

# Chapter 2

## Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods

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### Abstract

One of the traditional methods of cell viability analysis is the use of trypan blue dye exclusion staining. This technique has been the standard methodology used in academic research laboratories and industrial biotechnology plants. Cells were routinely counted manually with a hemocytometer. In recent years, modern automated instrumentation has been introduced to supplement this traditional technique with the efficiency and reproducibility of computer control, advanced imaging, and automated sample handling.

**Key words:** Cell viability, Cell counting, Trypan blue, Hemocytometer, Automation

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### 1. Introduction

Dye exclusion methods are traditionally used to assess cell viability, with trypan blue being one of the most common. Trypan blue is a vital stain that leaves nonviable cells with a distinctive blue color when observed under a microscope, while viable cells appear unstained. Viable cells have intact cell membranes and hence do not take in dye from the surrounding medium. On the other hand, nonviable cells do not have an intact and functional membrane and hence do take up dye from their surroundings. This results in the ability to easily distinguish between viable and nonviable cells, since the former are unstained, small, and round, while the latter are stained and swollen. The method does not differentiate between apoptotic and necrotic cells.

The traditional method of performing trypan blue cell viability analysis involves manual staining and use of a hemocytometer for counting (1). Recent advances in instrumentation have led to a number of semi- or fully automated systems that can increase

the throughput and accuracy of this technique. The Beckman Coulter Vi-CELL™ XR Cell Viability Analyzer (2) will be described here, but there are numerous other instruments available, including the Nexcelom Bioscience Cellometer Vision® (3), the Invitrogen Countess™ Automated Cell Counter (4), and the Roche Innovatis Cedex (5). In the case of the Vi-CELL™, cell viability can be calculated and reported in percentage, concentration, and cell count, and additional parameters, such as cell diameter and circularity, are also provided. This instrument has been validated against manual cell counting and shown to be highly effective (6).

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## 2. Materials

### 2.1. Manual Method

1. Trypan blue solution: 0.4% prepared in 0.81% sodium chloride and 0.06% dibasic potassium phosphate (see Note 1).
2. Bright-line hemocytometer and cover slip (Hausser Scientific, Horsham, PA) (see Note 2).
3. 70% (v/v) ethanol.
4. Microscope with 100× (10× eyepiece and 10× objective) magnification.
5. Micropipette and tips for 15 µL samples.

### 2.2. Automated Method

1. Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter, Inc., Fullerton, CA).
2. Vi-CELL™ XR Quad Pak Reagent Kit (Beckman Coulter, Inc.; see Note 3).
3. Vi-CELL™ Sample Vials (Beckman Coulter, Inc.).

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## 3. Methods

The traditional cell counting method uses a hemocytometer (originally used for counting blood cells), which is a thick, glass microscope slide with an indented chamber of precise dimensions. This allows a defined volume of cell suspension to be deposited in the chamber, where the cells can be counted.

### 3.1. Manual Method of Sample Preparation

1. Prepare a suspension of approximately  $1 \times 10^6$  cells/mL (see Note 4). Ensure that the sample is thoroughly mixed.
2. Make a 1:1 mixture of the cell suspension and the 0.4% trypan blue solution. The sample can be as small as 10 µL to several mL in volume. Gently mix and let stand for 5 min at room temperature.

3. Prior to use, wash the hemocytometer with 70% (v/v) ethanol and allow to dry.
4. Wash a coverslip with 70% (v/v) ethanol, allow to dry, and place on top of the hemocytometer counting chamber.
5. Apply 15  $\mu\text{L}$  of cell suspension to the edge of the chamber between the cover slip and the V-shaped groove in the chamber. Allow the cell suspension to be drawn into the chamber by capillary action (see Note 5).
6. Let sit for 1–2 min and then count (see Note 6).

### **3.2. Manual Method of Cell Counting**

The counting chamber of a hemocytometer is delineated by grid lines that identify the chamber areas to be used in cell counting. It has a depth of 0.1 mm and the four corner regions are typically used for cell counting. Each of these corner regions is 1 mm  $\times$  1 mm in dimension and is divided into 16 small squares in a four-by-four array (see Fig. 1). The volume of each of these four corners is thus 0.1 mm<sup>3</sup> or  $1 \times 10^{-4}$  mL.

1. Focus on the grid using the 10 $\times$  objective.

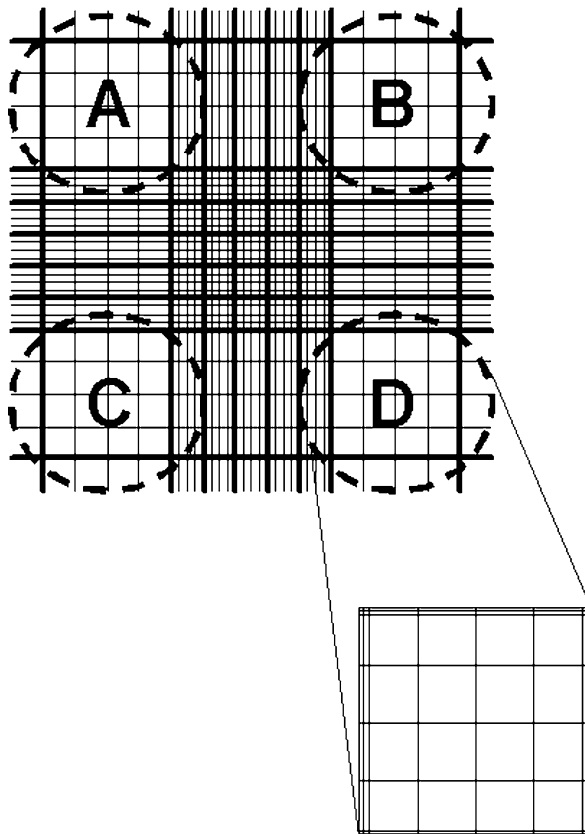


Fig. 1. The rulings on a hemocytometer.

- Count the number of cells – both viable (unstained) and nonviable (stained) in each of the four corner quadrants (A, B, C, D) (see Note 7). Take the average of these four readings and multiply by  $10^4$  to obtain the number of cells per mL in the sample applied to the hemocytometer.
- Multiply by two to take into account the 1:1 dilution of the sample in the trypan blue.
- Multiply by any dilutions in the original sample preparation of the cell suspension.

$$\begin{aligned} & \text{Number of cells (viable or nonviable)} \\ &= \frac{(A + B + C + D)}{4} \times 10^4 \times 2 \times \text{sample dilution} \end{aligned}$$

- The percentage of unstained cells represents the percentage of viable cells in the suspension.

$$\% \text{ Viable cells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}}$$

### 3.3. Automated Method

- Turn on the computer, instrument, and software program in this order.
- Pipette 0.5–2.0 mL of cell suspension(s) into the sample vial(s) (see Note 8). The Vi-CELL™ requires sample cell concentrations between  $5 \times 10^4$  and  $1 \times 10^7$  cells/mL (see Note 9).

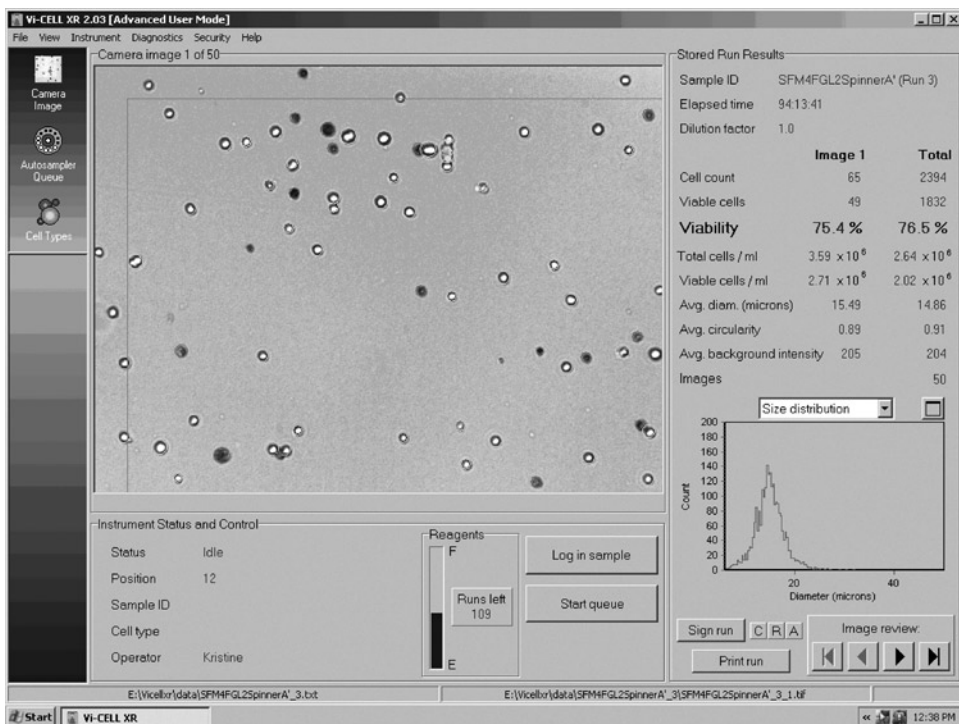


Fig. 2. The main window of the Vi-CELL XR 2.03 software program (Beckman Coulter) showing command icons, an image of the cell counting field, and a report of measured parameters.

3. Place the sample vials into vacant positions on the 12-position carousel.
4. Log in the sample on the software program.
5. Start the analysis by clicking on the “Start queue” icon (see Fig. 2).
6. Results of cell viability will be displayed on the computer screen (see Fig. 2).

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#### 4. Notes

1. Commercial preparations of trypan blue are usually filtered to remove debris. If the preparation is old or is made in the laboratory, it should be filtered through a 0.2- $\mu\text{m}$  filter before use.
2. There are a number of commercial sources of hemocytometers. One of the most common ones in use is the improved Neubauer type used in this protocol.
3. The Vi-CELL™ XR Quad Reagent Pak kit consists of four solutions that are automatically dispensed during analysis: 0.4% trypan blue, buffer, cleaning agent, and 70% isopropyl alcohol disinfectant.
4. Adherent cells must first be treated with trypsin to create a cell suspension. A concentration of  $1 \times 10^6$  cells/mL will result in an average of 50 cells per corner grid of the hemocytometer, which is a reasonable cell count for accuracy and precision. A concentration of  $1 \times 10^5$  cells/mL will be too low to produce an accurate count. This sample should be concentrated by centrifugation before counting. A concentration of  $1 \times 10^7$  cells/mL will be too high to count accurately and should be diluted before counting.
5. Some slight leakage out of the cover glass will ensure that the space under the cover glass is completely filled with cell suspension. Consistent application of the same (15  $\mu\text{L}$  or other) volume will also help to ensure consistent results.
6. Do not leave cells in trypan blue for more than 15 min, in order to prevent cell death due to trypan blue toxicity.
7. If cells lie on the outside grid lines demarking the four corner quadrants, a consistent counting strategy should be adopted. It is common to include cells on the top and left lines and to exclude cells lying on the bottom and right lines.
8. The Vi-CELL™ has been used successfully to count mouse lymphocytes, Chinese Hamster ovary cells, human peripheral blood mononuclear cells, and mouse A9 fibroblast cells.

9. One of the advantages of the Vi-CELL™ is its ability to handle samples of varying concentration. A disadvantage is the requirement for a minimum of 0.5 mL of sample.

## References

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