



Evaluation of the Automated Cell Density Examination System (Cedex®)

Show Ming Kwok^{1,4}, Wendy Ham^{1,4}, Meredith A. McGowan⁵, Ryo Ohashi⁴, Jose M. Otero³, and Jean-François P. Hamel²⁻⁴

(1) Department of Biology, (2) Department of Chemical Engineering (3) Division of Biological Engineering, (4) Biotechnology Process Engineering Center, (5) Dartmouth College

Correspondence: jhamel@mit.edu



Abstract:

Cell density and cell viability are essential in monitoring and controlling suspension cell cultures. Measurements of these parameters have been traditionally taken by tedious, time-consuming protocols. For example, to determine total cell count and cell viability of animal cells, a sample is treated with trypan blue, placed on a hemacytometer and counted manually. Recently, this assay has been automated to reduce the time and labor needed. The Cedex® method, based on optical recognition, is one such approach which integrates the steps from sample preparation to cell density and viability calculations. By identifying specific cell discrimination criteria, counting accuracy can be optimized for a given cell type. This study focuses on comparing Cedex® assay to traditional manual cell counting method for a hybridoma cell line. The data show that there is no significant statistical difference between Cedex® and manual cell counts ($p > 0.05$).

Introduction:

Viable cell density, total cell density, and viability are critical measurements in many biological processes including cell development, drug discovery, and bioprocess engineering. In a bioprocess, cell density and viability can be related to process robustness, product yield, and production efficiency. Typically, protein production scales linearly with viable cell density and viability, and they may serve as optimization parameters. The ability to measure viable and total cell density quickly, accurately, reproducibly, and cost-effectively, is essential for bioprocess development. Currently, the most widely used cell counting method is the trypan blue cell exclusion. Trypan blue staining is coupled with counting on a hemacytometer chamber under an inverted light microscope. Trypan blue allows visual analysis of the culture viability by distinguishing live cells (trypan blue negative) from necrotic cells (trypan blue positive) (see Fig. 3). This method is commonly utilized because of its simplicity and relatively low cost. However, manual counting can be time consuming and subjective to increasing human errors with increasing cell density. As a result, extensive research has been dedicated to finding an automated method. This study evaluates a new automatic cell counting system, Cedex®, by comparing its results in hybridoma cell density and cell viability with those from the manual counting method. Moreover, we conducted a study that compared the three kinds of hemacytometers (2 Reichert-Improved Neubauer hemacytometers and Fuchs Rosenthal Ultraplane hemacytometer) in terms of their consistency in cell density assessment.

Material and Methods:

- A combination of 9 samples consisting of 3 mouse-mouse hybridoma cell lines (1B4, 4F5, 3D6) arrayed with each of 3 media (DMEM, BD, BDSF) was used.
- The cells were grown in 75 cm² T-flasks for 4 days without passages. Samples were taken each day from each flask, providing measurements of heterogeneous viability and cell density range.
- Each sample was removed from the T-flask in the same manner as above, pipetting 10 times at each mixing. Each hemacytometer was prepared by the same person to reduce error. When the cell density was higher than 300 cells/chamber, the remaining sample would be diluted by 1x PBS in 1:1 and 1:2 volume ratios to aid the manual counting process
- 0.4% filtered trypan blue was used, prepared once every three weeks
- Images were taken using a microscope-mounted camera. Each image (see Fig. 1) captures one counting square from each of the three hemacytometers: Reichert – Improved Neubauer 1 (N1), Reichert – Improved Neubauer 2 (N2), and Fuchs Rosenthal Ultraplane (FR) (see Fig. 2).
- These images were saved on compact discs and counting was performed by three investigators on the computer screen.
- The same sample used for manual counting was fed into the Cedex® machine (at a setting of 20 images at a time, see Fig. 3) for Cedex counts.

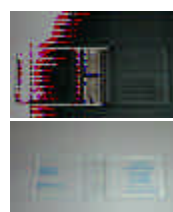


Fig. 1 Images of hemacytometers. Above: Reichert – Improved Neubauer 1, 0.1 mm deep, shorter channel, 9 squares (N1). Below: Fuchs Rosenthal Ultraplane, 0.2 mm deep, 16 squares (FR).



Fig. 2 Picture of Cedex® instrument along with the analyzing desktop computer. This system analyzes a sample's cell density and viability in 5 minutes.

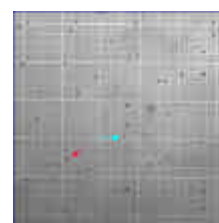


Fig. 3 Camera image (A4 8-7-01 N1-3) of a square in a hemacytometer chamber viewed from a computer screen. Blue arrow indicates a necrotic cells. Red arrow indicates a live cell.

Results:

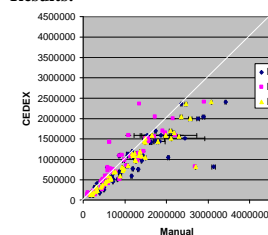


Fig. 4 Comparison Cedex® counts versus average manual counts. The manual and Cedex® counts are closer at lower cell densities. At higher cell densities, manual counts tend to be higher. The line models $y = x$.

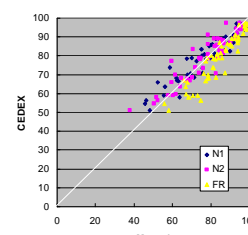


Fig. 5 Comparison of Cedex® viability versus manual count variability. The N1 and N2 manual count viabilities are higher than that of Cedex® count, while FR's manual count viability shows the opposite. The increased number of squares in the FR chamber results in a greater inconsistency in manual counts. The line models $y = x$.

Student's t-test Results:

Table 1. Student's t-test (two-tailed, two samples equal variance)

All of the t-test values are greater than 0.05, except for the value derived from comparing average FR dead cell count to Cedex® dead cell count (highlighted in yellow). A t-test value greater than 0.05 means that a difference is not statistically significant. In this case, no statistically significant difference is observed when comparing investigators' counts and when comparing the average of investigators' counts to Cedex® counts, except when FR was used to count dead cells.

	N1			N2			FR		
	Live	Dead	Viability	Live	Dead	Viability	Live	Dead	Viability
Manual A vs. B	0.718	0.725	0.710	0.883	0.805	0.904	0.929	0.805	0.805
Manual A vs. C	0.979	0.887	0.800	0.863	0.911	0.717	0.986	0.910	0.882
Manual B vs. C	0.922	0.619	0.529	0.751	0.715	0.629	0.905	0.714	0.887
Average Manual vs. Cedex	0.349	0.160	0.507	0.891	0.570	0.549	0.353	0.000825	0.964

	Average Cell Density	Viability
N1 vs. N2	0.219	0.991
N1 vs. FR	0.898	0.00584
N2 vs. FR	0.255	0.00488

Table 2. Two tails, unpaired, unequal variance Student's t-test resulting from the comparison between various hemacytometers. Viability obtained from FR count is statistically significantly different from that of N1 and N2.

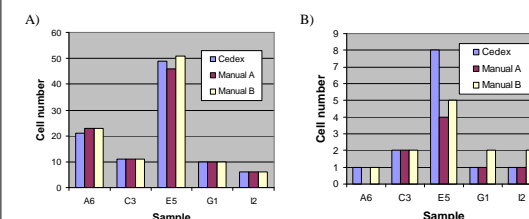


Fig. 6 Comparison of manual count and Cedex® count. Five images were randomly chosen from Cedex® generated images. Two investigators counted directly on the images (on the monitor), and the counts were compared to Cedex® counts. Count discrepancy increases with cell number, and the criteria for determining dead cells vary more significantly than those for determining live cells, between investigators and Cedex®. (Note: Cell number is the number of cell in each computer-generated image.)

Conclusions:

- There is no statistical difference between the Cedex® and manual counts of viable cell density.
- There is no statistical difference between manual and Cedex® viabilities in N2 and N1 hemacytometers.
- N1 and N2 enumerations are not statistically different; however, the viability values are statistically different from that of FR.
- Manual cell viability counts are less accurate than the Cedex® method since visual identification of dead cells was difficult.

Discussion:

- Cells have different color, contrast, and sizes making it challenging for the human eye to discern them consistently. The Cedex® has been designed to mimic the human capability for cell recognition and counting.
- There is a significant difference between manual counting and Cedex® counting in terms of analysis time. Cedex® can provide and analyze 20 images within 5 minutes, while manual procedures take at least 10 times longer to complete the same amount of work.

Acknowledgements:

The authors wish to thank Innovatis Company (Germany) for the loan of the Cedex® instrument, BD Biosciences (USA) for the donation of cell culture medium, and MIT's UROP Program for support, the National Science Foundation for support to the Biotechnology Process Engineering Center. The authors are also grateful to Delia Fernandez, Daryl St. Laurent, Joythi Joseph, and Helena Chia for their help during the course of this study.