

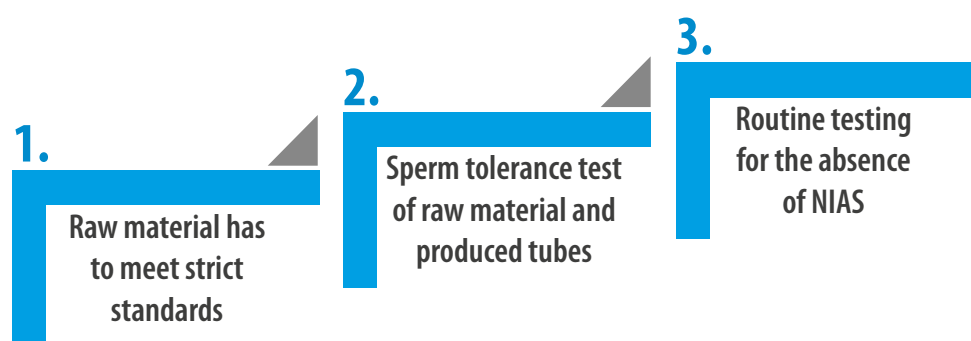


Quality Standard of Minitube's Boar Semen Tubes

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Pig production in the 21st century relies heavily on artificial insemination to ensure efficient breeding programs, fast genetic progress and high biosecurity standards. The benefits of A.I. are based upon the highly increased availability of superior genetics through the number of semen doses produced from one ejaculate, the storage time, and the improved logistics enabling shipment of semen conveniently to any place these genetics are needed.

Materials used to manufacture boar semen tubes must be tested and proven to be biologically inactive and sperm friendly. Minitube as a specialised and certified producer of boar semen tubes has a rigorous QC system in place, consisting of a three level concept for the quality assurance and testing of raw materials and final products.



1. Raw material standards

Each batch of the raw material Low Density Polyethylene (LDPE) assigned for the production of boar semen tubes at Minitube's manufacturing plant is accompanied by a batch specific certificate.

The raw material used in the production of boar semen tubes meets all of the following standards:

- EC Regulation 1907/2006/EC and amendments (referred to as REACH regulation), and further EC regulation 1272/2008/EC on classification, labelling and packaging of substances and mixtures
- EC Directive (EC) No 2033/2006 on good manufacturing practice for materials and articles intended to come into contact with food
- EC Directive (EC) No 2016/1416 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food
- EC Directive (EC) No 10/2011 on plastic materials and articles intended to come into contact with food
- Commission Regulation (EU) No. 10/2011 of January 14, 2011, including its amendments (EU) No. 1282/2011 of November 28, 2011, (EU) No. 1183/2012 of November 30, 2012, (EU) No. 202/2014 of 3 March 2014, (EU) No. 2015/174 of 5 February 2015, (EU) No. 2016/1416 of 24 August 2016 and (EU) 2017/752 of 28 April 2017
- Empfehlung III „Polyäthylen“ of Kunststoffe im Lebensmittelverkehr: Empfehlungen des Bundesinstituts für Risikobewertung (BfR) (former BgVV), latest amendment February 15, 2016 (BGBl. I S. 198)
- Code of Federal Regulations, issued by the Food and Drug Administration (FDA), paragraph 21 CFR 177.1520 (olefin polymers)

Pure LDPE is by definition free from any toxic ingredients like Bisphenol A, heavy metals, phthalates or adipates.



2. Sperm tolerance

Quality assurance tests are regularly performed at the Unit of Reproductive Medicine of the University of Veterinary Medicine Hannover Foundation, which is a DAkkS accredited GLP laboratory in accordance with the international ISO/IEC 17025 norm. Any new raw material has to pass the tests before it is released to enter the boar semen tube production chain.

For these tests, boar semen of at least 3 different boars is extended with Androstar Plus, divided into split samples and incubated in the boar semen tubes to be tested and in glass vials as a control. Semen is evaluated over a semen storage period of at least 6 days. All split samples are stored in a semen storage unit at 17° C. The following tests are performed:

- Motility: total and progressive (CASA) after 24 and 144 h
- Acrosome integrity (wet mount, phase contrast microscopy 1000x) after 24 and 144 h
- Membrane integrity (flow cytometry, FITC/PNA-PI) after 24 and 144 h
- Thermoresistance test after 144 h storage at 17°C (incubation at +38°C for 120 min), then analysis of progressive motility (CASA)
- DNA Integrity / SCSA (flow cytometry; acridine orange)
- Mitochondria membrane potential (flow cytometry; propidium iodide/ JC-1) after 24 and 144 h
- Membrane fluidity (flow cytometry, Yo Pro 1/ Merocyanin (M540)) after 24 and 144 h



3. Tests for the absence of non-intentionally added substances (NIAS)

NIAS are substances which are non-intentionally added to the raw material during production or transport and are potentially harmful. Though, substances like heavy metals, endocrine disruptors and plasticizers like Bisphenol A, phthalates and adipates must not be present in the material as per its formulation and certification, they can cause adverse biological effects in case they are added non-intentionally. Most importantly in the context of boar semen preservation, these substances can cause damage to the sperm during storage and hamper fertility and productivity of the sows inseminated.

Therefore in the third level of the Minitube quality assurance program, the absence of NIAS is controlled with routine testing of the raw materials for endocrine disruptors and plasticizers like Bisphenol A, phthalates and adipates as well as heavy metals.



Tube production facility at Minitube

It is a matter of principle for Minitube as a manufacturer of semen packaging material to take full responsibility of only using substances and raw materials which are not harmful to animals or sperm. This is only possible when the full production line is in control of the manufacturer, as is the case with Minitube's production of boar semen tubes in its own specialised production facility.