Counting blood cells with the Countess™ Automated Cell Counter

Introduction

Blood samples are frequently analyzed by laboratories and require standardized cell concentrations for most experiments. The hemocytometer is the most commonly used instrument for determining cell concentrations, and while it offers the researcher the flexibility of making immediate judgments and decisions about the sample analysis, the actual task is tedious, requires careful cleaning and handling of the hemocytometer parts, and is highly subject to inter-operator variation.

The Countess™ Automated Cell Counter introduces a great advance in cell counting. The counter uses the standard trypan blue technique for viability determination; digital image capture and a sophisticated image analysis program determine the cell count as well as the percent viability of the cell population. Automated counting bypasses tedious manual operations and calculations required with the standard hemocytometer while offering generally greater accuracy and precision. The Countess™ Automated Cell Counter also makes it practical to count multiple replicates and to design experiments with large numbers of samples for counting, as operator fatigue no longer compromises counting results.

The Countess™ Automated Cell Counter is capable of counting red and white blood cells from a wide range of collection media and processing techniques. Because the Countess™ instrument uses trypan blue stain, the viability of white blood cells (WBC) is easily determined, while platelets and noncellular debris are unstained and excluded from the count. Red blood cells (RBC) are also easily counted using the Countess™ instrument, although the instrument cannot determine RBC viability.

Overview

Sample preparation

Sample preparation for the Countess™ instrument is similar to that used for a hemocytometer. The instrument will give best results with fresh, homogeneous samples diluted to approximately 10^6 cells/ml in a clear, isotonic, low-protein buffer (e.g., PBS or PBS with 1% BSA):

1. Process the cells according to the protocol appropriate for your experiment (a few processing procedures are described below).
2. Prepare a dilution to an appropriate concentration (~1 x 10^6 cells/ml).
3. Mix an aliquot of cells with an equal volume of 0.4% trypan blue, and transfer 10 µl into "Side A" of a Countess™ chamber slide.
4. Insert the slide into the instrument, Side A first. The instrument reads only one chamber at a time. If Side B is loaded for analysis, remove, rotate, and reinsert the slide Side B first to read it.
5. Use the Zoom function and adjustment knob to focus the image and bring the objects into proper position (Figure 1).

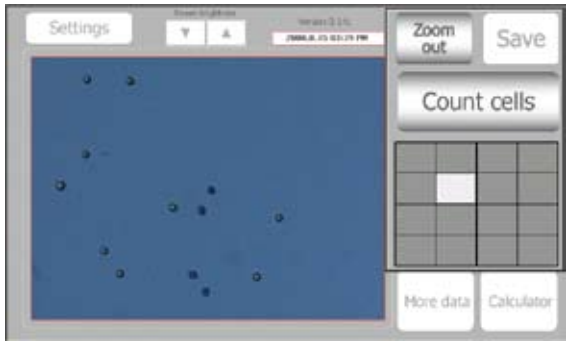

Figure 1—Appearance of live and dead cells in Zoom mode. With the Countess™ instrument in Zoom mode, adjust the image so that live cells have bright centers and well-defined edges, while dead cells have uniform centers and no defined edges.

6. Press "Count cells" to begin the process of acquiring and analyzing the image; the data readout will be given in total cells/ml, live cells/ml, dead cells/ml, and percent viability.
7. Press "Save" to archive the results. Enter a file name using the keypad buttons displayed on the Save menu.

Data analysis

The Countess™ image analysis system identifies objects in the field as cells based on their similarity to the average size of all objects in the image and their degree of circularity. Live cells are then identified as objects with bright centers and dark edges, whereas dead cells are objects that are dark throughout. This image analysis system is similar to that used by the human eye, but with the added benefit that the instrument does not tire as quickly as the human brain.

The acquired image and resulting data may be saved to a USB flash drive. Both the numerical data and the image are saved as a special Countess file and can be further analyzed using the Countess™ software (PC compatible), if desired. The Countess™ software can generate a printed report for archiving in a lab notebook. The image is also saved as a .jpeg file, and the numerical data in .CSV format can be compiled from many counted samples for analysis by any common spreadsheet program.

The Countess™ software provides a means to view how the Countess™ instrument evaluated the sample. This step is helpful in ensuring that the Countess™ instrument detected the desired objects and not debris. Although not required, this step may increase the user's comfort level with this counting technique:

1. Download the Countess™ software from the provided USB flash drive or from www.invitrogen.com/countess, noting the computer requirements and installation instructions.
2. Open the Countess™ software program and load the files to view by pressing "Open", select the location of the stored files, and choose "OK".

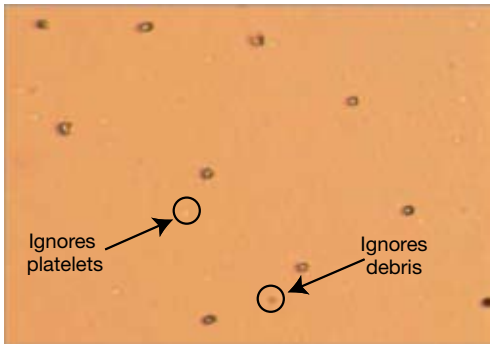

Figure 2—Whole lysed blood sample from a human donor. Sample analyzed on the Countess™ Automated Cell Counter using Countess™ software.

3. Choose the sample file to view, then choose one of the 16 grid squares to see a results view. The blue circles are the objects identified as live, the red circles are the objects identified as dead, and the black circles are objects recognized by the imaging algorithm but excluded from the entire analysis. The object classification may be changed or deleted by using the right mouse button to view the options, then to make a selection.
4. Press "Apply changes" at the bottom of the screen to update the data. Pressing "Save changes" will permanently change and save those edited data.

Cell preparation protocols

Protocol for whole, lysed blood samples

Materials

- 10X ammonium chloride lysis buffer
  - For 0.5 L:
    - NH_4Cl 40.1 g
    - $NaHCO_3$ 4.2 g
    - EDTA, disodium 10 ml
    - Deionized water to 500 ml
  - Dissolve components in 450 ml deionized water
  - Adjust pH to 7.35–7.45
  - Bring to 500 ml
  - Can be stored for 6 months at 2–8°C
- Whole blood sample with appropriate anticoagulant
- PBS
- Distilled or ultrapure water
- 50 ml (or appropriate size) conical centrifuge tubes
- Rocking incubator
- Centrifuge
- 0.4% trypan blue (Cat. no. T10282)
- Countess™ chamber slides (Cat. no. C10228)

Cell preparation

1. Collect blood sample with an appropriate anticoagulant.
2. Prepare fresh 1X ammonium chloride lysis buffer from the 10X stock using distilled water, and warm to 37°C.
3. Mix 1 part whole blood with 9 parts 1X ammonium chloride lysis buffer in a mixing tube (e.g., 5 ml whole blood with 45 ml 1X ammonium chloride lysis buffer).
4. Cap and incubate at room temperature for 20 min, with rocking.
5. Centrifuge for 5 min at 300 x *g* (or 1,500 rpm) at room temperature. Discard supernatant.
6. Resuspend cell pellet in 2 ml PBS; repeat centrifugation step #5.
7. Resuspend with 2 ml or an appropriate volume of PBS.

Counting

8. Mix 10 µl cells with 10 µl 0.4% trypan blue, and load 10 µl into a Countess™ chamber slide.
9. Insert the slide into the instrument loaded-side first. The instrument reads only one chamber at a time. If Side B is loaded for analysis, then the slide must be removed, rotated, and reinserted Side B first in order to be read.
10. Use the Zoom function and adjustment knob to focus the image and bring the objects into proper position (Figure 2).
11. Press "Count cells" to begin the process of acquiring and analyzing the image; the data readout will be given in total cells/ml, live cells/ml, dead cells/ml, and percent viability. If the total concentration is outside the optimal range, perform a 1:2 dilution from the stock using PBS and recount beginning at step #8.
12. Insert a USB flash drive into the instrument. Press "Save" to archive the results. Enter a file name using the keypad buttons displayed on the Save menu.
13. If desired, use the Countess™ software to ensure that the desired objects are included in the measurement.

Protocol for peripheral blood mononuclear cells

Procedure for use with BD Vacutainer® CPT™ tube

Materials

- BD Vacutainer® CPT™ tube
  - With sodium citrate (BD Cat. no. 362760)
  - With sodium heparin (BD Cat. no. 362753)
- PBS
- 15 ml conical centrifuge tube
- Centrifuge
- 0.4% trypan blue (Cat. no. T10282)
- Countess™ chamber slides (Cat. no. C10228)

Cell preparation

1. Collect blood in BD CPT™ tube; store at room temperature and use within 2 hr.
2. Centrifuge tube at 300 x *g* (or 1,500 rpm) for 30 min at room temperature.
3. Carefully transfer the whitish layer just under the plasma layer and above the gel layer to a 15 ml conical centrifuge tube.
4. Wash sample with PBS.
5. Centrifuge the cells at 300 x *g* (or 1,500 rpm) for 5 min. Discard supernatant.
6. Resuspend the pellet in 2 ml or an appropriate volume of PBS, and proceed with counting as described below.

Cell counting

7. Mix 10 µl cells with 10 µl 0.4% trypan blue, and load 10 µl into a Countess™ chamber slide.
8. Insert the slide into the instrument loaded-side first. The instrument reads only one chamber at a time. If Side B is loaded for analysis, then the slide must be removed, rotated, and reinserted Side B first in order to be read.
9. Use the Zoom function and adjustment knob to focus the image and bring the objects into proper position (Figure 3).
10. Press "Count cells" to begin the process of acquiring and analyzing the image; the data readout will be given in total cells/ml, live cells/ml, dead cells/ml, and percent viability. If the total concentration is outside the optimal range, perform a 1:2 dilution from the stock using PBS and recount beginning at step #7.
11. Insert a USB flash drive into the instrument. Press "Save" to archive the results. Enter a file name using the keypad buttons displayed on the Save menu.
12. If desired, use the Countess™ software to ensure that the desired objects are included in the measurement.

Procedure for use with Ficoll-Paque® gradient

Materials

- Ficoll-Paque® PLUS (GE Healthcare Cat. no. 17-1440-02))
  or
  Accuspin™ System Histopaque® 1077 (Sigma Cat. no. A0561)
- Blood sample with appropriate anticoagulant
- 10 ml centrifuge tubes
- Syringe and needle to remove Ficoll-Paque® PLUS from bottle
- PBS or HBSS or any balanced saline solution
- Centrifuge
- 0.4% trypan blue (Cat. no. T10282)
- Countess™ chamber slides (Cat. no. C10228)

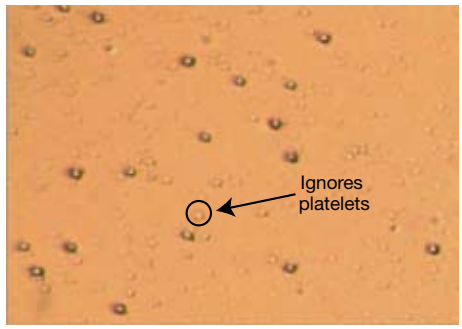

Figure 3—Peripheral blood mononuclear cells (PBMCs) prepared by gradient centrifugation with Ficoll®-Hypaque®. The sample was analyzed on the Countess™ Automated Cell Counter. The Countess™ software is able to distinguish platelets and debris from white blood cells.

Cell preparation

1. Collect whole blood sample with an appropriate anticoagulant.
2. Add an equal volume of balanced salt solution to anticoagulated whole blood and mix well.
3. Invert Ficoll-Paque® PLUS bottle several times to ensure thorough mixing.
4. Using aseptic technique with the syringe and needle, transfer 3 ml Ficoll-Paque® PLUS to a 10 ml centrifuge tube.
5. Carefully layer 4 ml diluted whole blood from step 2 onto the top of the Ficoll-Paque® PLUS, taking care to not allow the blood and Ficoll-Paque® PLUS to mix.
6. Centrifuge at 400 x *g* (1,700 rpm) for 30 min at room temperature.
7. Aspirate and discard the upper plasma layer; transfer the mononuclear layer to a clean centrifuge tube.
8. Wash with balanced salt solution; centrifuge at 300 x *g* (1,500 rpm). Discard the supernatant.
9. Resuspend the pellet in 2 ml balanced salt solution, and proceed with counting as described below.

Cell counting

10. Mix 10 µl cells with 10 µl 0.4% trypan blue, and load 10 µl into a Countess™ chamber slide.
11. Insert the slide into the instrument loaded-side first. The instrument reads only one chamber at a time. If Side B is loaded for analysis, then the slide must be removed, rotated, and reinserted Side B first in order to be read.
12. Use the Zoom function and adjustment knob to focus the image and bring the objects into proper position (Figure 1).
13. Press "Count cells" to begin the process of acquiring and analyzing the image; the data readout will be given in total cells/ml, live cells/ml, dead cells/ml, and percent viability. If the total concentration is outside the optimal range, perform a 1:2 dilution from the stock using PBS and recount beginning at step #10.
14. Insert a USB flash drive into the instrument. Press "Save" to archive the results. Enter a file name using the keypad buttons displayed on the Save menu.
15. If desired, use the Countess™ software to ensure that the desired objects are included in the measurement.

Figure 4—Red blood cells read on the Countess™ Automated Cell Counter are counted as beads, and will not give a viability reading.

Protocol for red blood cells

Red blood cells (RBCs) are large enough objects for the Countess™ Automated Cell Counter to identify. While an undiluted sample of whole blood is too concentrated for the instrument to work properly, blood diluted ~1:10,000 may fall within the optimal object concentration range.

For best results counting isolated RBCs, set the instrument to "Bead mode". The algorithim for object and viability determination does not recognize intracellular objects, so differentiating infected vs. uninfected RBCs is not possible.

Instrument setup

1. Press "Settings" on the main screen.
2. Choose "Bead mode".
3. Close the screen by pressing "Close"; the "Count cells" button will now read " Count beads".
4. Proceed with counting as described below.

Counting

5. Mix RBCs with a balanced salt solution or other appropriate diluent (1:1000–1:10,000).
6. Mix 10 µl cells with 10 µl 0.4% trypan blue, and load 10 µl into a Countess™ chamber slide.
7. Insert the slide into the instrument loaded-side first. The instrument reads only one chamber at a time. If Side B is loaded for analysis, then the slide must be removed, rotated, and reinserted Side B first in order to be read.
8. Use the Zoom function and adjustment knob to focus the image and bring the objects into proper position (Figure 4).
9. Press "Count beads" to begin the process of acquiring and analyzing the image; the data readout will be given in total beads/ml. If the concentration is outside of the optimal range, prepare another dilution and return to step #5.
10. Insert a USB flash drive into the instrument.
11. Press "Save" to archive the results. Enter a file name using the keypad buttons displayed on the Save menu.
12. If desired, use the Countess™ software to ensure that the desired objects are included in the measurement.