



Sperm-TIC®

1:20

Single Tests for Quick, Simple, Clean and Precise Counting of Spermatozoa.

Product information for quantitative visual microscopic counting of spermatozoa.

Principle

Microscopic counting of spermatozoa after dilution and demobilisation. The cells don't move or move only a few, so Sperm-TIC® allow quick, uncomplicated, clean and precise counting. Dilution is 1:20. Minimum cell counting is 200.

Reagents

Sperm-TIC® are ready for use and have a shelf life at room temperature (+15... +25 °C) up to the imprinted expiry date. Remove tube only for use. Store tubes on a dark place (closed box) and upright in the package. Do not use if reagent is not clear and free of particles or if there are any crystallisations.

Risks and Safety

Please observe the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling. It is important to ensure effective protection against infection according to laboratory guidelines. Use a capillary holder for volume capillaries.



For additional safety information please refer to the information on the label and the corresponding Safety Data Sheet (SDS). Download by QR code or link: www.sds-id.com/100027-3

Contents/Main Components

003604-4380	Cont.	1% oxalate buffer pH = 6.25, NaCl, Eosin Y C.I. 45380, <0.002% NaN ₃ .
003604-6100	KIT	Sperm-TIC® 1:20 plus • Single test with capillaries
003604-4380		1. 100x380 µL Sperm-TIC® 1:20
ETE020		2. 100x 20 µL End-to-end volume capillaries
KFK		3. 100x Chamber filling capillaries.
003604-6005	SET	Sperm-TIC® 1:20 • Single test w/o capillaries
003604-4380		1. 5x 380 µL Sperm-TIC® 1:20

Optional:

TIC-CP20	SET	TIC 20µL Capillary pack, containing:
ETE020		1. 100x 20 µL End-to-end volume capillaries
KFK		2. 100x Chamber filling capillaries.

Do not use other capillaries which are not approved for this TIC test kit.

Additionally required or recommended materials

099920-0001	Capillary holder *
CC-NEUBI	Neubauer "improved" counting chamber *
	Microscope for use in biomedical laboratory.

* Available from Bioanalytic GmbH.

Reference Ranges

	[x 10 ⁶ /ml]	[Total/Ejaculate]
Humans [1]:.....	> ... 20	> ... 40
Animal species:.....	Sperm cell range is depending on species, age and other influences. Please see official literatures.	

Sample Material

Process the fresh semen within 1 hr after collection.

Liquefaction

After ejaculation into the collection vessel, the fresh semen is typically a semisolid coagulated mass. Within some minutes at room temperature, the semen usually begins to liquefy and becomes thinner fluid. But a heterogeneous mixture of lumps will be seen in the fluid. As liquefaction continues, the ejaculate becomes more homogeneous and quite watery. The complete sample usually liquefies within 15... 30 minutes at room temperature. Sometimes it may take up to 60 minutes or more.

If complete liquefaction does not occur within 60 minutes, this should be recorded!

Semen samples collected at home or by condom will normally have liquefied by the time they arrive in the laboratory.

Normal liquefied semen samples may contain jelly-like granules (gelatinous bodies) which do not liquefy. These do not appear to have any clinical significance. The presence of mucus strands, however, may interfere with semen analysis.

Continuous gentle mixing or rotation of the sample container on a two-dimensional shaker is recommended to produce a homogeneous sample. Either at room temperature or in an incubator set at 37 °C.

If the semen does not liquefy within 30 minutes, do not start semen analysis. Wait for another 30 minutes. If liquefaction has not occurred within 60 minutes, enzymatic digestion may be necessary (method on request).

Procedure

Use the Sperm-TIC® at room temperature of 18...25 °C. Prepare two Sperm-TIC® for each sample.

Using capillary pipettes

Use liquefied fresh homogeneous mixed semen within 30... 60 minutes after collection.

Fill a 20 µL end-to-end volume capillary bubble-free with semen from end to end. We recommend using a capillary holder for this (see ordering information). Remove remaining semen from the outside of the capillary with lint free tissue without drawing semen out of the capillary. Give the filled volume capillary into the opened tube, close and shake very well until all semen has been removed out of the capillary. Wait 5 minutes for immobilizing of sperms is complete. Don't remove the capillary out of the tube.

Shake the tube once more before loading the counting chamber. Fill the chamber filling capillary about a quarter to half its length by capillary action and seal the upper end with your finger. Touch the tilted capillary (narrow angle) against the edge of the cover slip and load the counting chamber. Count cells immediately..

Using automatic micropipette

Only appropriately trained laboratory staff should use this method!

Instead of end-to-end and chamber filling capillaries use an adequate automatic micropipette. Proceed as outlined above for the capillaries. Flush pipette tip sufficiently with the reagent solution. Shake the tube once more before loading the counting chamber.

Generally:

Only if necessary (depending on microscope depth of field) incubate the counting chamber in a humidity chamber for maximum 5 minutes for sedimentation of the sperm cells. Count immediately.

Examination/Calculation

Microscopic counting with phase-contrast optics or with transmitting light at 100×; better 200× or 400× magnification.

Count only whole spermatozoa (with heads and tails). Whether or not a spermatozoon is counted is determined by the location of its head.

Neubauer "improved" or Neubauer counting chamber

Count the sperm cells of the 4 large corner squares of each 1 mm² surface, consisting of 4 × 4 squares. If the Neubauer „improved“ counting chamber is used count cells up to the center line.

If the total count of the 4 large corner squares is lower than 200 cells, count all 9 squares of chamber.

Spermatozoa concentration

$$\frac{\text{Standard-Dilution } 1:20}{\text{Total count of the 4 big corner squares} \times 50'000} = \text{Sperm per ml}$$

$$\frac{\text{Standard-Dilution } 1:20}{\text{Total count of all 9 big squares}} \times 22'222 = \text{Sperm per ml}$$

Spermatozoa per ejaculate

$$\text{Sperm/ml} \times \text{Semen volume [ml]} = \text{Spermatozoa per ejaculate} \\ = \text{Total Sperm Number}$$

Basis of assessment

Sperm cells should be counted in both chambers of the haemocytometer of each Sperm-TIC® of the same ejaculate sample. If the two values agree sufficiently, the aliquots taken can be considered representative for the sample.

Calculate the sum and difference of the two numbers.

$$\frac{(|N1-N2|)}{(\sqrt{N1+N2})} < 1.96$$

The difference between independent counts is expected to be zero, with a standard error equal to the square root of the sum of the two counts. Thus should be < 1.96 by chance alone for a 95% confidence limit.

Note:

Assessing both chambers filled from a single dilution is not true replication, as this will not allow detection of errors of sampling, mixing and dilution.

Capability Characteristics

Limitations

Strongly decreased sperm cell values can complicate a correct cell counting. Count a minimum of 200 sperm cells per each counting grid.

If there are too few spermatozoa per field at the recommended dilution, prepare another, lower, dilution.

If there are too many overlapping spermatozoa per field at the recommended dilution, prepare another, higher, dilution.

Notes

Classifications

Not for human diagnostics.

Disinfection of Counting Chambers

Soak the counting chambers and cover slips in Bioanalytic *µSlide Cleaner/Disinfectant* or similar disinfectant overnight. Rinse off disinfectant with water.

Support/Information service

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de.

Periodically check for updates of this product information on our website.

Feedback

Information from users can be reported to support@bioanalytic.de.

Suggestions for further developments will be considered.

Waste Management

Please observe your national laws and regulations.

Used and expired solutions must be disposed of in accordance with your local regulations.

Inside the EU, national regulations apply that are based on the current, amended version of Council directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Decontaminated packaging can be disposed of as household waste or recycled, unless otherwise specified.

Literature & Footnotes

Legends for the graphic symbols and tags used follow relevant norms or are available on our internet pages.

- [1] Thomas L., Labor und Diagnose, (1992) 4. Aufl., Die Medizinische Verlagsgesellschaft Marburg; S. 1799.
- [2] WHO Laboratory manual for the Examination and processing of human semen; Fifth Edition; 2010
- [3] Handelsman DJ et al. (1984). Testicular function in potential sperm donors: Normal ranges and the effects of smoking and varicocele. International Journal of Andrology, 7:369-382.
- [4] WHO (1987). (prepared by Comhaire F et al.) Towards more objectivity in diagnosis and management of male infertility. International Journal of Andrology, (Suppl. 7): 22-24.
- [5] Behre HM et al. (2000). Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, eds. Andrology, male reproductive health and dysfunction. Berlin, Springer: 92.