TECHNICAL REPORT
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Comparison of counting chambers
Minitube chamber and Leja chamber

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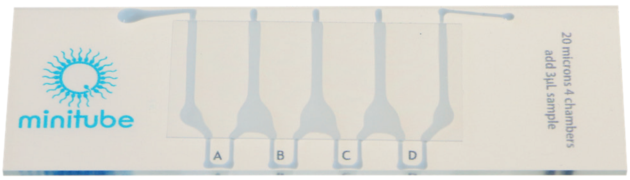
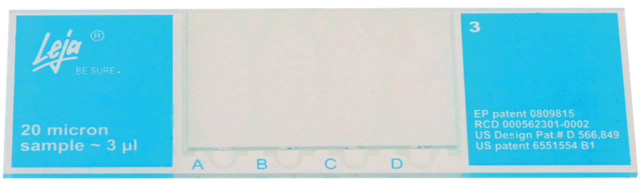
Introduction

Minitube offers two types of disposable counting chambers for the analysis of motility and concentration with the AndroVision® CASA-system - the **Minitube chamber** and the **Leja chamber**. Both types of chambers have the same physical properties: a chamber depth of 20 µm and 4 chambers on each slide.
The focus of this technical report is a comparison of the two counting chamber types, regarding all important parameters which represent the quality and functionality of counting chambers.

Minitube chamber



Leja chamber



1. Chamber depth and sperm concentration

The accuracy of the depth at any point of the chamber is the most important factor of manufacturing precision, because it correlates directly with the accuracy of the concentration measurement. Therefore Minitube examines the chamber in random samples of every new batch of counting chambers to confirm that the physical conditions for precise concentration measurement are fulfilled.

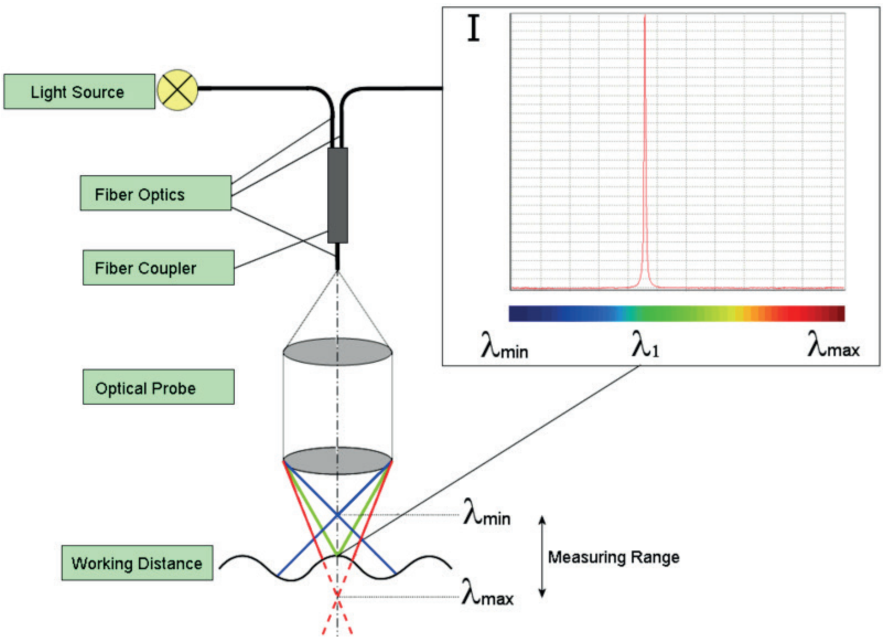


Fig. 1: Chromatic confocal principle of distance measurement

Chamber depth measurement – how it works:

A white light source is used to illuminate the surface of the object being measured. Based on the wavelength of the reflected light, a very precise distance measurement can be taken up to 66 times per second. The optical probe determines the measuring range, or focal depth of the spectrum. Because of the high numerical aperture of the probes and dynamic range of the sensor, it is possible to measure on nearly all materials.

minitube

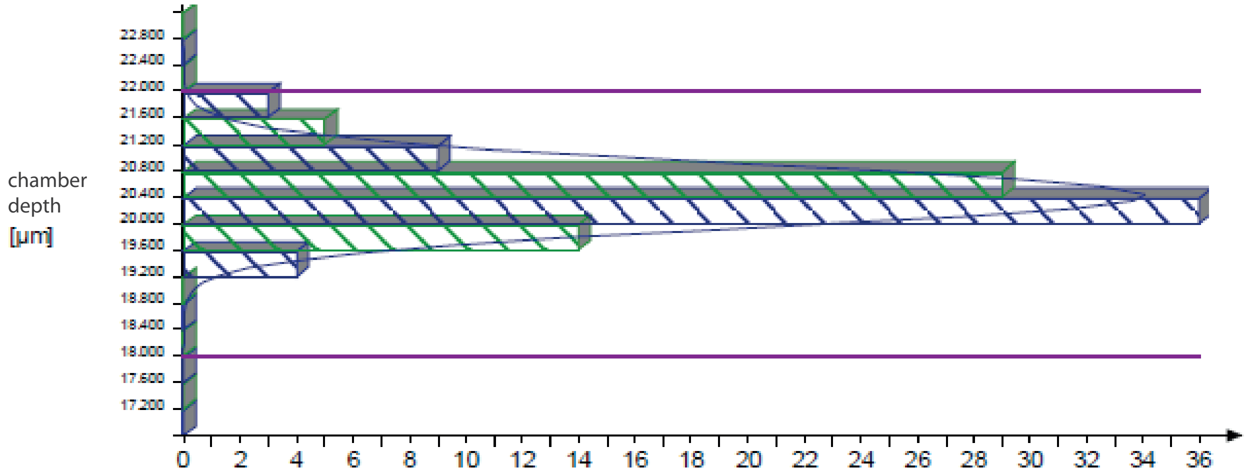


Fig. 2: Distribution (Histogram) of the chamber depth of Minitube chambers (n=100); the depth of all chambers is within the manufacturing tolerance of ± 10%

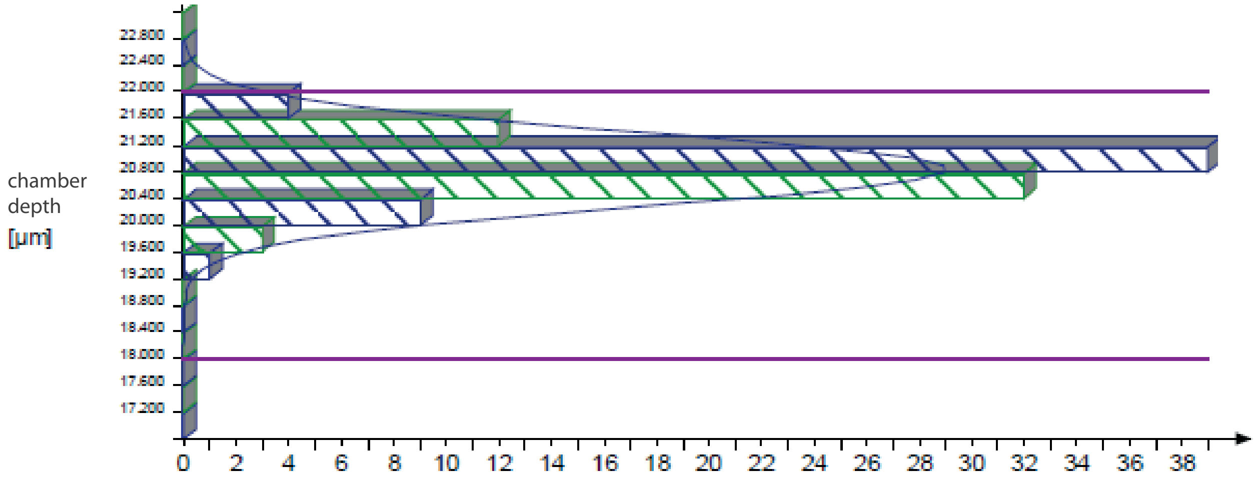


Fig. 3: Distribution (Histogram) of the chamber depth of Leja chambers (n=100); the depth of all chambers is within the manufacturing tolerance of ± 10%

2. Filling behavior and sperm concentration

In addition to the chamber depth, the filling process and the sperm cell distribution within the counting chamber influence the correctness of concentration measurement. Therefore a comparison with a reference method of concentration measurement (NucleoCounter) is performed to exclude any negative effects on the precision of the concentration measurement with both types of counting chambers.

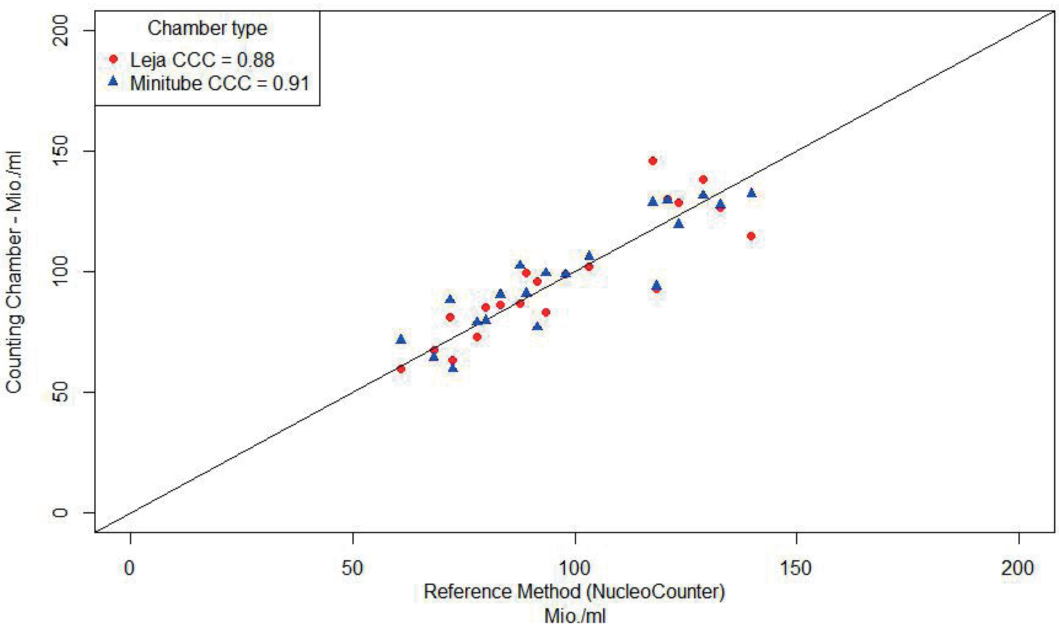


Fig. 4: Semen concentration measurement of boar ejaculates with the Minitube chamber and the Leja chamber in comparison to the reference method NucleoCounter (n=20)

Each dot in this graph represents one measurement. The black line in the graph is the 45-degree line - the line of perfect concordance. An indicator of the degree of concordance is the distance of the measurements to the black line. As can be seen from the graph, nearly all measurements are close to the line which indicates a very good agreement between both counting chamber measurements (with AndroVision®) and NucleoCounter.

Visual evaluation of the measurement agreement on a graph is easy, but subjective. Therefore mathematical coefficients should be calculated to describe the measurement agreement numerically. The Concordance Correlation Coefficient (CCC)[1] can be used for this purpose. The closer the value approaches 1.0, the better is the agreement between the two measurement methods. The CCC for the Minitube chambers is 0.91, the CCC for the Leja chambers is 0.88. In the literature, a CCC of 0.81 or higher is described as "almost complete" agreement[2].

3. Toxicity test and motility analysis

Sperm motility is the second analysis parameter counting chambers are used for. Parallel to the examination of the chamber depth, a sperm toxicity test is performed at Minitube for every batch. For this test, diluted and pre-warmed (+38°C) semen is filled in also pre-warmed counting chambers. The diluted semen is incubated in the counting chamber for up to 8 minutes at +38°C. Every minute a motility analysis with AndroVision® is performed. To exclude any toxic effect of the chamber on the sperm cells, the total and progressive motility must be stable over the total incubation time within 10%.

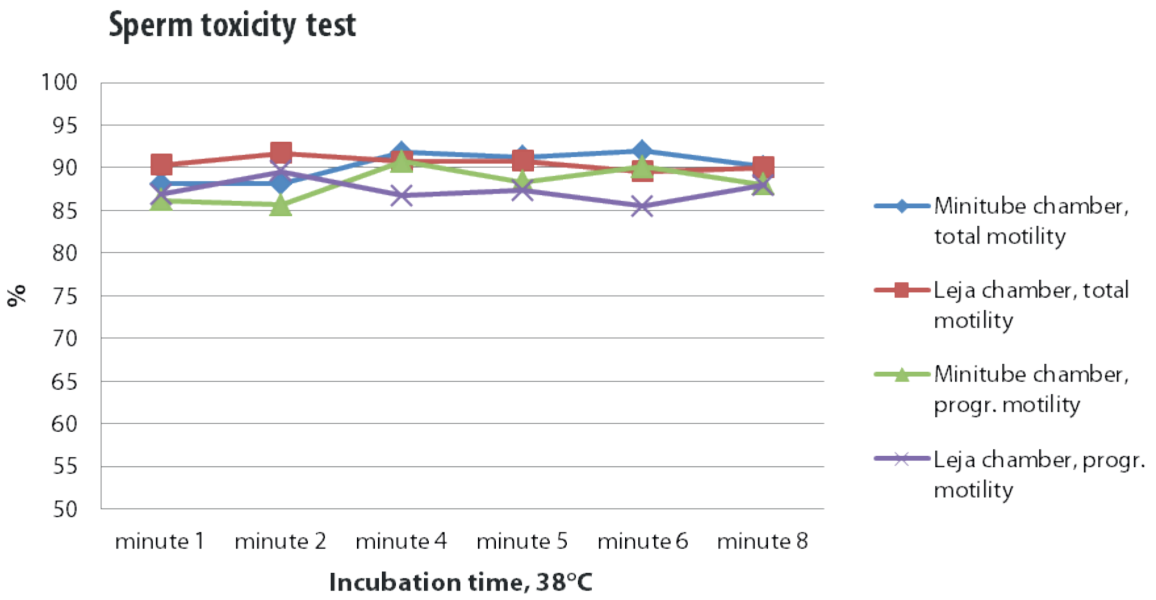


Fig. 5: Measurement of motility with AndroVision® of both chamber types

The sperm toxicity test shows no difference between the two chamber types, neither for total nor for progressive motility. The variation of the values - between 88-92% for total motility and 85-88% for progressive motility - is in the standard range of measurement accuracy of a CASA-system.

4. Conclusion

In summary, the quality of the Minitube chamber and of the Leja chamber is concordant and consistently at a very high level. Statistically, the agreement is almost complete regarding concentration measurement. Sperm motility is not influenced negatively and the accuracy of manufacturing of both chambers proves to remain on a high level in our regular batch controls, ensuring accurate sperm concentration measurements.

References

[1] Lin, L. I. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45, 255–68 (1989).

[2] Koch, R. & Spörl, E. Statistische Verfahren zum Vergleich zweier Messmethoden und zur Kalibrierung: Konkordanz-, Korrelations- und Regressionsanalyse am Beispiel der Augeninnendruckmessung. Klin. Monbl. Augenheilkd. 224, 52–57 (2007).