

The international AI news from Minitube

Sperm Notes

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Minitüb GmbH
Hauptstrasse 41
84184 Tiefenbach – Deutschland
Telefon: +49 (0) 8709 9229 0
Fax: +49 (0) 8709 9229 39
E-Mail: minitube@minitube.de



Foreword



Dear SpermNotes readers,

2010 really was an exciting and successful year for Minitube. In June Minitube celebrated its 40th anniversary with friends from all over the world and arranged the Distributor Meeting in a beautiful and historical horse stud near Berlin. And in November Minitube exhibited at EuroTier 2010, the leading Fair about animal husbandry.

EuroTier once again brought new record figures: more than 140.000 visitors poured into the fairgrounds in Hanover between November 16th and 19th. Minitube can report a very successful Fair participation. A large number of visitors from all over the world learned at the booth in Hall 12 about the latest innovations from Minitube.

The new hygiene concept for laboratories found as much approval as the latest versions of Sperm Vision™ – the CASA system for all

requirements. For the first time, an integrated solution for the semen freezing process was presented to the visitors with the TurboFreezer. And so the Minitube team can only agree with the positive conclusion of the DLG referring to a very successful EuroTier 2010 with 8 % more visitors.

I hope you enjoy the following articles about quality control, hygiene and new products. And as you know, previous issues can be found on our web page: www.minitube.com. We would be very pleased if you checked our website. For comments and any ideas you are welcome to send an email: minitube@minitube.de or call us: +49-8709-9229-0.

Minitube wishes you a very successful year 2011.

Christa Simmet
Director of Sales and Marketing

Impressions from the EuroTier 2010



Quality Control of Porcine Semen Extenders - the safe way.

Boar studs rely on Minitube extenders, because of the certainty that their quality is not compromised. Production standard, the choice and source of ingredients and the quality control of Minitube extenders are unmatched in the industry.

1. Extenders are only produced with best raw materials meeting a specific high quality standard. The risk of impurities, inconsistencies and contamination is eliminated as technological and quality prerequisites are set for the production and control of a top product.

2. Consistent standard: only a strict selection and a positive quality control of all raw material batches, as well as of the mixed ingredients, guarantee a continuing outstanding quality standard.

3. Traceability: in every production step, Minitube can re-trace and document the flow path of the media - from the production and testing of the raw materials through to the final testing and release of the final product.

The quality of raw materials

Sugars and antibiotics are crucial ingredients of today's state of the art semen extenders. For antibiotics to be effective, they have to show long term stability. The stability has to be guaranteed for the full shelf life of the extenders.

Table 1 illustrates the quality characteristics, analyzed and guaranteed by certificate, for glucose and antibiotics used in Minitube extenders.

Glucose	Antibiotics
Purity	Purity
Particle size	Particle size
Microbiological contamination	Microbiological contamination
Foreign particles	Foreign particles
Endotoxin level	Endotoxin level
Heavy metals	Heavy metals
Water content	Consistency within batches
Consistency between batches	Minimum shelf life over 20 months

Table 1: Overview of characteristics analyzed and documented in raw material batches

The Quality Control Process

The extensive quality control is bound to the strong GMP⁽¹⁾ and DIN ISO 9001:2008 standards in all production steps. GMP demands considerably higher standards than the DIN ISO norms, which are:

- hygienic design of installations & machines
- hygienic design of the facility
- clean room conditions Class D/C (e.g. Hepa filters, 10 fold air exchange/hour)
- reliable production procedures
- SOPs (Standard Operating Procedures)
- documentation (for traceability reasons)
- trained staff

The production site of Minitube extenders meets the strict regulations of the GMP guidelines and of production of pharmaceuticals for human medicine. The production machines are fully automatic. Thus, sources of errors are excluded and a high sanitary standard is guaranteed.



⁽¹⁾GMP is the abbreviation for "Good Manufacturing Practice", meaning that the production methods meet strict regulations. Manufacturing procedures must be exactly described and documented at every stage of production. This quality system ensures a seamless tracing of all raw materials.

⁽²⁾Pharmacopeia represents international documented quality standards and guarantees for chemicals and drugs. Depending on their country of origin, they are named Ph Eur (Europe), BP (Great Britain) or USP (USA).

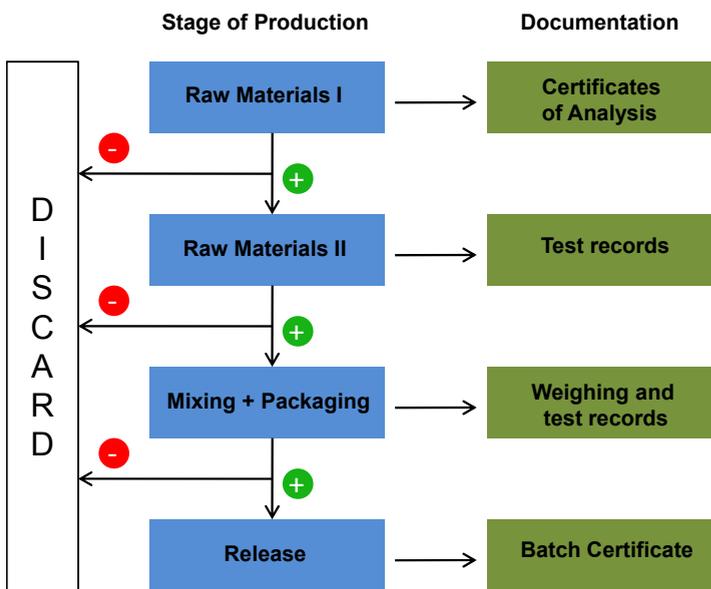


Diagram 1: Quality Control Steps in the extender production process

Quality control of raw materials

Certificates of Analysis document the Pharmacopeia⁽²⁾ of the raw materials used for Minitube semen extenders.

Some of the raw materials are additionally analyzed beyond pharmacopeia regulations, as shown in Diagram 2.

Every single component batch is then tested for its suitability for semen preservation under veterinarian supervision:

- physico-chemical suitability
- spermatological suitability

Batch release of raw materials for extender production is only given after fulfillment of all requirements.

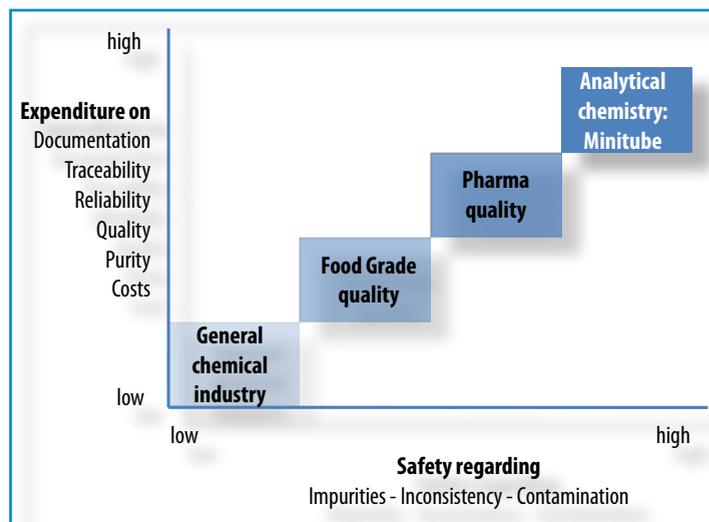


Diagram 2: The grade of raw materials used in Minitube semen extender production

Quality control of extender batches

Quality control of each batch is performed under veterinarian supervision of the University of Hanover and by commercial AI centers with EU certification. As shown in Table 2, sensitive analyzing methods are applied to split samples over a specific storage period for each extender.

Quality parameters	Methods
semen motility	checked daily during the maximum storage time applicable to each extender by means of the objective CASA system (Sperm Vision™)
acrosome integrity and morphology	microscopic evaluation after 72 hours of storage
acrosome and plasma membrane integrity	Flow cytometric analysis with FITC/PNA and PI fluorescent stains after 24 hours and 144 hours of storage

Table 2: Quality parameters and methods used to determine extender characteristics

Release

All products undergo a final check to ensure that performance and purity requirements according to Minitube Quality Control guidelines are met. Final approval, is only given when the extender has successfully passed all quality control procedures. Proof hereof is provided by a certificate that accompanies each batch.



Conclusion

Three factors determine the consistently high quality of Minitube preservation media for boar studs:

- (1) The source of raw materials: Minitube only relies on suppliers that have proven to meet the highest quality standards over a long period of time.
- (2) The GMP production standard
- (3) Safety: Minitube's quality ensuring procedures set an unmatched industry standard.



Minitube's extender production GMP plant



The pictures show the fully automatic production facility of Minitube in Tiefenbach, Germany that meets the strict regulations of the GMP guidelines.

Hygiene and antibiotics

– effective tools to produce high quality boar semen

by Prof. Karl-Fritz Weitze and Prof. Dagmar Waberski, Unit of Reproductive Medicine, University of Veterinary Medicine Hanover

High quality semen doses provide fertile sperm cells and avoid the transmittance of pathogens into the female genital tract. During storage, extended boar semen is highly susceptible to bacterial growth. **Strict hygienic measures at all stages of semen collection and processing are therefore fundamental in order to minimize the bio burden of extended semen.** As hygienic measures only often cannot ensure a bacteria free semen production, single antibiotics or antibiotic cocktails are used in boar semen production to control microbial growth in the extended semen.

It is important that in the extended semen the minimum inhibitory concentration required for these antibiotics to effectively control bacterial growth is always maintained throughout the desired storage time. Otherwise at any point during semen production or storage antimicrobial resistance can and will appear. A number of publications dealing with the efficacy of antibiotics in extended boar semen support this statement by indicating the frequent occurrence of bacteria with resistance against one or even several antibiotics.

The fight between bacteria and AI stud personnel is permanent and tends to escalate, whenever a new resistance appears.

Once a boar stud is confronted with the presence of resistant bacteria in its production system, too often the consequence is to use another antibiotic or - even more questionable - to use antibiotic cocktails of unknown composition, unknown antimicrobial efficacy and questionable sperm friendliness.

In contrast to this practice, **good laboratory practice would require a detailed analysis of the source and nature of the contaminating bacteria as well as the development of a plan to eradicate the problem.** It is worth to mention that sometimes immediate action is needed in order to keep the production afloat. The immediate use of an effective antibiotic is then required, without having accompanying hygiene measures in place. But the most important task is to identify the source of the problem.

Only limited information exists on the bacterial growth dynamics in extended semen. In general terms, bacteria and sperm cells have very similar requirements with regards to temperature, nutrients, pH and osmolarity of the medium. This is the main reason why bacteria develop so well and grow so rapidly once they develop resistance to the included antibiotics. The growth rate of bacteria in extended semen develops in an exponential way over storage time which allows fast growing bacteria to reach their detrimental threshold value even within a few days with severe negative effects on sperm fertilizing capacity.

As a general rule and based on different field studies, it is recommended that extended boar semen should contain less than 10^4 colony forming

units/ml. The adequate sampling routine should cover 1% of the daily collected and processed ejaculates or 4 samples per week, whichever is greater. Samples of freshly extended semen do not provide any information about the potential growth of bacteria; therefore it is important to assess the semen doses after storage for 48 h.

As the uncontrolled use of antibiotics or antibiotic cocktails with unknown antimicrobial efficacy can have severe consequences for semen quality and the development of antimicrobial resistance, it is of utmost importance that commercially available extenders declare the type and amount of antibiotics included. Without this information, it is impossible to establish an effective hygiene and biosecurity management in a boar stud.

Due to the fact that bacterial contamination during boar semen production is very difficult to avoid, the above mentioned facts must be recognized when a targeted semen quality control programme is established. Besides, the prudent use of antimicrobials, the daily general cleanliness, personnel hygiene as well as targeted hygiene and sanitation procedures along the complete semen production chain are the essential fundamentals for a healthy and sustainable boar semen production.

Hygiene measures must be based on:

1. Adequate facility design, including boar barn and laboratory
2. Establishment of sanitation guidelines
3. Training of personnel in individual and general hygiene
4. Definition of critical control points
5. Regular monitoring of the bio burden in these critical control points
6. Enforcement and validation of corrective measures
7. Adequate and prudent use of antibiotics

Ideal semen collection starts with the separation of the semen collection area from the boar barn in order to have less airborne particles and bacteria in the semen collection area. The boars should be clean, dry and free of straw and other particles when used for semen collection.

Hygiene during semen collection includes the use of clean boar dummies with smooth and intact surfaces, clean single-use gloves, sterile semen collection bags and filters as well as an appropriate semen collection technique avoiding the contamination of the ejaculate with fluid from the preputial cavity or the first jets from the pre-secretion of the ejaculate.

The use of automated semen collection systems is advantageous, since the semen is not exposed to air during collection. Before and during transport to the laboratory, semen vessels have to be kept closed in order to prevent contamination by airborne micro-organisms.

Once arrived in the laboratory, any materials coming into contact with the semen extender or extended semen represent further major potential sources of contamination.

Laboratories should be strictly separated from the collection area including air transmission. Laboratory work flow from semen examination to filling and sealing the semen tubes should be in-line (straight), thereby avoiding cross-traffic between work stations. Only single use disposable wipes should be allowed to prevent cross-contamination. **Laboratory desks, floors and walls should be as empty as possible. Any material or equipment is a potential source of contamination and impairs cleaning, especially where corners and niches are formed.**

Prepared extenders may become contaminated when stored overnight at room temperature. It is recommended to avoid the usage of extender prepared the day before. If it is necessary then the leftovers should not be mixed with the freshly prepared extender but used as a separate batch.

Semen dyes used in some AI stations to identify different lines of boars have been identified as a major source of bacterial contamination in extended semen.

Antibiotics are helpful to control bacterial growth in extended semen, however, they can not compensate for hygienic deficiencies in semen collection or processing. Special care needs to be taken to avoid the development of resistant bacteria in laboratory sinks or drains. Extender or extended semen expose bacteria present in sanitary installations to antibiotics and create a perfect environment for the selection of resistant bacteria. Therefore, a strictly enforced procedure for the disposal of extender or extended semen must be established without using laboratory sinks and drains.

Automated semen processing allows a hygienic production of semen doses. However, daily care of the machine and especially the tubing is mandatory. Repeated use, cleaning, sterilization and even simple aging affect surface properties of silicone tubing which may ultimately evolve in the formation of biofilms as a source of persistent contamination.

Water and extender tubing are particularly susceptible to the formation of biofilms. Once created, biofilms are tenacious and hard to remove. It is therefore recommended as preventive measure that all tubing coming into contact with semen must be cleaned and sanitized daily and changed regularly at intervals of 2 to 4 weeks, all tubing used for extender must be changed every 4 to 8 weeks.

All surfaces within the laboratory must be easy to reach and easy to clean. Special benches with rounded edges and partially integrated equipments

are available. The surface of the bench material is totally sealed and can effectively be disinfected.

The boar stud personnel must be instructed and involved in the realization of a hygiene targeted semen quality control plan in order to create a high consciousness in hygiene.

Sources of contamination at semen collection

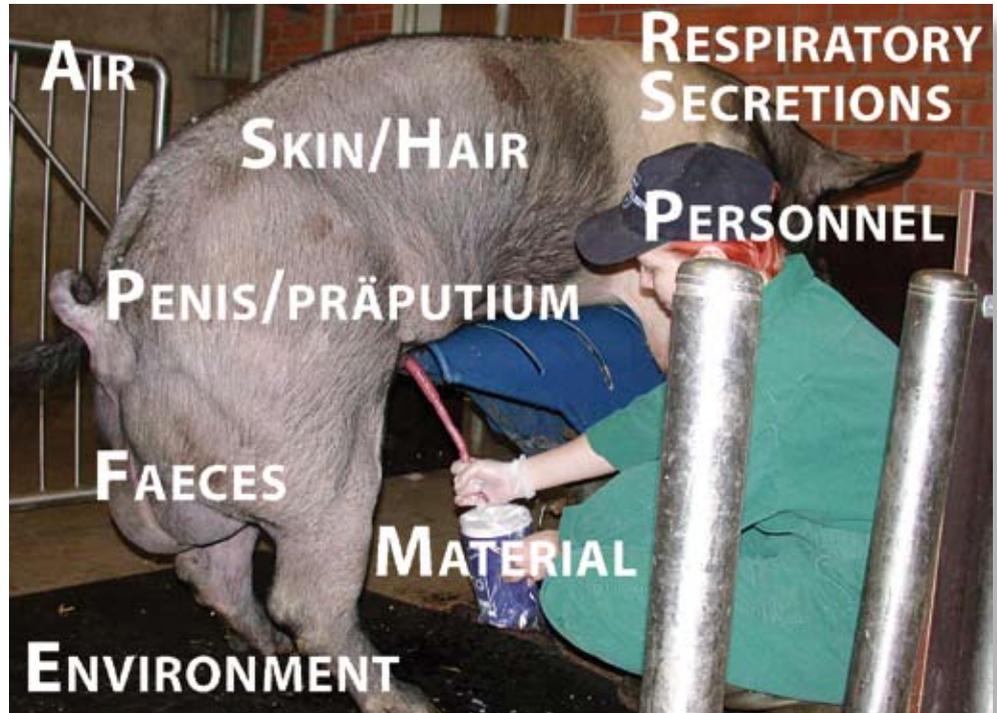


Foto: Waberski

The instructions for cleaning and disinfection must be clear and should cover the following points:

1. **What is to be cleaned and disinfected (structure, equipment, utensils coming into contact with water, extender or semen)**
2. **What chemicals and equipment are to be used**
3. **The method of cleaning or disinfection**
4. **What protective clothing should be worn and what precautions are to be taken**
5. **When it is to be cleaned/disinfected (after use, daily, weekly, monthly)**
6. **Who will carry out cleaning/disinfection**
7. **Monitoring arrangements**

Concluding, the application of adequate hygienic guidelines with regards to the design and maintenance of the semen collection area and the laboratory as well as preventive action by the AI stud personnel are fundamental. A profound hygienic consciousness of AI staff has to be created and trained regularly.

The use of antibiotics has to be careful, responsible and as conservative as possible. Antibiotics are a necessary tool to secure the production of high quality semen doses. It should be the aim of AI stud management to make sure that the industry will be able to benefit from this tool as long as possible on the lowest level of escalation.

Laboratory - Design for Hygiene

SpermNotes talked to engineer Thomas Langer, Minitube Technical Development & Service about the Minitube-concept for the new generation of laboratory benches without angles and edges, optimized for the daily disinfection routine and complying with the latest hygiene standards.



SPERMNOTES: Mr. Langer, how did the idea to develop a new hygienic concept for laboratories actually occur?

LANGER: Well, hygiene is a very essential issue for laboratories. We recognized again and again during installations and service visits at our customers that there were too many cables and single devices on the laboratory bench to assure optimal conditions for a high hygienic standard. This observation inspired us to find a solution for this problem. Our aim was to develop the ideal working station for the analysis and preparation of semen with integrated devices like the semen scale or warming plates, control units and a hidden cable duct. In addition, the surface should be as easy as possible to disinfect in order to achieve the best hygienic standard in the laboratory.

SPERMNOTES: Which material do you use to gain such a good hygienic effect?

LANGER: Our choice was a non-porous synthetic material that is very sturdy and durable. A further advantage of this material which is already in use in human medicine e.g. in dental laboratories, is that it is eminently smooth and that it can take any shape.

SPERMNOTES: Mr. Langer, could you please tell our readers why this kind of laboratory bench is so much easier to keep clean?

LANGER: Well, the big achievement is the elimination of all 90° angles, gaps in the benchtop surface, hidden corners and rough surfaces. That means that all areas are easily accessible and all corners and edges are transformed into smooth curves with seamless transitions. Also the surface of the bench material is sealed to allow effective disinfection. The integration of heating plates into the intact benchtop surface area helps to maintain a tidy and clear workplace. Our podiums for the microscope, scale, photometer and other devices prevent spilled liquids to creep

under these devices which makes it very easy to clean up. And last but not least, the cable tangle finally disappears. That's what we understand by hygienic design: Built to last - built to be clean.

SPERMNOTES: Is it possible to adjust this concept also to the customer's individual needs?

LANGER: Yes of course. This is no standard concept - we individually customize each bench to the special needs of our customer. And it is important to know, that it is also possible to integrate existing laboratory equipment into the newly designed laboratory benches.

SPERMNOTES: And what does the customer have to do to get such an individual laboratory bench design?



Thomas Langer,
Minitube Technical Development & Service

LANGER: Please contact one of our sales representatives. He will advise you in detail and develop your special solution. You get an individual offer on the basis of a rough sketch of your laboratory layout.

CLEARGLIDE™

well known product in a new shape



The well-known catheter ClearGlide™ from Minitube is now provided with a unique Sealing Flange for an even more secure fit during insemination.

Once inserted, the ClearGlide™ tip locks firmly into the sow's cervix due to this unique flexible collar that flips back during insertion. Once in place, it naturally creates a firm grip. The new Sealing Flange reduces semen back-flow down to a minimum.

The soft and slippery ClearGlide™ material allows for gentle insertion and removal requiring minimal lubrication.

ClearGlide™ is easy to use and also available as SafeBlue® catheter: top quality insemination catheters, prelubed and single packed. Better insemination hygiene improves insemination outcomes.

**ClearGlide™, with handle, 25/bag
500/box**

[REF.:17106/4026](#)

**ClearGlide™, without handle, 25/bag
500/box**

[REF.:17106/3026](#)

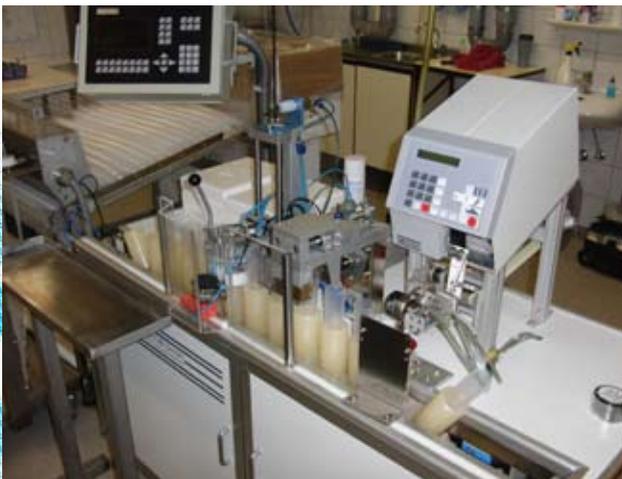
**SafeBlue ClearGlide™,
with handle, 100/package**

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**SafeBlue ClearGlide™,
without handle, 100/package**

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Time to say goodbye - Old lady retires after 18 years



It's time to say goodbye now to the BSP in Cloppenburg-Bethen - a machine that worked very hard and reliably during the last 18 years with an incredible output of more than 9 million tubes.

Her career began in September 1992 in Cloppenburg-Bethen, one of the Boar Studs of Weser-Ems e.V. At that time she was a real pioneer in the field of automatic boar semen processing. The BSP was tirelessly working everyday with very little need of maintenance or repair. A real proof of quality!

Now the old lady went into her well deserved retirement. We are proud of this outstanding performance and wish her successor in this big semen production unit, the latest model of the MiniBSP, all the best and as many years of service as this BSP achieved.



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