TECHNICAL REPORT
02 | 2013

Water quality in the boar semen production laboratory

Minitüb GmbH

Purified water is needed in semen production for two purposes:

- Preparing extenders
- Cleaning and rinsing glassware, filling water bath and vapor sterilizer

Tap water doesn't contain only pure water (H2O), it contains also minerals, like calcium carbonate, magnesium carbonate, nitrates, phosphates and other ions. It may contain even variable levels of chemical waste, organic compounds, microorganisms and endotoxines. In many areas, low level of chlorine and fluorine are added to tap water for human health prevention.

In order to obtain appropriated tap water for semen laboratory use, water must go through a purification process. Particularly **semen doses** require a very high grade purified water.

Purification systems for water

1. Distillation

With the distiller, water is vaporized by heat and condensed and collected as distilled water. Distilled water is practically sterile and to a high degree deionised. However, volatile organic compounds will remain in the distillate.
For water distillation, mono- or bi-distiller equipments are required and frequently a previous step of deionisation is used. "Hard" water has to be deionised before entering the distiller. Otherwise, the distillation process will be very time consuming and the tubing inside the distiller must be scaled frequently. The process of distillation requires an important additional amount of tap water for cooling.Distillation is an excellent option when a relative small volume is needed for preparing extenders, i.e. up to 30 liters each day. The distiller has to be properly serviced and quite often scaled. An improperly serviced distiller doesn't produce properly pure water.
For medium or large boar semen production laboratories, the distiller is usually not rapid enough.

2. Demineralization

Also called deionization, removes ionic compounds from the water, such as Ca2+ or Mg2+. It works with resin, a component of small particles electrically charged, contained in cartouches where water flows through. Ionic components are retained in the resin. After some months (1-3 depending on the system and the needs of purified water), resins have to be replaced or recycled. The process of purification is pretty rapid and requires little energy.
It is important to have a conductivity measuring device connected to the system, indicating the moment the process begins to be ineffective. This conductivity measuring device has to be checked regularly and, when the measured value is exceeding, the cartouche has to be replaced immediately by a new one.
Deionized or demineralized water is not sterile. Bacteria contained in the water will pass through the process. Resins are not sterile and bacteria frequently grow rapidly inside the cartouches, which may deliver an even more contaminated product than tap water. For this reason, it is important to use the same cartouche for no more than 3 months, even when the measured conductivity remains below the limit.

In order to control bacteria, an additional treatment of the demineralized water is necessary. It can be done by distillation or with a bacterial filter (pore size 0.2 µm) or using ultra-violet light.
The demineralization combined with the bacterial control method is a very convenient choice for laboratories having a very high quality of tap water, a good supply of cartouches or recycling system and the capacity of sustaining continuous costs. This solution is especially recommended for boar semen production laboratories needing high volumes of purified water, more than 50 liters each day.

3. **Reverse osmosis**
In reverse osmosis purification systems, pressure is applied to a container with tap water pushing the tap water through a semi permeable filter which retains the undesired components. After passing the filter, water comes out purified, being retained by the osmotic pressure up to 98 % of solved components. However, the contained gases are not retained.
Reverse osmosis is quite efficient removing bacteria. If tap water is "hard", having high conductivity, reverse osmosis is not efficient enough in demineralizing, being necessary to install a second process. For this reason, reverse osmosis is frequently combined with demineralization and ultra filtration. Reverse osmosis needs an important additional volume of water. Only 20 to 30 % of the used water is purified.
Reverse osmosis is frequently used in semen laboratories with high requirement of water volume (100 liters or more each day). Combination of demineralization and reverse osmosis is very efficient, frequently accompanied by UV light treatment.

The most utilized and efficient system is the combination of reverse osmosis, deionization, filtering with coal filter and UV light exposition.

The chosen method for water purifying will depend on the tap water quality of the AI-Center and the volumes of purified water needed each day. In every case, quality parameters have to be fulfilled.

For classifying quality of purified water, the ASTM-System has been adopted, which recognizes 4 different types of water (Table 1).

Table 1. Classification of water types

Parameter	TYP I	TYP II	TYP III	TYP IV
Conductivity (micro siemens/cm)	0.056	1.0	2.5	5
Electric resistance (Mega Ohm/cm)	18	1.0	4.0	0.2
Bacterial growth (UFC/ml)	0 (A)	10 (B)	100 (C)	100 (C)
TOC (ppb)	10	50	200	-
Sodium (ppb)	1	5	10	50
Chloride (ppb)	1	5	10	50
Silicates total (ppb)	3	3	500	-
Heavy metals (mg/l)	0.01	-	-	
Endotoxins	0.03	0.25		

- ASTM (American Society for Testing and Material)

For **preparation of semen extenders, water quality following ASTM Typ II** is recommended. It has to comply with following **minimum requirements**:

- **Conductivity**: below **5 µS/cm**. Conductivity is a value showing presence of ions or salts in the water. If the value is too high, water will damage the semen, because osmolality of the medium is increased. Calcium has to be completely eliminated, since percentage of capacitated sperm will increase after time. Water with a higher conductibility (up to 20µS/cm) may be used, if compensated with higher concentration of sperm cells in the seminal doses.
- **Bacterial content: ideally 0 and maximum 1 CFU** (=1 colony forming unit) per 10 ml. Bacterial content is harmful and tends to proliferate in the semen extender, which is an excellent medium for bacterial growth. Bacteria often motivate the reduced survival of extended semen, the reason why only a very low bacterial content in the water is acceptable. However, specific pathogenic organisms (bacteria and virus causing specific diseases in animals) have to be excluded. Presence of endotoxins must be low, because they are highly toxic for sperm cells.
- **TOC (total organic components)**: **maximum 50 ppb**. This is a parameter for the total content of organic carbon in water. TOC reflects the presence of organic compounds produced by industrial house and agriculture chemical waste. This factor is not extremely important, but its value should not be exceeded, being a good indicator for the general quality of water.

The **requirements for water used for rinsing glassware** or other material getting in contact with semen are lower. For this purpose demineralized water will be sufficient. However, rinsing has always to be done very throughly, in order to eliminate minerals or detergents adhered to surfaces which could get in contact with semen or extenders.

It is important to remember, that quality of purified water deteriorates with the time, depending on the storing system. As a norm, **storage for more than one week is not recommended**.

Recommendations

1. **Before deciding the system** for purification of water, it is recommended:
   - **Tap water analysis**: calcium carbonate contents, bacteria, organic contamination, conductivity and osmotic pressure.
   - **Definition of requirements**: How much purified water should be produced each day? And in what period of time this volume of water will be needed?

2. **Care of purified water**. Purified water may easily deteriorate before it is used. Immediately after production it may flow through non sterile tubing, containing even growing bacterial colonies as a biological film that contaminates constantly the water. This happens frequently and is particularly critical in relatively old systems. Containers and bottles for purified water storage are frequently not sterile, especially if they are not kept close. If exposed to light, bacteria may proliferate. Some bacteria, for example, the pseudomonas group, can use the plastic compounds of tubing for their metabolism. Purified water should not be stored, it should be used immediately for best. Tubing, faucets and containers should be sterilized frequently.

3. **Alternative: buying water**. If there is no water purifying system available, the best choice is purchasing purified water. However, deionised water for domestic use or batteries is not sufficiently pure for semen extender preparing. Purchased water should be sterile, deionised (max. defined conductivity 5 µS/cm) and pyrogen free.