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**LETTER TO THE EDITOR**

Semen Analysis

# Sperm concentration measurement with a disposable counting chamber

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Dear Editor,

In its latest edition, the World Health Organization (WHO) provides guidelines regarding the standardization of techniques for the measurement of sperm concentration[1] and recommends use of a 100 μm-deep hemocytometer. However, these instructions are not always followed correctly.[2] Furthermore, although hemocytometers provide more accurate and consistent results, they present several drawbacks: the chamber must be properly assembled prior to its use so as to ensure that the counts are correct and it needs to be cleaned thoroughly to remove all traces of sperm, which risks breaking the glass coverslip. A disposable counting chamber (Kova) exhibits several advantages: low cost, ease of use (*e.g.*, no cleaning, no need for mounting the coverslip, and a fixed device); disposability, which is particularly advantageous with sperm infected with virus; and the possibility of processing 10 samples. Here, we sought to validate the Kova chamber as a reliable device for the measurement of sperm concentration, according to the ISO 15189 standards.

Samples consisted of latex beads (Qwik Check®, Theradiag, Marne-la-Vallée, France) and semen samples from infertile patients collected by masturbation in the laboratory, after having provided informed consent (Bichat Hospital, APHP, Paris, France). Two levels of latex beads were used: level 1 ($(22 \pm 5.5) \times 10^6$ beads ml$^{-1}$) and level 2 ($(46 \pm 11.5) \times 10^6$ beads ml$^{-1}$). After the samples were mixed, they were diluted using positive-displacement pipettes in a 0.35% (*v*/*v*) formalin solution (dilutions ranged from 1:2 to 1:100). A 10 μl diluted aliquot of either latex beads or patient sperm sample was then loaded in a counting chamber (Kova® Glasstic® Slide 10 [CML, Nemours, France] and/or an improved Neubauer chamber [Dutscher, Brumath, France]) depending on the nature of the experiment. The counting chamber was left for 10–15 min and counting was then performed using positive phase-contrast optics with a magnification of ×400, according to the instructions provided by the WHO.[1] In light of the absence of a state of the art definition in spermiology, our results were compared with data from the literature (when available) and with the criteria defined by the biological variations calculated on within-subject and between-subject biologic variation (Ricos).[3]

All of the determinations of concentrations were done blinded by four different operators on 60 sperm samples (concentrations ranging from $0.4 \times 10^6$ ml$^{-1}$ to $382.5 \times 10^6$ ml$^{-1}$). Differences in Bland–Altman plots[4] showed concordance of sperm concentration values measured with the Kova and the Neubauer devices (**Figure 1a**). Three points of discrepancy were above the 95% limit of agreement, without any clinical impact given the high values. The correlation between the two methods was studied with Tessier's least rectangle regression, by fitting a linear equation to the observed data. Statistical correlation revealed a coefficient of determination $R^2 = 0.982$ (**Figure 1b**), indicating correct linearity of the regression and thus a strong association between the values determined with the Kova and the Neubauer devices.[5] Differences in accuracy,[5] evaluated for four levels of selected clinical interest ($5 \times 10^6$ ml$^{-1}$, $30 \times 10^6$ ml$^{-1}$, $100 \times 10^6$ ml$^{-1}$, and $300 \times 10^6$ ml$^{-1}$), were 13.8%, 1.9%, 4.1%, and 4.7%, respectively. The mean relative difference between both of the methods was 3.6%, with a 95% confidence interval that ranged from 1.7% to 8.9%. All of these values were lower than the limit of inaccuracy B (15.6%).[3] The statistical power of comparison between both methods, calculated with a threshold of 10% and a risk of 5%, was 99.5%, assuming that there is no statistical difference between both methods for sperm concentration measurement.

Repeatability was expressed as the coefficient of variation (CV). Each sample was counted ten times for each calibrated bead and five times for each semen sample by the same operator. The CVs were 9.2% and 5.7% for level 1 and level 2 bead solutions, respectively. They were 8.6% and 7.9% for the low ($20 \times 10^6$ ml$^{-1}$) and high ($200 \times 10^6$ ml$^{-1}$) sperm level samples, respectively. All of the CVs were lower than the reported intratechnician CV, which averaged 12.5% for hemocytometer counts,[6] and they were similar to that reported for technicians with daily practice (9.8%).[7]

Reproducibility was expressed as the CV. To our knowledge, there is no consensus in regard to the method to obtain reproducibility.

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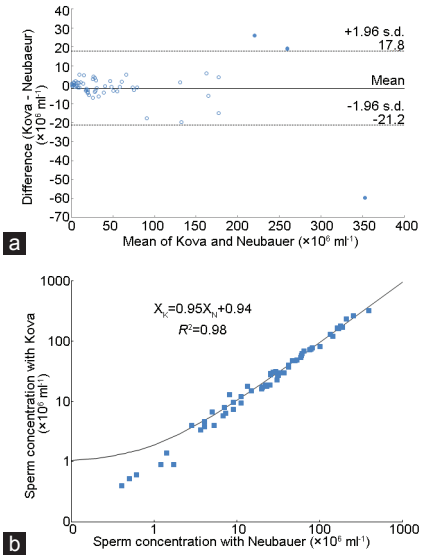


**Figure 1:** Comparison of Kova and improved Neubauer for sperm concentration measurement. A total of 60 different sperm concentrations were measured with the two methods. (**a**) Bland–Altman plot of difference in the concentration ($10^6$ $ml^{-1}$) measured with Kova chamber and improved Neubauer hemocytometer against the mean of the 2 values (circles). The solid line represents the mean of differences. The dotted lines indicate the 95% confidence limits (mean ± 1.96 × s.d.). (**b**) Linear regression of the log-transformed concentrations ($10^6$ $ml^{-1}$) estimated with Kova chamber 10 (y-axis) and improved Neubauer hemocytometer (x-axis). The solid line represents the Tessier's linear regression ($X_K = 0.95X_N + 0.94$).

Each sperm sample was analyzed the same day with the same batch of pipettes, the same 0.35% formalin solution, and the same Kova chamber. The only difference was the operator. These procedures were performed ten times by two different operators for three semen samples at different concentrations ($1 \times 10^6$ $ml^{-1}$, $15 \times 10^6$ $ml^{-1}$, and $90 \times 10^6$ $ml^{-1}$). Individual CVs of two operators were 15.1% and 14.4% for the low level, 14.0% and 9.0% for the medium level, and 12.3% and 13.6% for the high level. All of the CVs were close to inter-trained operator comparisons (i.e., 8.1%, 21.8%, and 22.9%).[7–9]

Thanks to participation in two external quality control (EQC1 and 2) programs, it was possible to determine the inaccuracy and expanded measurement uncertainty.[5] Fixed semen samples were sent by the French quality control institution (Biologie Prospective, Nancy, France). Sperm concentrations were measured with a Kova device by all of the operators in the laboratory. Inaccuracy was calculated by the formula, $100 \times (X - V)/V$, where X is the mean value obtained by all of the operators in our laboratory and V is the target value defined by the EQC program. The inaccuracies were 13.2% and 13.0% for EQC1 and 2, respectively, and less than the limit of inaccuracy B defined by Ricos (15.6%).[3] Expanded measurement uncertainty U associates the random error (reproducibility) with the systematic error (inaccuracy) and was estimated to be $\pm 5.0 \times 10^6$ $ml^{-1}$, i.e. 39.0%, or close to the limit coefficient of variation TE defined by Ricos (37.7%).[3] This outcome is acceptable for high and medium concentration values but not for the low sperm concentration values. It is certainly a limitation of this method. There is, however, no data in the literature for comparison with other devices for the measurement of sperm concentration. It is likely that they will yield similar results and that referent clinicians should also be informed about this uncertainty.

Although it is obvious that manual methods are less reproducible than automatic ones[10] and that the Kova chamber does not comply with the WHO guidelines, we have proven that this easy-to-use and inexpensive manual method is as accurate and efficient for measuring sperm concentration as the reference method.

## AUTHOR CONTRIBUTIONS

ML and CP designed the experiments. ML, MALdeR, AB, and XF performed the experiments. ML, JD, XF, and CP analyzed the data. ML and CP wrote the manuscript together with input from JD, MALdeR, XF, FE, SE, and AB. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing interests.

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