

Counting of Sperm Cells with Hemocytometer

Equipment Needed:

1. Platelet Unopette, (Becton-Dickinson; B-D: #5855)
2. Hemocytometer, (Exodus Breeders Corporation; Exodus #185)

Sperm Counting Technique:

1. Mix raw semen thoroughly.
2. Fill the Unopette capillary tube in the horizontal position. It will fill on its own when placed in the semen.
3. Wipe excess semen off outer sides of capillary tube.
4. Apply pressure to the Unopette Canister.
5. Insert capillary tube into the canister.
6. Release pressure on the canister. The semen will be aspirated from the capillary tube.
7. Mix diluted semen well.
8. Put Hemocytometer on flat surface with the cover slip in place.
9. Fill the Hemocytometer chamber. Place the chamber in a covered Petri dish with moistened filter paper for 10 minutes in order for the spermatozoa to settle.
10. The Hemocytometer is divided into nine (9) large squares. Count all sperm heads in the large center square (1mm X 1mm X 0.1mm). In regard to the heads on the lines of the squares, count only those on the left and top lines of each square. Do not include those on the right and bottom lines.
11. The number of sperm counted in this large center square multiplied by 10^6 (1,000,000) is the concentration per ml of raw semen.
12. The concentration of sperm per ml X the total number of ml of raw semen = the total number of sperm in the ejaculate.

HEMATOLOGY A SAFER SPERM COUNT

A study was conducted to develop a method for a “safer” sperm count. As a result, this laboratory now performs all sperm counts using a Unopette Brand System.

For many years mouth pipetting has been forbidden in the clinical laboratory. However, the technique of specimen aspiration into a white or red blood cell diluting pipette using a mouthpiece and aspiration tubing is still being used for counting cells in some body fluids. The concern, of course, is the possibility of exposure to infectious agents such as HIV and Hepatitis B virus. Although no evidence of HIV was shown in “health care workers with intense exposure” to infectious agents, the presence of this virus has been demonstrated in many body fluids as well as blood.¹

The manual pipetting technique can be replaced by the use of premeasured diluting reservoirs such as the Unopette Brand Systems.² These are available for many types of cell counts on blood and can be used for counting blood cells in many body fluids. None has been developed for the performance of sperm counts, leaving the standard manual dilution the only method available. With the recent increase of fertility testing in our area and the concern of exposure to HIV in semen³ as well as other infectious agents, a need arose for the development of a safer sperm-count method.

Thirty-six specimens for sperm counts or semen analysis were available during the time of this study. A standard manual count was performed on each specimen using a 1:20 dilution with a white blood cell diluting pipette, distilled water, and a Neubauer Hemocytometer (the established procedure). A second count was performed on each specimen using a Platelet Unopette with a dilution of 1:100 and a Neubauer Hemocytometer.

The data in this study demonstrates a Pearson correlation coefficient of 0.994 with good linearity between counts of 2 to 300 million per cubic millimeter. The counts ranged from a low of 2 million sperm per cubic millimeter to 438 million sperm per cubic millimeter.

Since correlation of Unopette counts with manual and automated methods have previously been studied,² accuracy of the Unopette Brand System was not in question here. Rather the correlation of sperm counts diluted manually in distilled water versus those diluted in a Platelet Unopette was the concern. The effect of 1% buffered ammonium oxalate on sperm identification was unknown.

When compared, the traditional and Unopette methods have a correlation of 0.994 for sperm counts. As a result of these findings, our laboratory now performs sperm counts using the Platelet Unopette as a “safer” diluting method. Considerations for further studies include: (1) a larger population of counts above 300 million to determine linearity in the above normal range and (2) use of WBC Unopette with 1:200 dilution.

References

1. Gerberding JL, Bryant-LeBlanc CE, Nelson K et al. Risk of transmitting the human immunodeficiency virus, cytomegalovirus, and hepatitis B virus to health care workers exposed to patients with AIDS and AIDS related conditions. *Infect DIS* 1987;156:1-8.
2. Becton Dickinson Vacutainer Sysems. Laboratory procedures using the Unopette Brand System. Becton Dickinson; 1977.
3. Zagury D, Bernard J, Leibowitch J et al. HTLV III in cells cultured from semen of two patients with AID. *Science* 1984;226:500-3

Hematology/Chemistry Test

Description	Dilution	Pipette	Shipping Box	Unit Case	Reorder Numbers
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Hematology Tests, Manual Methods

WBC/platelet, 1.98 mL 1% ammonium oxalate	1:100	20 µL	----	1000	365855
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