This is accomplished by electric heating elements in equipment such as microscope stages, extender vats, and warming platforms/blocks. The heating element will be set to a certain temperature, but this is no guarantee that the set temperature is at the targeted temperature. Heat will be lost to the environment when there is a large surface and therefore it may be necessary to adjust the temperature setting to compensate. At the same time the temperature setting on the equipment may be inaccurate. Therefore, it is important to check the actual temperature on a daily basis with a calibrated thermometer.

Many boar studs use infrared (i.e. laser) thermometers because they are quick, practical and reduce risk of contamination versus traditional stick-type thermometers that need direct contact with the object or liquid being measured. However, infrared can be both less accurate and less repeatable than traditional methods such as the contact or dip thermometer. The accuracy and repeatability of infrared is very dependent on how the device is used, the distance to the object/liquid, the angle of use, and the surrounding ambient temperature. A boar stud that uses an infrared thermometer should follow the method of operation as described in a standard operating procedure. It should also be tested against a calibrated thermometer.

CONDUCTIVITY/TOTAL DISSOLVED SOLIDS (TDS)-METER

The water used for extender preparation must be purified (Type I water). As an integral part of a boar stud's QA program, the correct ratio of extender powder and water must be tested before each production day by measuring the conductivity or TDS. Test the water first prior to introducing the extender powder. After that, mix extensively and test the prepared extender. As conductivity is affected by temperature, most meters have an automatic correction for this variable. However, it is still wise to check the correct functioning of the meter with a standard solution before actually testing water and extender samples.

CALIBRATION OF CONDUCTIVITY/TDS-METERS:

1. Ensure that the standard calibration solution is at the same temperature as the solution to be tested to minimize errors from the temperature effect.
2. Pour enough (≥2.5cm filling level) standard calibration solution into a clean and dry vessel.
3. Remove protective end cap from the meter, exposing the stainless steel electrodes.
4. Turn the meter on and dip electrodes into the standard calibration solution so they are fully submerged. Do not trap any air bubbles around the electrodes to avoid measurement errors.
5. Keep electrodes in the standard calibration solution until the reading stabilizes.

6. Adjust calibration buttons so that digital display indicates the same value as the standard calibration solution.
7. Rinse a meter's electrodes with a volume of the liquid to be tested. Do not use the rinse portion as a test sample to minimize carry over contamination from the calibration solution. This process eliminates the need to dry the meter's electrodes. If this is not practical, rinse the electrodes with distilled water and blot dry using a clean wipe.

The meter is now calibrated and ready to measure the TDS of the water and extender samples. Different meters may have slightly different methods of operation. Read the manufacturers instruction manual carefully before using the meter.

PH-METER

Every extender has its own acceptable pH range. The proper mixing/quality of the extender should be tested with a calibrated pH-meter. Calibration is typically performed by measuring a series of reference standards, so-called pH buffers, that have known and accurate values at different temperatures. Testing can be performed as follows:

BEFORE CALIBRATION:

Properly clean the electrode prior to calibration. If you have stored your electrode, make sure it is clean and ready for use per the manufacturer recommendations.

THE MAIN STEPS FOR A GENERIC PH CALIBRATION PROCEDURE:

1. Turn the pH-meter on and allow adequate time to warm up (check operating manual).
2. Select two pH buffers that bracket the expected sample pH (for boar semen extenders pH ranges between 6.8 and 7.2). The first buffer should be pH 7.00 (zero point adjustment). The second buffer should be near the expected sample pH.
3. Ensure that the sensor and buffer solution are at the same temperature. If not, allow time for temperature equilibration.
4. Pour the required amount of buffer solution into individual glass vials or beakers. Buffer solution will remain stable in a glass beaker for a maximum of 2 hours. Do not calibrate the electrode directly in the buffer storage container to minimize contamination. Keep the buffer containers closed to avoid CO2 absorption. Do not pour used buffer back into the storage bottle.
5. Place electrode into the first buffer. When the reading becomes stable, set the pH-meter to pH value of the first buffer at the measured temperature. Most modern pH-meters have an "auto-read" function for early detection of a stabilized reading.
6. Between different buffers, rinse the electrode with distilled water followed by the second buffer. Alternatively rinse electrode with distilled water and gently blot dry with lint-free tissue. Avoid rubbing or wiping electrode bulb.
7. Repeat step 5 for the second buffer solution.
8. When pH-meter calibration is done, rinse the electrode with the sample fluid and then place the electrode into the sample and take your pH measurement.

Tips

- Store pH electrodes in storage solution between use; store TDS and conductivity probes in safe packing material per reference manual instructions.
- Use instruction manuals from all your technical devices as reference materials.
- Have the most important spare parts on hand for each piece of critical equipment.
- For critical equipment and procedures, practice one time per month with alternative methods to have a backup routine in place should the unexpected happens.

WATER QUALITY

For the production of semen extender, type I A (ASTM standards) water quality is required. Table 3.5 shows the chemical and microbiological requirements for Type I A water.

TABLE 3.5: REQUIREMENTS WATER QUALITY FOR EXTENDER PRODUCTION:

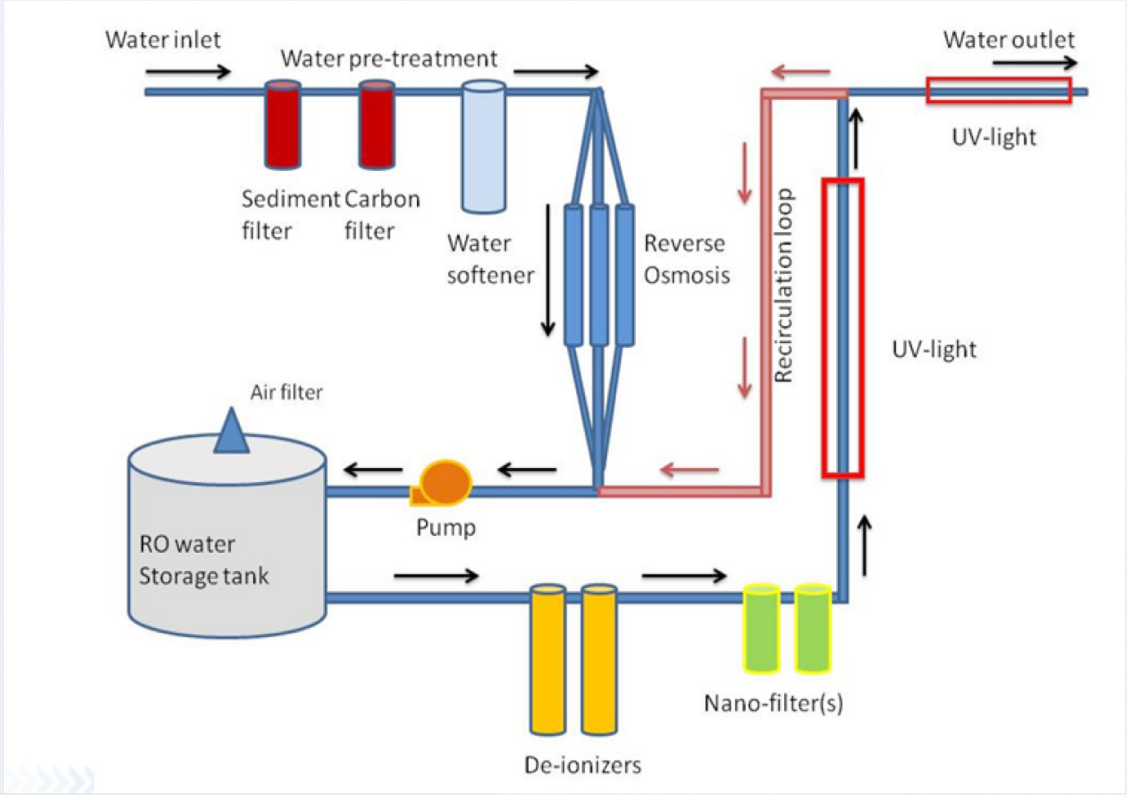
CHEMICAL REQUIREMENTS	TYPE I
Resistivity (MΩ -cm) at 25° C	>18
Conductivity (µS/cm)	< 0.056
Total Organic Carbon (ppb)	<50
Sodium (ppb)	<1
Chloride (ppb)	<1
Total Silica (ppb)	<3
MICROBIOLOGICAL REQUIREMENTS	**TYPE A**
Heterotrophic Bacteria Count (CFU/ml)	<1
Endotoxin (units per ml)	<0.03

WATER PURIFICATION SYSTEM

The selection of a water purification system is often based on factors such as return on investment, local water quality, risk assessment, local availability of vendors, and routine service/repairs.
In general, the choice should be made based on the quality of the system, the local service available, the capacity needed (water produced in liters per hour) and ease of use including ease of sanitization.
There are several principles that should be followed for installation of water purification systems:

- Prevent biofilm formation:
  - Avoid dead ends. Install a re-circulating loop for continual water movement
  - Use flexible hose/tubing to limit the number of elbows
  - Limit the number of connections and valves
  - Install a conical water storage tank
- Keep distance from the system to the tap point as short as possible
- Keep UV-sterilization and 0.2 micron (if available 0.1 micron) filters (for eliminating microbiological growth) as near to tap point as possible before and after the storage tank
- Install specialized recirculation faucets within the re-circulating loop (faucets are commercially available)
- Use an easy accessible and cleanable conical water storage tank
- Follow proper cleaning and disinfection procedures
  - Frequency of cleaning and disinfection is typically based on microbiological status but should be performed at least 4x/year (including water storage tank and RO-membrane)
  - Create a whole system sanitation procedure in collaboration with manufacturer or supplier or have it be performed by a local specialist
  - Use special cleaning and disinfection products for reverse osmosis membranes (product should be compatible with RO-membranes)
  - Connect an induction tank to system for introduction of cleaning and disinfection products in a safe manner
  - Flush the system after sanitation to prevent the lingering presence of residues and use test strips to assure effectiveness of the procedure
- Conduct microbiological monitoring through independent 3rd party testing
- Replace filters after sanitation
- Replace de-ionizing resins after sanitation
- If a hose at the tap point is needed, use a silicone hose that can be easily sterilized after use
- Before filling the extender vat, let the tap run for a while to flush out any bacteria that may be residing in the faucet

GRAPHIC 3.1: PRINCIPLES OF A RE-CIRCULATING WATER PURIFICATION SYSTEM DESIGN



BACKUP OR CONTINGENCY WATER

The boar stud should have a source of backup type I A water in case the primary system fails. The volume of backup water should be determined by management and needs to include consider production volume, production days per week (i.e. weekend versus weekday and number of production days in a row), availability of local water system parts and service, ability to replenish contingency water quickly if the primary system remains broken for an extended period of time, and the estimated length of time the primary system may be "down" before being fixed (i.e. "worst-case-scenario").

The backup water can be bought from an outside source and stored onsite until expiration at which point it should be replaced. Another alternative would be to maintain two independent water systems onsite, each being used to produce water of the same quality. Even though the secondary system may be of reduced output in liters per hour it can substitute for the primary system for several production days if required.

SOURCE WATER QUALITY

The quality of the source water, also called "raw water" (the water that is used to produce the purified water) is another important factor that determines if you need to purchase water or have an onsite purification system is. We recommend frequent testing to determine and monitor the source water quality. Although many minerals and substances can be filtered from water, poor source water quality may jeopardize the production water and thus the extender quality. This will impact the semen quality in a negative way. If the source water meets the quality requirements for potable water it is qualified to be used for boar stud water purification systems. There are drinking water regulations available on the internet with extended tables and maximum permissible contaminants. Often suppliers provide the test results of public drinking water. When a private well is used for the source water it is necessary to frequently test the water quality.

There are three main quality indicators for source water:

- Total Coliform count
  If the total coliform count is high, it is likely that harmful viruses, bacteria, and parasites might also be found in the water.
- Fecal Coliform or Escherichia coli (E. coli) presence
  Fecal coliform bacteria are a specific kind of total coliform. The feces (or stool) and digestive systems of humans and warm-blooded animals contain millions of fecal coliforms. E. coli is part of the fecal coliform group and may be tested for by itself. Fecal coliforms and E. coli are usually harmless. However, a positive test may indicate that feces and harmful germs have found their way into your water system.
- pH level
  The pH level describes how acidic or basic water is. The pH level of the water can influence how water looks and tastes. If the pH is too low or too high, it could damage pipes or cause heavy metals such as lead to leak out of the pipes into the water.

An example of water quality requirements (EPA-Environmental Protection Agency) can be found in Table 3.6. Check with your local health/environmental department for contaminants to test for.

TABLE 3.6: LIST OF NATIONAL SECONDARY DRINKING WATER REGULATIONS (USA-EPA)

INORGANICS	LEVEL
Aluminum	<0.05 to 0.2 mg/L
Chloride	<250 mg/L
Color	<15 (color units)
Copper	<1.0 mg/L
Corrosivity	<noncorrosive
Fluoride	<2.0 mg/L
Foaming Agents	<0.5 mg/L
Iron	<0.3 mg/L
Manganese	<0.05 mg/L
Odor	<3 threshold odor number
pH	<6.5-8.5
Silver	<0.10 mg/L
Sulfate	<250 mg/L
Total Dissolved Solids	<500 mg/L
Zinc	<5 mg/L
ORGANICS (DISINFECTION BY-PRODUCTS/PESTICIDE CONTAMINATION)	**LEVEL**
Trihalmethane TTHM - total	<80 mg/L
Haloacetic Acids HHA5 - total	<60 mg/L
Chloroacetic acid	<1 mg/L
Bromoacetic acid	<0.01 mg/L

CLEANING AND DISINFECTION

A cleaning and disinfection plan contains the frequency and the method of cleaning and disinfection. There are general cleaning and disinfection procedures for barn, laboratory (including ceilings, walls, floors, countertops, and cabinets) and boar housing. There are more specific cleaning and disinfection procedures for collection pens and dummies in the barn and for materials and equipment in the laboratory. All cleaning and disinfection procedures follow a set of simple, but important steps:

1. Remove organic material
2. Clean by use of a detergent (soaking)
3. Scrub the surface with a brush, sponge or cloth to detach organic matter and dissolve biofilm
4. Rinse with the objective to dilute and remove detergent, grease, and proteins
5. Dry
6. Disinfect or sterilize to kill remaining microorganisms
7. Clean/disinfect or replace cleaning materials (brush/sponge/cloth) after each usage

Proper cleaning is the most important part of the above described procedure. Disinfection of surfaces without proper cleaning doesn't kill microorganisms as organic matter will protect them against disinfectants.

The use of detergents and disinfectants require the correct concentration, water temperature, and exposure time to be effective. Strictly follow the manufacturer's instructions and assure sufficient rinsing to remove any residue. Furthermore, it is important to use disinfectants that are effective against the bacteria that are found in the environment to be treated. Keep in mind that bacteria can gain resistance against disinfectants over time, which is a good reason to use several equally effective commercially available products.

Cleaning and disinfection of materials is not as simple as it seems. If not executed properly there is a risk that materials get contaminated or that detergent/disinfectant residues get left behind. If material with residues comes in direct contact with semen, the semen quality is compromised. This is a critical point, yet is difficult to monitor which is the reason many boar studs decide to use disposable instead of reusable materials.

As mentioned in Section II, chapter "Laboratory Setup" , cleaning and disinfection should be made as easy as possible by choosing the right design and materials for the walls, ceilings, countertops, and other furniture. Use smooth, solid and non-corrosive materials for walls (i.e. poured epoxy/resin flooring, stainless steel, solid petroleum based composites and many others), floors and countertops as much as possible. The areas that need daily cleaning (the production lab, the collection area and dummy) should only contain essential items required for production. For example: Store excess consumables in a storage room and not in the laboratory, thereby eliminating the need for laboratory cabinets. Do paperwork in a separate office. Use a wheel cart and only take consumables into the laboratory that are needed for that day's production. Use stainless steel tables on wheels that are easy to move around the production laboratory or remove the tables entirely for a complete cleaning of walls and floors.

DETERGENTS

There are many choices for detergents. The detergent must be effective in removing organic matter, must leave no residues after rinsing, and must be non-toxic. Safe choices would be to use lab ware detergents, cleaning detergents for the feed industry, or even liquid detergents for manual dish washing. A well known detergent for cleaning lab ware and surfaces is Decon 90®. Make sure all surfaces and materials are well rinsed after using the detergent. For the ceiling, walls, and floors, heavy duty detergents can be used such as Simple Green®. Follow the instructions on the label. Do not use spray bottles with the cleaning product as vapor can easily spread to areas that cannot be rinsed. We recommend preparing a cleaning solution that can be administered on the surface with either a sponge or cloth.

DISINFECTANTS

Disinfection is needed if sterilization is not an option for re-usable materials. At the same time, equipment and work counters have to be disinfected after cleaning at the end of each production day. Table 3.7 shows a list of active ingredients for disinfectants.

TABLE 3.7: ACTIVITY LEVELS OF SELECTED LIQUID GERMICIDES

PROCEDURE/PRODUCT	AQUEOUS CONCENTRATION	ACTIVITY LEVEL
DISINFECTION		
Glutaraldehyde	Variable	high to intermediate
Ortho-phthal-aldehyde (OPA)	0.50%	high
Hydrogen peroxide	3-6%	high to intermediate
Formaldehyde	1-8%	high to low
Chlorine dioxide	Variable	high
Peracetic acid	Variable	high
Chlorine compounds	500 to 5,000 ml/l free available chlorine	intermediate
Alcohol (ethyl, isopropyl)	70%	intermediate
Phenolic compounds	0.5 to 3%	intermediate to low
Iodophor compounds	30-50 mg/l free iodine to 10,000 mg/l available iodine 0.1-0.2%	intermediate to low
Quaternary ammonium compounds (QUADS)		low

Source: Center for Disease Control and Prevention (CDC)

There are a few disinfectants that work well for boar studs:

- 70% Isopropyl alcohol (preferred)
- Germicidal Bleach

Clean and disinfect all equipment used during semen dose production after each use. This includes CASA systems, computers, keyboards, dispensers, microscopes, telephones, pipettes, etc. Do not forget to clean and disinfect the walls up to a height of 35 cm/14inch from counter top after each production day. Make sure to disassemble devices as much as possible and clean all parts. Ease of cleaning should be an important buying criteria when you purchase equipment.

Clean from high surfaces to lower surfaces, use cleaning materials that are disposable. We recommend using cleaning carts for the laboratory as this is a self-contained mobile platform on which you can place cleaning buckets. This avoids placing buckets directly on the laboratory tables. Contamination can be spread by the underside of buckets from a dirty area to a clean area. Also, a cart and its contents can be completely removed from the laboratory after cleaning.

Make a schedule for daily, weekly and monthly cleaning. Table 3.8 shows an example of such a schedule. Contemplate hiring professional cleaners for general rooms such as showers, break room, hallways, restrooms, offices, and other general areas so that boar stud staff can focus on the cleaning and disinfection of the production areas.

TABLE 3.8: EXAMPLE OF A CLEANING SCHEDULE FOR LABORATORY AND GENERAL ROOMS

Where	What	Daily	2x weekly	Weekly	Monthly	Quaterly	Procedure no.	Product	who
Laboratory	Floor-sweep-mop		X						professional
Laboratory	Anti fatigue mats		X						professional
Laboratory	Ceiling-Walls rotational (1 part every week)-vents - door			X					professional
Laboratory	Pass through windows: ceiling walls bottom and windows (except barn side)		X						professional
Laboratory	Reception window	X							Barn staff
Laboratory	Countertops + aisle	X							lab staff
Laboratory	Cabinet doors and drawer fronts		X						professional
Laboratory	Cabinets inside						X		Lab-staff
Laboratory	Specific lab equipment-machines-materials	X							Lab staff
-	Autodiluter	X							Lab staff
-	Microscope	X							Lab staff
-	Scales	X							Lab staff
-	Autodispensers	X							Lab staff
-	SPS-11	X							Lab staff
-	Conductivity meter	X							Lab staff
-	Heat sterilizer			X					Lab staff
-	100 liter extender vats	X							Lab staff
-	Extender vat scales/underneath					2X			Lab staff
-	Manual Sealer	X							Lab staff
-	Dish washer			X					Lab staff
-	MOFA storage cabinet			X					Lab staff
-	Refrigerator			X					Lab staff
Laboratory	Underneath equipment and machines (describe in procedure)			X					Lab staff
Laboratory	Office type equipment-machines (computer/keyboard/telephone/copier)		X						professional
Laboratory	Stools/chairs		X						professional
Laboratory	Glass and plastic ware / pitchers / lids (non disposables)	X							lab staff
Laboratory	Sinks	X							Lab staff
Laboratory	Trash cans (small)	X	X						Lab staff
Laboratory	Clean and disinfect racks		X			X			Lab staff
Laboratory	Clean and disinfect carts			X		X			professional
Other rooms	Cool room		X						professional
Other rooms	Shipping room		X						professional
Other rooms	RO-water production system-room (floor and whatever is realistic to clean)		X						professional
Other rooms	Hallway from lab towards the break room		X						professional
Other rooms	Showers			X					professional
Other rooms	Rest rooms			X					professional
Other rooms	Break room			3X					professional
Other rooms	Entry hall-bench (dirty side)					X			staff
Other rooms	Clean Break room Refrigerator					X			staff
Other rooms	RO-water production system					X			Water professional

Make sure to write specific cleaning and disinfection/sterilization procedures for rooms, equipment and devices. Review manufacturer instructions which detergents and disinfectants can be used.
If sterilization is required for items that come into direct contact with semen (i.e. packing machine hoses), we recommend using an autoclave or high temperature drying oven. Special indicator tape or strips are commercially available to check if the sterilizer reached the proper temperature and exposure time in order to kill microorganisms. Table 3.9 shows examples of different sterilization methods, temperatures, and exposure times.

TABLE 3.9: EXAMPLES OF STERILIZATION EQUIPMENT, TEMPERATURE AND EXPOSURE TIMES

TYPE OF STERILIZER	TEMPERATURE	EXPOSURE TIME	DRYING TIME
Gravity displacement steam sterilizer	121°C (250°F)	30 min	15-30 min
Gravity displacement steam sterilizer	132°C (270°F)	15 min	15-30 min
Pre-vacuum steam sterilizer	132°C (270°F)		15-30 min
Steam flush pressure pulse steam sterilizer	132°C (270°F)		15-30 min
Dry heat sterilizer	170°C (340°F)	60 min	
Dry heat sterilizer	160°C (320°F)	120 min	
Dry heat sterilizer	150°C (300°F)	150 min	
Boiling in water	100°C (212°F)	20 min	15-30 min

A lot of detailed information about cleaning and disinfection methods, detergents and disinfectants can be found on the Centers for Disease Control and Prevention (CDC) website:
https://www.cdc.gov/infectioncontrol/guidelines/Disinfection/index.html

INTERNAL QUALITY ASSURANCE AND CONTROL

QC monitoring is vital to ensure high semen production standards are being maintained at a boar stud. The semen dose (end product) should continually be monitored on a pre-determined schedule for the number of cells, post-production motility, and temperature. Laboratory processes and components that contribute to the end product are also important aspects of a QC monitoring program. This program should include the post-dilution motility testing of prepared extender at the start of the processing day. In general, the objective of internal or external monitoring is that 95% or more of the tested samples of the end product meet the semen dose quality criteria.

EXTENDER QUALITY CONTROL

Each (prepared) extender has specifications for pH and conductivity. As described previously, both can be tested with measurement devices to assure that extenders are prepared in the correct way. These measurements should be performed after the prepared extender has been well mixed and is stabilized. The required stabilization time differs between extenders. Keep a log of the instrument calibration and measurements results for reference.

POST-DILUTION MOTILITY EVALUATION

A post-dilution motility evaluation is performed on each vat of extender, directly after dilution (2-3 samples) and 2 hours after dilution of the same samples. If there are quality problems with the extender, these will be detected early and a new extender can be prepared without a major loss of productivity. When there is more than one vat being used, test each one at the beginning of production. Encountering a problem early-on will allow enough time to prepare fresh extender for later use.

POST-PRODUCTION MOTILITY EVALUATION

A post-production motility evaluation can be used to assess the sperm motility over several days after production. The reduction in motility per 24 hours is a good indicator to what extent the semen dose production has been performed correctly. It also indicates that sperm cell quality is likely to be maintained at a sufficient level until the expiration date. The goal for total motility is to remain above 70% of the total sperm cells at expiration. Boars with semen quality issues should be identified prior to dilution (i.e. trashed raw ejaculates; on-hold and possible culling if problem persists) or through frequent post production motility evaluation of individual ejaculates (i.e. history of long-term viability issues). The frequency of individual boar testing should be based on a risk assessment. Testing at least every 4th ejaculate per boar is necessary to detect any problems with post-production motility. The number of samples to test is dependent on the total number of doses produced on a production day (see Table 3.10). The evaluation of a representative number of samples is needed to monitor the quality of the production process for internal reference and should be used to detect and address problems in order to prevent issues in the future. Finally the system has to ensure that produced semen doses still meet the minimum motility values by at expiration date.

TABLE 3.10: NUMBER OF SAMPLES FOR DIFFERENT CONFIDENCE LEVELS AND ERROR RATES

# DOSES WEEK	95%	99%	95%	99%	95%	99%	← CONFIDENCE LEVEL
	10.0%	10.0%	5.0%	5.0%	2.0%	2.0%	← DEVIATION LEVEL
100	25	35	44	59	77	90	
150	26	38	48	67	94	94	
200	27	39	51	72	105	117	
250	27	40	52	75	112	149	
300	27	41	53	77	117	159	
400	27	41	54	80	124	174	
500	28	42	55	82	128	183	
750	28	42	56	85	134	194	
1,000	28	43	57	86	138	204	
1,500	28	43	57	87	142	212	
2,000	28	43	58	88	143	215	
3,000	28	43	58	88	145	219	
4,000	28	44	58	89	146	222	
5,000	28	44	58	89	146	223	
6,000	28	44	58	89	146	224	
8,000	28	44	58	89	147	225	
10,000	28	44	58	89	147	225	

PROCEDURE:

- Save a representative number of samples (based on Table 3.10) from batches or single sire collections in 5 ml sample tubes (1 tube for each test day). In addition save a sample in the tube (last one of batch/ejaculate) or bag used for packaging.
- Re-activate samples for evaluation according to directions provided by manufacturer of extender. Re-activation steps:
  - Invert semen sample tubes until all cells are re-suspended homogeneously.
  - Incubate samples in a heat block at 38°C/100°F
- Note that incubation time is dependent on the type of extender and should be based on the extender label/instructions or guidance directly from the supplier. Perform post-production motility checks on at least the expiration day and a day between production (day 0) and expiration day. Most common is to perform the additional check on day 1.
- If the motility score is less than the pass/fail cutoff percentage on the test day, retest the sample to confirm the result.
- If difference in motility between this analysis and the former (normally day 0 or day 1) analysis is more than 3% per 24 hours, re-analyze sample to confirm result.

Minimum criteria are listed in Table 3.11.

MONITORING NUMBER OF CELLS PER DOSE

The monitoring of sperm cell number needs to be performed to assure the minimum threshold is being met and to determine the variation between doses. The monitoring needs to be done on a representative number of samples. The preference here is to use accurate equipment such a Nucleocounter® or a Flow Cytometer. This equipment is expensive and is not widely used in commercial AI laboratories.
If you don't have the equipment, the testing can be outsourced to a professional 3rd party.
As discussed previously, another accurate option is to use a counting chamber (hemocytometer) and microscope. However, this is a time consuming process and requires a skilled and experienced technician.

TEMPERATURE MANAGEMENT

Some extenders may protect against sperm damage due to temperature fluctuations. However, temperature management during collection, evaluation, processing, storage and shipping remains important. Temperature stress should be minimal during the production, storage and shipping of semen doses. Critical temperature management aspects for the semen production process include:

- Monitor ejaculate temperature during collection and transition (time) from collection to dilution. Measure semen temperature at different points during the transition from collection to reception to dilution. This provides an indication of the required temperature for materials and extender.
- Note that ejaculate temperatures may differ between seasons and boar studs. Therefore frequent measurements are important to adjust material and extender temperatures accordingly.
- Allow a maximum temperature difference of ± 2°C/3.6°F from the semen temperature for materials or extender.
- Use a calibrated thermometer or check the thermometer used against a calibrated thermometer to ensure accuracy of readings.
- Check the temperature setting on the heating device (if present). Note that temperature settings are not always accurate and should be checked with a thermometer regularly.
- Microscopes to evaluate motility should have a heated stage with a temperature set (and checked/confirmed) at 38°C/100°F for semen to express optimal motility.
- After dilution, cool semen gradually to a temperature of 17 ± 2°C/ 63±3.6°F prior to shipping. Although commercial extenders with temperature fluctuation protection are available, we recommend to target for this storage and shipping temperature until sufficient scientific evidence is available that shows otherwise.

MICROBIOLOGICAL TESTING

Microbiological testing is normally performed by an external specialized laboratory but can also be performed in house. Sample the end product water, extender and environment such as dummies as well as the countertop and sink/drain. For more information refer to Section 3, Pharagraph “External Quality Control” or consult PIC Technical Services.

EXTERNAL QUALITY CONTROL

External quality control of the end product is required for a variety of reasons. It assures that equipment is calibrated correctly and dose quality is within acceptable limits. Routine external monitoring to evaluate cell numbers, motility, and morphology at an independent, certified andrology laboratory is one of the cornerstones of a QC program. Preferably, such an andrology laboratory uses a CASA system for motility evaluation and an accurate and validated method to determine the concentration, e.g. by using a Nucleocounter® or flow cytometer. The preferred morphology scoring would be with fixed or stain sperm cells evaluated manually at 1,000x magnification with all specific classifications of abnormalities reported. In Table 3.11 the minimum criteria for a good quality semen dose can be found.

TABLE 3.11: MINIMUM CRITERIA SEMEN DOSE AT EXPIRATION DATE

SEMEN VARIABLE	MINIMUM CRITERIA
Volume	< ±1 ml off target
No. of sperm cells / dose	<± 5% off target
Motile sperm cells	> 60%
Progressive motile sperm cells	> 50%
Loss of (progressive) motility per 24 hours	< 3%
Agglutination	< 30%
Abnormal sperm cells	< 30%
Cytoplasmic droplets (as part of abnormal cells)	< 20%
Bacterial contamination*	< 1CFU/ml

*Measured after 48 hrs aerobic incubation at 37°C

MICROBIOLOGICAL TESTING

Bacteria in semen doses can deteriorate the quality of the semen. Semen extenders contain antibiotics that should inactivate bacteria in the ejaculate. However, if the contamination is large, the antibiotic concentration may not be sufficient to inactivate all bacteria. Antimicrobial resistance in bacteria is another cause of bacterial contamination of semen doses. The microbiology testing of semen doses provides an insight into the hygiene level of semen production. Extender is prepared with purified water and accounts for at least 75% of the semen dose volume. Therefore, it is important to test the water and extender on a routine basis. Most bacteria found in semen doses are from animal, human or environmental origin and thrive best at moderate temperatures between 20°C/68°F and 45°C/113°F under aerobic conditions. Therefore, microbiological testing can be performed best at a temperature of 37°C/99°F under aerobic conditions.

- Microbiological testing should be performed at least 24 hours after extender dilution to give antibiotics time to inactivate bacteria
- Due to competition, certain bacteria multiply slower and only if others are inactivated. Therefore, testing a portion of the samples at a later age may show bacterial growth, even though growth wasn't observed in fresh samples
- If bacteria are found on a regular basis:
  - Identification is necessary to find the source of the contamination
  - Testing susceptibility against a panel of antibiotics including the extender antibiotics will give information about resistance development

HACCP

HACCP stands for Hazard Analysis Critical Control Point. HACCP is a systematic preventive approach used in food production to ensure food safety from biological, chemical, and physical perspective. It analyzes hazards in production processes that can cause the finished product to be unsafe and it designs measurements to reduce the risk level. This methodology can very well be used for semen production. The 7 HACCP principles are as follows:

1. Conduct a hazard analysis:
   Find the hazards that cause substandard semen dose quality. Think of cold shock, bacterial contamination, errors in extender preparation, water quality, cross contamination, temperature variation, toxic materials, etc.
2. Identify the critical control points:
   Analyze each step and all materials used from semen collection to semen packaging, storage, and transportation. Examples of critical points in semen production are: Water quality (both chemical and microbiological), extender quality (pH and conductivity), antibiotic effectiveness, contamination points, and non intentional hazardous substances in materials that come into contact with semen such as latex or powdered gloves, bleached cotton gauze, detergent residues in hoses, etc.

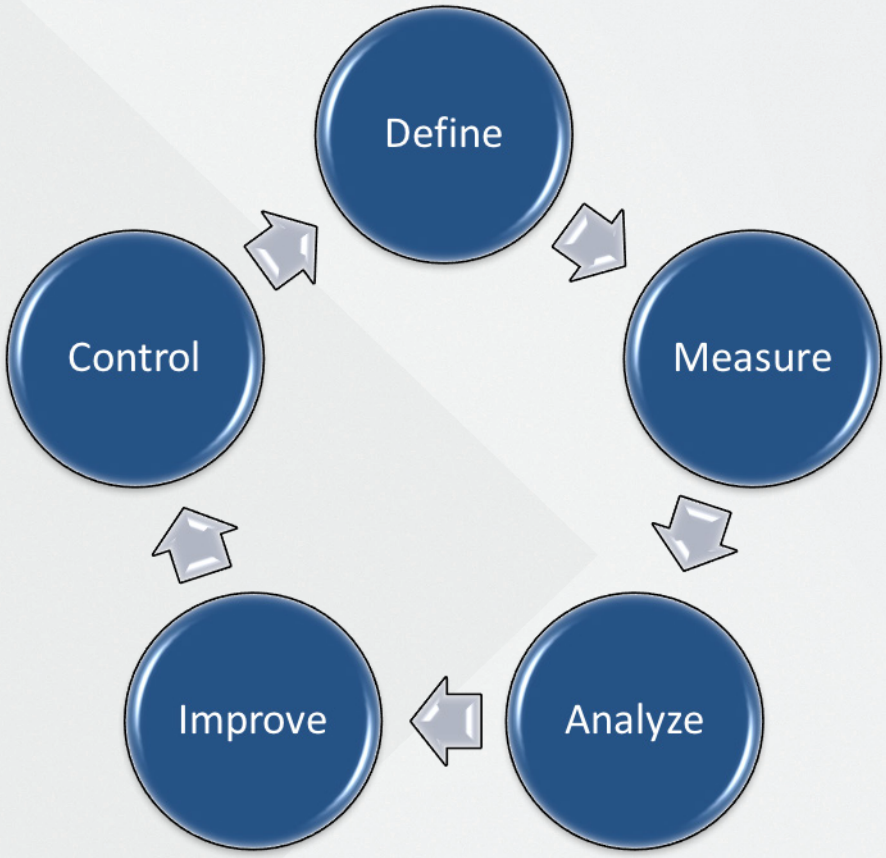
3. Establish critical limits:
   For each control point set up critical limits. For example:
   a. Microbiological status:
      ii. Semen dose: <1 CFU/ml
      iii. Countertop surface after cleaning: < 3 CFU/cm2
      iv. Extender: <1 CFU/ml
      v. Purified water (for extender preparation): <1 CFU/ml
      vi. Source water: Coliform bacteria <1 CFU/100ml
      vii. Dummy surface after cleaning: < 10 CFU/cm2
   b. Temperatures:
      iii. Collection cup: 38°C ± 2°C/100°F±3.6°F
      iv. Heating plate and block for microscope slides, cover slips and sample tubes: 38°C ± 1°C/100°F±1.8°F
      v. Heated microscope stage: 38°C ± 1°C/100°F±1.8°F
      vi. Plastic liners, tubes and bags: assure sufficient warming time if taken out of the storage so the temperature is at least room temperature (20°C/68°F) and not warmer than the diluted ejaculate
      vii. Extender: ejaculate temperature before dilution ± 2°C/3.6°F
      viii. Cool room: 17°C ± 2°C/63°F±3.6°F
4. Monitor CCPs:
   Once you have identified CCPs and established critical limits, perform relevant tests on a representative number of samples.
   e. For water used to prepare extender, measure the conductivity.
      For extender, measure conductivity and pH.
   f. Take the temperature of ejaculates at arrival in the lab and check the temperature of extender.
   g. Have the water source (at inlet) analyzed for heavy metals and organic matters frequently (quarterly).
   h. Test the water, extender and semen doses for microbiological contamination.
   i. Test the contamination of surfaces after cleaning to check the effectiveness of the sanitization procedures.
   j. Test the scales used in the lab with standard weights before use.
   k. Test the temperatures of heating devices every time before production.
5. Establish corrective actions:
   Where there is a deviation from established critical limits, corrective actions are necessary.
   An important purpose of corrective actions is to prevent the sales of substandard semen doses which may affect fertility in a negative way. Corrective actions should include the following elements:
   - Determine and correct the cause of non-compliance
   - Determine the disposition of the non-compliant product
   - Record the corrective actions that have been taken

   Examples:
   - Purified water with bacterial contamination:
     - Sanitize the water purification system
     - Change resins/filters
     - Cleanout the water storage tank
     - Check the UV-filters and replace if necessary
     - Use purchased water to produce extender or consider boiling the water (depending on volume needed)
     - If contamination returns, you may want to consider re-designing the water purification system by re-circulating water, replacement of the water storage tank, or changing/upgrading the UV-light capacity

   Record the deviation, the corrective actions and the test results in a log/journal after the changes have been completed.

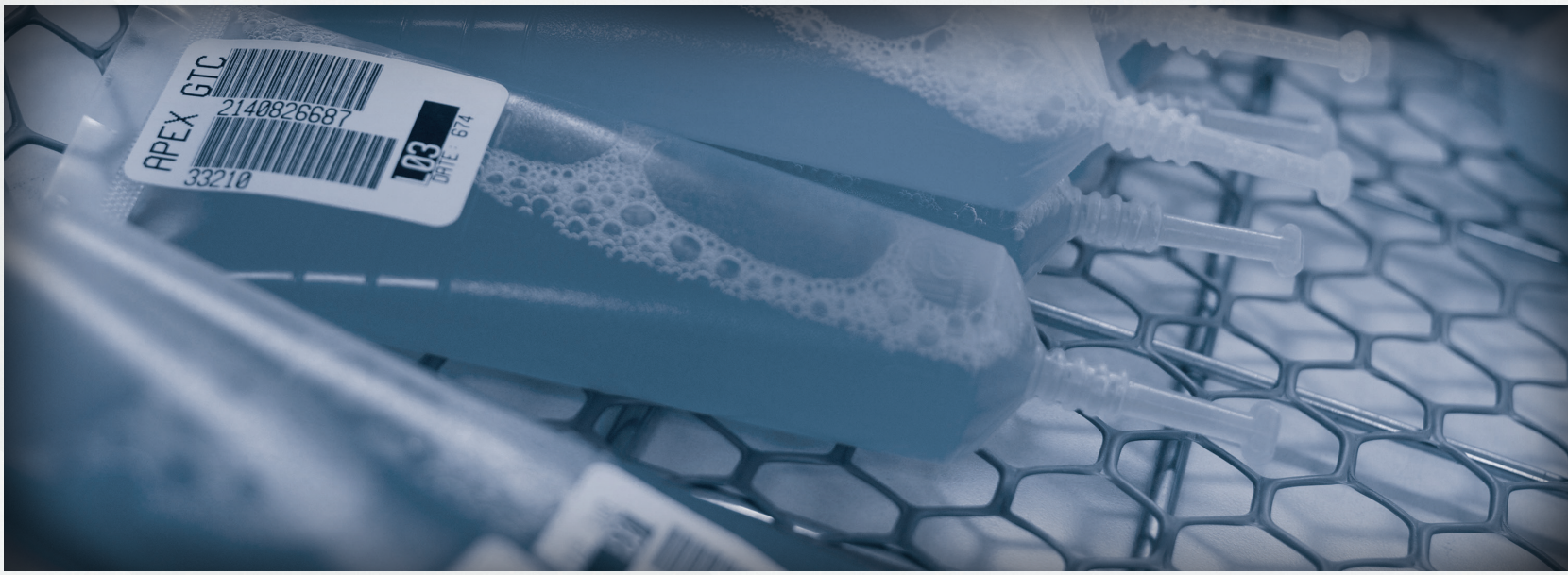
6. Establish and verify procedures for ensuring the HACCP methodology is working as intended: Verification procedures may include activities such as review of HACCP plans, CCP records, critical limits (based on monitoring results), number of samples for microbial testing, and testing frequency.
7. Keep records
   Records are important. Test results should be recorded, results should be analyzed, and the information obtained be used to improve procedures. This process should result in the resetting of the minimum criteria in order to improve the control you have over the production and reduce the hazard risks.

GRAPHIC 3.2: HACCP PRINCIPLE



SECTION 4

GENETIC CONSIDERATIONS



In this section we will provide an overview of the importance of genetic management followed by some recommendations and the presentation of tools PIC has developed to support stud managers in their decisions.

WHY GENETIC POTENTIAL OF THE HERD MATTERS

Each dose of semen shipped from the boar stud greatly impacts the performance (and therefore profit) potential of the pigs it produces. If we consider an average of 30 doses produced per collection the potential impact could up to ~50,000 market pigs (Table 4.1).

TABLE 4.1: POTENTIAL MARKET PIGS BORN/INFLUENCED PER COLLECTION

TERMINAL BOAR	196 MARKET PIGS
GP Boar	4 select Camborough gilts per litter → 60 market pigs per Camborough per lifetime → 3,360 market pigs per collection
GGP Boar	4 select pure-line gilts per litter → 15 Camborough-gilts per pure-line gilt per lifetime → 60 market pigs per Camborough Per Lifetime → 50,400 market pigs per collection

PIC boars are selected based on the performance-potential of their progeny in commercial production. The selection program is focused on maximizing profit potential of PIC genetics in our customer's operations and PIC's Index represents an animal's genetic potential across all profit-drivers. The PIC Index value shows that each index point of improvement for a terminal sire is worth about $.10 / progeny in additional profit potential. On the maternal side, each index point is worth approximately $.05 / progeny in additional value potential. This makes genetic-index a key consideration in boar stud inventory management. Continuing from our previous example, a 1-point boost in the index of a collection would provide a boost in value to the production system of up to $3,000 per collection (Table 4.2).

TABLE 4.2: ADDED VALUE EXAMPLE FOR 1 POINT INDEX BOOST

SIRE	VALUE TO PRODUCTION SYSTEM
Terminal Boar	*$19.20 per collection!*
GP Boar	*$187 per collection!*
GGP Boar	*$2,808 per collection!*

MANAGING THE GENETIC POTENTIAL OF THE STUD

As the genetic potential of a stud increases over time, so does the value of the market pigs produced by the active inventory. Practically, this is managed by introducing high-indexing boars, and using those to replace lower indexing ones. Two strategies to optimize the boar stud's contribution to the pork production systems are:

1. Proactively placing boar orders based on optimal projected replacement rates
2. Actively managing cull-boars based on genetic merit (after necessary production culls)

While genetic potential is a primary contributor to a stud's impact on the economics of pork production, the costs to operate a stud are also an important consideration. Recommended replacement rates are based on balancing the improved genetic value that younger boars contribute to market-pig flows with the reduction in production costs per dose as a boar ages. Typically, these replacement rate targets are approximately:

- 130% for GGP boars
- 100% for GP boars
- Up to 95% for CBV+/Profit+, Max boars (the highest-indexing terminal boars available)
- 70% for standard AI terminal boars.

OPTIMUM BOAR LIFE (OBL)

PIC's OBL-tool helps stud-managers prioritize culling-decisions based on the value each individual terminal boar is contributing to the production system. This calculation is primarily driven by the current genetic merit of the boar and his age (utilized to evaluate remaining productive life and expected semen output). Throughout a boar's lifetime in stud, his total profit contribution is a combination of these two factors, however the relative impact of each to total-system-profitability shifts as the boar ages:

- Entry into working boar inventory:
  - Genetic merit: The boar represents new/high genetic potential to the system and the market pigs produced from his doses will have higher performance-potential through grow/finish, relative to older boars in stud. This is the primary value-driver for young, high-indexing boars.
  - Semen output: The first few weeks in stud represent the lowest semen output levels for a boar. As a result, the cost per dose produced is the highest when the boar first enters the stud.
- After a few months in stud:
  - Genetic merit: Index of the boar starts to decline. Since the genetic merit of the active-boar is compared to replacing him by a younger boar from PIC's genetic farms, this reflects that new boars entering the stud would produce market pigs with higher performance-potential, relative to the aging boar.
  - Semen output: The boar is reaching the peak of his semen production curve, and he is providing increased value to the production system through the lower cost per dose. At this stage, the stud's cost-advantage balances his declining genetic-value.
- At the end of a boar's optimum stud-life:
  - Genetic merit: Index of the boar has continued to decline, indicating the boost in performance-potential that would be seen in market pigs sired by a new and younger boar continues to get larger.
  - Semen output: The boar has reached the plateau in his semen production curve. The lower production cost per dose of this older boar no longer balances the difference in genetic potential that a new boar would bring. At this time, the production system would get more value from replacing the older boar with a new, higher-indexing boar.

The use of Optimum Boar Life ensures that robust genetic improvement at PIC's genetic farms results in realized product differentiation in commercial closeouts.

FURTHER TOOLS

PIC is continuously searching for opportunities to use the best available information to further improve services and products dedicated to the boar studs. On a regular basis different customer studs deliver semen quality data that get used as part of the overall index calculation with the goal to continuously improve boar semen quality over time. Furthermore different analysis tools for boar studs in PICtraq® are available. For more information please contact your PIC Genetic Services representative.

APPENDIX A:

WATER GUIDELINES FOR PIGS

ITEM	PPM (PARTS PER MILLION)
Calcium	<1,000
Chloride	<400
Copper	<5
Fluoride	<2-3
Hardness (Calcium Carbonate)	< 60 soft > 200 hard
Iron	<0.5
Lead	<0.1
Magnesium	<400
Manganese	<0.10
Mercury	<0.003
Nitrites	<10
Nitrates	<100
Phosphorus	<7.80
Potassium	<3
Sodium	<150
Selenium	<0.05
Solids dissolved	<1,000
Sulfate	<1,000
Zinc	<40
Total viable bacterial count (TVC) per ml 37 °C/99 °F	Low but more important no fluctuation between samples, target < 200 TVC/ml
22 °C/72 °F	< 10,000 TVC/ml
Coliforms/100 ml	Zero

Adapted from NRC (2012) and Task Force on Water Quality Guidelines, 1987, Canadian Water Quality Guidelines, Inland Waters Directorate, Ottawa, Ontario.

EVALUATION OF WATER QUALITY FOR PIGS BASED ON TOTAL DISSOLVED SOLIDS (NRC, 2012).

TOTAL DISSOLVED SOLIDS (MG/L)	RATING	COMMENTS
< 1,000	Safe	No risk to pigs
1,000 – 2,999	Satisfactory	Mild diarrhea in pigs not adapted to it
3,000 – 4,999	Satisfactory	May cause temporary refusal of water
5,000 – 6,999	Reasonable	Higher levels for breeding stock should be avoided
> 7,000	Unfit	Risky for breeding stock and pigs exposed to heat stress

APPENDIX B:

FEED LEVEL IN RELATION TO BODY WEIGHT OF THE BOAR[1]

BODY WEIGHT, KG	BODY WEIGHT, LB	MCAL ME/D	MCAL NE/D	FEED, LB/D	FEED, KG/D
<159	<350	7.2	5.3	5.0	2.3
159	350	7.9	5.9	5.5	2.5
205	450	8.6	6.4	6.0	2.7
250	550	9.5	7.0	6.6	3.0
295	650	10.4	7.7	7.2	3.3
341	750	11.2	8.3	7.8	3.5

Adapted from PIC Technical Memo 142, Assumes ambient temperature of 17-18oC/62-65°F, Based on a dietary energy density of 2350 kcal NRC NE/kg,

APPENDIX C:

USE OF REFRACTOMETERS FOR SEMEN EXTENDER QC

Preparing liquid semen extender is one of the most vulnerable points in semen processing. Wrong water-extender ratios can have a negative effect on the viability of preserved semen cells. Depending on the degree of inadequate mixing, this could lead to minimal negative effects or up to a 100% loss of semen cell function in the dose. Refractometry can be used as a cheap and easy to use tool for double-checking on proper extender preparation.

REFRACTOMETER

The best choice is a Brix 18 refractometer. Is has a scale from 1 to 18% Brix, divided into 1% graduations. Calibration - Calibrate the refractometer every week according to the user manual. In most cases purified water is used to set at 0% Brix.

SETTING YOUR BENCHMARK

The Brix-value varies between extenders, dependent on their ingredients. Water quality also influences the value. To set your target it is required to know about your specific value which should be between 4 and 5% Brix, in most cases. Measure your Brix value on five production days in a row to define your acceptable range. This procedure has to be repeated every time you change extenders or extender ingredients.

USING THE REFRACTOMETER

Make sure your device is calibrated. Use a pipette to put a drop of extender on the blue measurement area. It is important that the entire area is covered with fluid. Look trough the ocular and read the Brix value. If the value is out of range (more than ± 0.5) make sure that your refractometer is calibrated and used in the right way, then repeat the measurement. In case the measurement remains out of range do not use the extender for semen preservation and prepare a new vat.

CRITICAL POINTS:

- Refractometer should be calibrated on a regular basis
- Brix value can change if extender has changed or other ingredients are added
- Brix value can change if water quality changes
- Measurement should be done at similar extender temperatures every time
- The entire blue measurement area has to be covered with fluid

APPENDIX D:

EXAMPLE FOR SEMEN PACKAGING

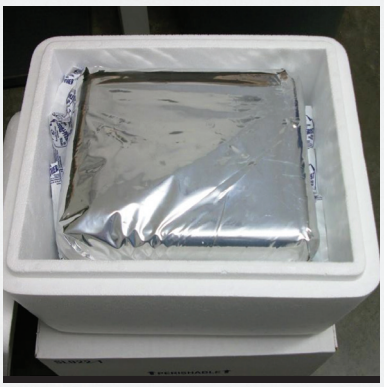
1. Prepare liners and coolers.

2. Layer doses inside Thermalast bag within inner cooler.

3. Add a room temperature gel pack.

4. Put on the lid and seal with tape.

5. Wrap inner cooler with Thermalast bag.



6. Put inner cooler inside outer cooler and add gel packs (warm or cool depending on season).

7. Put on the lid and seal with tape.

8. Place in box for shipping.

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