USER MANUAL

Disposable counting chambers

General company and product information

Manufacturer:

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Leja is certified ISO 9001

General product information

Intended use

Leja® slides are designed for microscopic quantitative and qualitative evaluation of cells in suspension. These can include blood cells, cells in culture and spermatozoa. Leja slides are manufactured within strict tolerances for fixed and uniform chamber height for the purpose of accurate and precise cell counting, motility and morphology analyses. Leja slides are single-use devices to be used by a person trained in their use. Please read this instruction manual prior to using Leja slides.

General description

The Leja slide is a high optical quality microscope slide (75 x 25 x 1 mm) with sawed, blunt edges to prevent injury to the user.
On top of the slide is a fixed cover slip of 0.7 mm in thickness. The two glass plates of the Leja slide are at a fixed and controlled distance to form uniform chambers with 10, 12, 20 or 100 microns of height. Leja slides may contain 2, 4 or 8 independent chambers.

Leja slides should be used with a microscope that may be integrated in a Computer Assisted Semen Analysis (CASA) system. Leja slides with chamber heights of 10, 12 and 20 microns are suitable for the analysis of motile spermatozoa in combination with 10x or 20x magnification. The Leja slides with chamber height of 100 microns are designed for the analysis of small numbers of cells. For advice on which type of Leja slide and chamber height to use, please contact your local distributor or Leja directly.

As clean glass can be a reactive surface, Leja slides are coated. The coating prevents the sticking of the cells to the glass surface and prevents the formation of air bubbles during the filling process.

Leja slides are produced in a clean room to reduce the presence of dust particles inside the chambers.

The pertinent details of each Leja slide are printed on the left and right margins of the slide.

Handling of the slides

Leja slides are single-use devices. They cannot be washed and re-used.

To handle the slides, manipulate only the coloured sides; avoid touching the base or coverslip of the chambers with fingers or other objects. Eliminate dust from stage heaters or heating plates before placing Leja slides on them.
Warming the slides before use may be recommended when working with certain cells such as sperm cells. Only slides that are to be used for evaluation within a period of 30 minutes should be pre-warmed. Repetitive warming and cooling of a box of slides is not recommended.

Product testing, customer feedback, and history of similar products, support a customer performance optimum use within 12 months from the date of manufacturing for this product as long as this product is stored in its original packaging in an environment below 27°C (80 degrees F) and protected from light sources. For use beyond this timeframe, it remains the responsibility of the user to assess the risk of using such product.
In storage, a haze may form inside the counting chamber. Filling will wash this haze away without affecting the functionality of the chamber.

Handle Leja slides carefully as they are made of glass, which can break and create sharp edges. When working with body fluids, always use personal protective equipment (gloves, goggles, etc.). Handle used Leja slides as infectious waste and discard used Leja slides according to local guidelines for the handling of infectious waste.

Preparation of microscope

Please consult our website www.leja.nl for information and instructions on

- Segre-Silberberg effect compensation factor
- Microscope calibration
- Calculations on sperm concentration and sperm motility by hand counting

Working with Leja slides for semen analyses

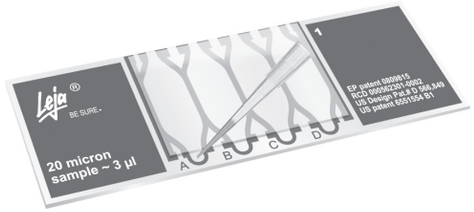
Sample preparation

If needed, as with human sperm samples, allow the semen to liquefy completely (about 30 minutes at room temperature). Motility patterns of sperm cells are sensitive to temperature. To get repeatable and comparable results the temperature regime during storage and assessments have to be well controlled. Temperature “shock” of cells should be avoided. If sperm samples are cooled down to room temperature or to lower temperatures, motion patterns can be impeded or completely blocked. The restoration of motion patterns needs time. Treat semen samples according to a standard protocol and measure motility patterns at a fixed time interval after collection and preparation of the sample.

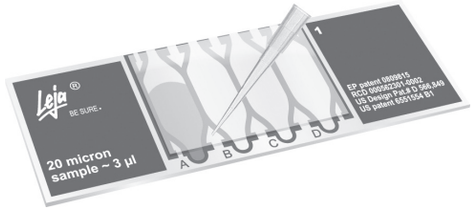
It is advised to keep human and porcine semen samples in a closed tube at 37° C for up to 30 minutes before evaluation. The slides must be warmed on a heating plate set at 37° C for minimum 2 minutes and maximum 30 minutes before filling.
For direct analyses on fresh semen the semen should be diluted with pre-warmed extender to approximately 25 million cells per ml for analysis in 20 µm slides.

Washed sperm samples
Because all Leja slides have been specially coated, sperm cells will generally not adhere to the glass surfaces of the slides. If the slides are properly filled, air bubbles will generally not form. For sperm cells that have been removed from their protein-based environment through sperm washing and that are diluted in standard extenders or in egg-yolk based extenders, addition of Motixcell or Easybuffer (IMV Technologies) is advised for representative analyses.

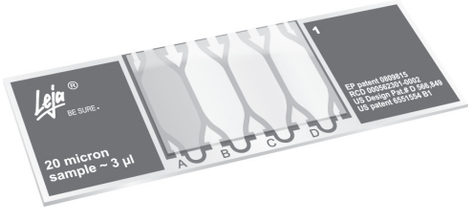
Loading of Leja slides
Load the sample into the chamber using a positive displacement pipette. Homogenize the sample before pipetting slowly from the centre of the tube.



Hold the pipette at an approximate angle of 45º and slowly deposit the indicated volume in the entry port depicted A, B, .. etc..



Allow the sample to fill the chamber by capillarity – do not push liquid in the chamber with the pipette. Remove excess fluid from the entry port with a cotton swab. Do not overfill the counting chamber as this will yield in false results.



If you need to calculate your specific sample's Segre-Silberberg correction factor then keep a stopwatch ready. Start the stopwatch when you start the liquid displacement on the pipette. When the front of the liquid (meniscus) reaches the far end of the chamber (opposite the entry port), stop the stopwatch and record the filling time.

Using Leja slides in CASA
Leja slides are designed for use with CASA systems. The CASA system must be properly set up and calibrated, according to its manufacturer's instructions. The reading positions of the CASA system must be set at a minimum distance of 3 fields from the resin track.

Procedure
After filling the slide, place it in under the CASA system's optical mechanism and follow the CASA system's manufacturer's instructions to obtain the readings. Count at least six different fields and 800 – 1000 cells for accuracy. Expected precision P for N measured cells is

$$P = 1/\sqrt{N}$$

Repeat the process on a different chamber loaded with the same sample to get a second reading.
Verify whether the assessments are a correct representation of the entire semen sample by doing the following calculation:

$$\left|TC_1 - TC_2\right| \le 2 \cdot \sqrt{TC_1 + TC_2}$$

Take the sum of the counted cells (TC_1 for total count in chamber 1 and TC_2 for total count in chamber 2) and multiply the square root of this by 2. Also take the absolute difference of the two counts.

When this formula holds, the assessments have resulted in two observations within the limits of the 95% confidence interval and the assessments are accepted. If the formula does not hold, start the process again, completely homogenizing the sample, loading the chambers and counting again.

To correct for the Segre Silberberg effect you will need to integrate the correction factor S_x that is depending on the filling time. See instructions on our website www.leja.nl on how to obtain this factor. If the CASA system cannot correct for the S_x factor, you will have to do this manually.
Now calculate the final concentration C_f (c_1 is the first concentration reading of the CASA system, c_2 the second)

When the CASA system has a build-in correction for the Segre Silberberg effect, you just have to average the two obtained concentration values:

$$C_f = \frac{(c_1 \cdot S_{x1}) + (c_2 \cdot S_{x2})}{2}$$

References
Douglas-Hamilton DH, Smith NG, Kuster CE, Vermeiden JP, Althouse GC.Particle distribution in low-volume capillary-loaded chambers. J Androl. 2005 Jan-Feb; 26(1): 107-14.

Douglas-Hamilton DH, Smith NG, Kuster CE, Vermeiden JP, Althouse GC.Capillary-loaded particle fluid dynamics: effect on estimation of sperm concentration. J Androl. 2005 Jan-Feb;26(1):115-22.

Disclaimer

LOT	lot number
REF	Product reference
	See instructions for use
	Single use