



## Post-vasectomy

When performing a post-vasectomy check, you expect no sperm cells. It is however, statistically impossible to “count” 0. Each number counted has a margin of error and has to be reported with a 95% or 99% confidence interval. For example, when you do not see any sperm cells, the 99% confidence interval tells us that the actual concentration of sperm cells in the sample lays between 0 and 184 cells ml<sup>-1</sup>.

Everybody understands the importance of counting every possible sperm cell in a post-vasectomy sample. There is a tendency to centrifuge these samples. Research has shown that this is not the best approach<sup>1,2</sup>. After centrifugation, sometimes no cells were counted in the pellet, while they were detected in the supernatant. Also, the same research has shown that 50% to 70% of the cells are lost in the procedure and may thus give a false sense of security.

As a doctor, this should be discussed with the patients. Also, in order to avoid liability claims, the best available counting method should be used. A patient should be aware of the limits of “counting” 0. When you are aware of the errors produced by the different counting methods, you will only trust the results of the large volume counting chambers, like the Leja 100 micron chamber.



Possible number of spermatozoa per ml if no sperm are observed per chamber				
Makler	Wet preparation	Neubauer	Neubauer (9 fields)	Leja 100 micron
370,000	92,500	37,000	4,111	148
Lower limit of detection (what is the minimum concentration per ml in order for one sperm cell to show up in the counting chamber.)				
Makler	Wet preparation	Neubauer	Neubauer (9 fields)	Leja 100 micron
100,000	25,000	10,000	1,111	40

Since it is also important to report motile sperm in a post-vasectomy sample, we suggest the following approach. This will provide you the best available level of security.

- Do not centrifuge the sample.
- Look for sperm cells in a standard Leja 20 micron counting chamber.
  - When motile sperm cells are seen, report a negative result.
  - When only non-motile sperm cells are seen, this might yield a negative result depending on the number.
  - When no cells are seen, or very little non-motile cells, use a Leja 100 micron chamber.
- The Leja 100 micron chamber will give the best reliability about low sperm counts, when no cells are seen here, or a concentration lower than your national standard's threshold, a first positive result can be reported.

A fluorochrome may be added to increase sperm cell detection in the 100 micron chamber.

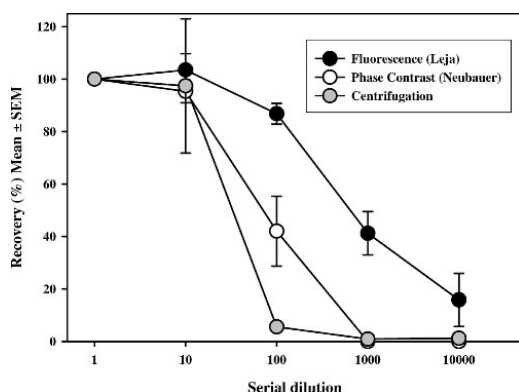
References can be downloaded from [www.leja.nl](http://www.leja.nl):

1: T.G. Cooper *et al.*, Azoospermia: Virtual Reality or Possible to Quantify?; Journal of Andrology, Vol. 27, No. 4, July/August 2006

2: M. Corea *et al.*, The diagnosis of azoospermia depends on the force of centrifugation; Fertility and Sterility, Vol. 83, No. 4, April 2005.

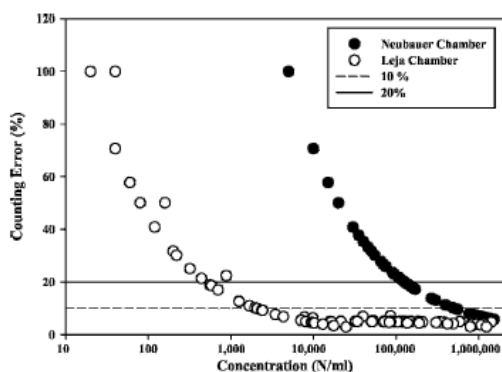


## Evidence form the research



Analysis of 5 different samples serially diluted (10-fold) from about 1 million  $\text{ml}^{-1}$  revealed that spermatozoa could be observed in the first 3 dilutions by using phase contrast microscopy only (Neubauer chamber), whereas spermatozoa were observed at all 5 dilutions by the fluorescence method (Leja chamber)<sup>1</sup>.

This shows that the greatest dilution ( $\sim 100 \text{ ml}^{-1}$ ), still yielded visible cells in the Leja chamber and not in the Neubauer nor a wet preparation after centrifugation.



This figure shows the anticipated increase in counting error with fewer sperm counted in samples of low concentration, and that with the Neubauer chamber concentrations below  $100,000 \text{ ml}^{-1}$  cannot be estimated with precision below 20%. Intercepts of the curves with the 10% and 20% error axes revealed sensitivities (spermatozoa  $\text{ml}^{-1}$ ) of 500,000 and 150,000 for the Neubauer chamber, 2,000 and 500 for the Leja chamber<sup>1</sup>.

Hence, much smaller concentrations can be more precisely counted with the Leja chamber.

Number of supernatant samples (n=25) from non-azoospermic patients containing sperm after centrifugation at various forces<sup>2</sup>.

Centrifugal force	No. of supernatant samples with sperm
500 x g	25
1,000 x g	25
3,000 x g	23

This shows that even after centrifugation with 3,000 x g, sperm cells can be seen in the supernatant and thus centrifugation does not yield in a better sperm count.

Sperm motility after centrifugation of non-azoospermic semen specimens at various forces<sup>2</sup>.

Centrifugal force	Sperm motility (range / mean)
500 x g	7-87 / 37%
1,000 x g	0-93 / 28%
3,000 x g	0-93 / 29%

This shows that the motility of the sperm drastically decreases by the centrifugation procedure, which might yield in false positive patient clearances.