

Accuracy of semen counting chambers as determined by the use of latex beads*

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Objective: To assess the accuracy of the Hemacytometer (Hausser Scientific, Horsham, PA), Makler (Sefi-Medical Instrument, Haifa, Israel), Cell-VU (Millennium Sciences Inc., New York, NY), and Micro-Cell chambers (Conception Technologies, San Diego, CA) counting chambers.

Design: A solution containing a known concentration of latex beads was used as the standard to perform counts on the four different counting chambers.

Main Outcome Measures: Bead counts for the four different chambers were compared with the bead counts of the standard solution. Variability within chambers also was determined.

Results: Mean bead concentrations for both the CEU-VU and Micro-Cell chambers were consistently similar to the bead concentration of the standard solution. Both the hemacytometer and the Makler chambers overestimated the actual bead concentration of the standard solution by as much as 50% and revealed significant interchamber variability.

Conclusions: Our data revealed marked differences in the accuracy and reliability of the different counting chambers tested and emphasized the need for standardization and quality control of laboratory procedures. *Fertile Sterile* 1996;66:662-5

Key Words: Sperm count, quality control, latex beads, Makler, Micro-Cell, Neubauer, Cell-vu

Semen analysis, particularly the sperm count, remains the essential test for evaluating male fertility potential. Despite its importance, little is actually known about the accuracy and reliability of the different commercially available counting chambers used to perform the sperm count. Early studies used semen samples to compare the accuracy of different counting chambers, but unfortunately the data were

difficult to interpret because no standard solution was used (1).

Commercially available solutions containing known concentrations of particles have been introduced for use in laboratory quality control in an effort to raise the level of accuracy and reliability of counting procedures. The purpose of this study was to evaluate the accuracy of the four most frequently used counting chambers by performing counts from a commercially available solution containing a known concentration of latex beads. By using the standard solution only, we attempted to remove possible confounding sources of error that may be inherent in semen samples, such as variations in sperm density because of variations in viscosity or because of sperm clumping and the presence of immature forms.

MATERIALS AND METHODS

We used three hemacytometers (Hausser Scientific, Horsham, PA), three Makler chambers (Sefi-

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Table 1 Testing Conditions

No. of Chamber depth	No. of chambers tested	counts per chamber	Dilution
0.1 <u>mm</u>	3	5	1:20
0.01 <u>mm</u>	3	5	None
0.02 <u>mm</u>	5	1	None
0.02 <u>mm</u>	5	1	None

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Medical Instrument, Haifa, Israel), five Cell-VU disposable chambers (Millennium Sciences Inc., New York, NY), and five Micro-Cell disposable chambers (Conception Technologies, San Diego, CA). A suspension of latex beads, 3 in diameter (Accubeads; Hamilton Thorne Research Inc., Beverly, MA), at a concentration of 35×10^6 beads/mL, was used as the standard solution. The suspension was vortexed before each measurement as directed by the manufacturer.

Testing conditions and chamber characteristics (as provided by each manufacturer) are presented in Table 1. All counts were performed by one technician (E.F.S.) Chambers were loaded in standard fashion with a P20 Pipetman (Baxter Diagnostics, Deerfield, IL) and examined using a light microscope with a 40X objective. To ensure consistent bead counting, a minimum of 200 beads were counted for each of the chambers used. A description of chamber loading follows.

Hemocytometer

The hemacytometer consists of a thick glass slide with an H-shaped trough forming two counting areas. The edges of the trough are raised to support a clear coverslip 100 μ m from the bottom of the chamber. A 3×3 mm ruled area, divided into smaller squares, is located on the glass slide.

A 1:20 dilution of the bead suspension with distilled water was prepared and chambers were loaded with 10 μ L of sample. Counts were performed as directed by the World Health Organization manual (2); in brief, all chambers were allowed to settle for 3 minutes before bead counts were performed. All beads within the five blocks of 16 squares each were counted, including those touching the lower and right sides of each block of 16 squares. This process was repeated a second time; the average of the two counts represents the number of beads $\times 10^6/\mu\text{L}$.

Five separate determinations were performed on each of the three hemacytometers (2). Chambers were cleaned between counts with 70% ethanol solution and wiped with absorbent towels. Chambers were allowed to dry before reloading.

Makler

This chamber was designed specifically for determination of sperm concentration and percent motility of undiluted semen. It has a reported staged depth of 10 μ m, one tenth the depth of an ordinary hemacytometer. The chamber is constructed from two pieces of optically flat glass; the upper layer serves as a cover glass, with a fine grid one mm in the center subdivided into 100 squares of 0.1×0.1 mm each.

The Makler chambers were loaded with a standard volume of 5 μ L as indicated by the manufacturer's directions (Sefi-Medical Instrument). To ensure a tally of ≈ 200 particles, beads in 50 small squares within the grid area were counted and the number divided by 5 to obtain the count in $10^6/\text{mL}$. Five separate determinations were performed on each of the three Makler counting chambers tested. Chambers were cleaned between counts with 70% ethanol solution and wiped with absorbent towels. Chambers were allowed to dry before reloading.

CELL-VU

CELL-VU consists of a dual-chamber glass slide patterned from a printed inert surface. The surface supports a 0.5 mm thick coverslip containing a laser-etched grid on the reverse side. The grid area is 1×1 mm, divided into 100 smaller squares each measuring 0.1×0.1 mm. The chamber has a reported depth of 20 μ m.

The Cell-VU disposable, chambers were loaded with a standard volume of 4 μ L as described in the manufacturer's directions. (Millennium Sciences Inc.). To be consistent with other chamber counts, 30 boxes were counted to ensure a minimum tally of 200 beads per count. This count was divided by six to obtain the bead count in $10^6/\text{mL}$.

CEU-VU chambers are designed for one-time use and therefore only one count was performed on each chamber. Five chambers were tested.

Micro-CeD

The Micro-CeD chamber contains two independent chambers and uses a 0.5-mm fixed coverslip. Chamber depths are reported to be 20,4 μ m. The Micro-CeH chamber has no grid and thus requires a reticle for

Table 2 Results: Bead Concentrations.*

	Bead concentration per chamber type	Bead concentration per Chamber
	<i>x</i> _{all} .L	<i>x</i> ₀₁ /?nL
Hemocytometer 1	51.4 ± 5.0	45.7 ± 3.0 63.5
Hemocytometer 2		5.6 45.1 ± 1.8
Hemocytometer 3		54.3 ± 2.2 43.5
Makler 1	51.2 ± 4.2	5.4 55.9 ± 8.0
Makler 2		Not applicable
Makler 3		Not applicable
Cell-VU Micro-cell	35.1 ± 2.2	
	36.2 ± 3.2	

Values are means SEM. A standard solution containing 35 x 10⁶ beads/mL were used for all chambers.

manual use. Undiluted semen samples are loaded at each end of the slide and enter the chambers by capillary action.

The Micro-CeR chambers were loaded with a standard volume of 5 ML and excess fluid was removed. As indicated in the manufacturer's directions, 200 particles were counted, using an eyepiece reticle (Conception Technologies). NEcro-CeU chambers are designed for one-time use and therefore only one count was performed on each chamber. Five chambers were tested.

Statistical Analysis

Mean counts and standard errors for all chambers were calculated. Differences between counts from different chambers and types of chambers were compared using one-way analysis of variance (ANOVA).

RESULTS

A comparison of the mean head concentrations of the four chamber types is presented in Table 2. The mean bead concentration per chamber for the three hemacytometers tested revealed significant inter-chamber variability (P < 0.001, one-way ANOVA). For the Makler chamber, the mean bead concentration for the three chambers tested also revealed significant interchamber variability (P = 0.02, one-way ANOVA).

The mean bead concentrations per chamber type were similar for both the hemacytometer and Makler and both were approximately 50% higher than the standard. These values were significantly higher than the value of the standard by examination of 95% confidence intervals. The mean bead concentrations per chamber type for CELL-VU and Nficro-CeU were similar and neither was significantly higher than the standard.

DLSCUSSION

The sperm count is the essential component of se- men analysis and, as such, is the cornerstone of the male fertility evaluation. However, even when done properly, the analysis is subject to many sources of error. Errors may be inherent or technical in nature. Inherent errors are errors caused by the non-uniform and random distribution of particles in solution as described by Poisson's principle (1). These errors can be lessened by counting larger numbers of particles. Technical errors usually are thought to be caused by personnel or procedural differences but also may be attributed to inaccuracies of the counting chambers themselves.

The Hemacytometer is one of the oldest and most frequently used chamber for assessing sperm concentration. In 1964, Freund and Carol (1) described counting spermatozoa from diluted semen samples using a Hemacytometer chamber that originally was designed for counting red blood cells and leukocytes from peripheral blood. Using techniques similar to those described in the WHO manual (2), they found that sperm counts varied tremendously and attributed much of the variation to inaccuracies of the Hemacytometer.

In 1978, Makler introduced a different type of chamber to minimize the variation in Hemacytometer measurements. He attributed variation in sperm counts to lack of volume control in traditional Hema- cytometers. Theoretically, the Makler chamber only permitted a standardized volume of semen for analysis. Although Makler reported very high accuracy, other authors report a lack of correlation between results of the Makler chamber and hemacytometer (3-5).

Ginsberg and Armant (5), to the best of our knowledge, were the first to use a solution containing a specific concentration of particles to compare results of different counting chambers. Counts and standard deviation for the Makler chamber were found to be higher than the counts for both the hemacytometer and NECRO-Cell chambers.

In 1993, Peters et al. (4) reported on the use of latex heads that were added to semen for quality assurance in sperm counting. They observed a statistically significant discrepancy between sperm counts performed on the Hemacytometer and Makler chamber. Mean Makler sperm concentrations were found to be 1.6 times greater than mean Hemacytometer counts. These authors suggested that corrections be made between the different chambers by using a factor derived from ratio of heads counted in the two chambers (4).

Commercially available solutions containing a known concentration of latex beads are now available for use as standards for quality control purposes. We used such a solution to evaluate the accuracy and reliability of the Hemacytometer, Makler, Micro-CeR, and CEU-VU counting chambers. Our results showed that the disposable CELL-VU and Micro-Cell chambers are more accurate and less variable than the Hemacytometer and Makler chambers. Using the Cell-VU and Macro-Cell chambers, bead counts were similar to the bead counts of the standard solution. Both the Hemacytometer and Makler chambers overestimated the actual bead concentrations of the standard solution by as much as 50% and revealed significant interchamber variability. We realize that different laboratories use different counting chambers and, therefore, results of semen analysis may be markedly different from laboratory to laboratory. We suggest that an effort be made to

Standardize counting chambers and procedures for all laboratories, with the ultimate goal of improving accuracy and reliability of results.

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