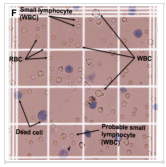

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Cell counting with a haemocytometer (with mouse bone marrow example)

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1 *Works for me* dx.doi.org/10.17504/protocols.io.btqgnmtw

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ABSTRACT

Cell counting is a critical step to determine the number of live leukocytes (white blood cells, WBC) and erythrocytes (red blood cells, RBC) per femur, and is accurately performed using a microscope and haemocytometer. This step allows enables the distribution of a specific number of cells per well in the staining plate. Some researchers prefer to perform an erythrocyte (or red blood cell, RBC) lysis prior to counting, as some researchers are concerned about misidentifying erythrocytes as leukocytes during counting. This RBC lysis removes all erythrocytes prior to counting, resulting in easier counting of leukocytes. However, we have found that differentiating leukocytes from erythrocytes is straight forward at a higher magnification based on size (erythrocytes are smallest), colour (erythrocytes are red in colour), and birefringence (leukocytes exhibit birefringence, erythrocytes do not). Additionally, RBC lysis prior to cell staining will have a small impact on CD115 staining levels (see protocol notes). To circumvent the need for RBC lysis prior to staining, we count the number of leukocytes (and erythrocytes) in the centre square only, at a high magnification. Alternatively, an automated cell counter can be used to speed up counting. However, care must be taken to ensure that any automated cell counter is accurately performing counts, as most automated cell counters are calibrated to human blood cells.

EXTERNAL LINK

https://link.springer.com/protocol/10.1007/978-1-4939-9454-0_12

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Analysis of the murine bone marrow hematopoietic system using mass and flow cytometry. 2019. TM Ashhurst, DA Cox, AL Smith, NJC King. Mass Cytometry, 159-192.

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MATERIALS TEXT

- Sample tubes
- Phosphate buffered saline (PBS)
- FACS buffer (PBS, 5 mM EDTA, 5% FCS)
- 0.4% Trypan blue solution for cell counting
- Cell suspension
- Pipette and tips
- 96-well plate (or tubes)
- Haemocytometer
- Microscope

SAFETY WARNINGS

Precautions need to be taken when handling potentially infectious samples, including operation within a biosafety cabinet class II.

DISCLAIMER:

> This protocol utilised a modification of the traditional method for counting cells on a haemocytometer.

BEFORE STARTING

Our protocol begins after isolating cells. Please ensure adequate ethics approval has been obtained.

In the protocol below, we describe the counting of mouse bone marrow cells extracted from the femur, resuspended in 1 mL of FACS buffer. The same can be applied for cells isolated from other tissue, with modifications to the suspension concentration. See the associated guidelines for recommendations.

Isolate cells

1 Keep cell suspension on ice on in a refrigerator where possible.

> Info
> It is established that some cellular markers, such as surface expression of M-CSFR (CD115), are downregulated when cells are removed from their biological niche. Storing cells at 4°C or on ice prevents this downregulation.

Prepare cell suspension 1m

2 Mix the cell suspension by inverting the tube numerous times, add 10 µL of the cell suspension to 40 µL of FACS buffer (1/5 dilution), then add 10 µL of this solution to 10 µL of 0.4% trypan blue (1/2 dilution factor), resulting in a final dilution factor of 1/50.

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Counting cells 4m

3 Take 10 µL of this mixture and load onto the haemocytometer.

4 Place haemocytometer under the microscope and find the counting grid.

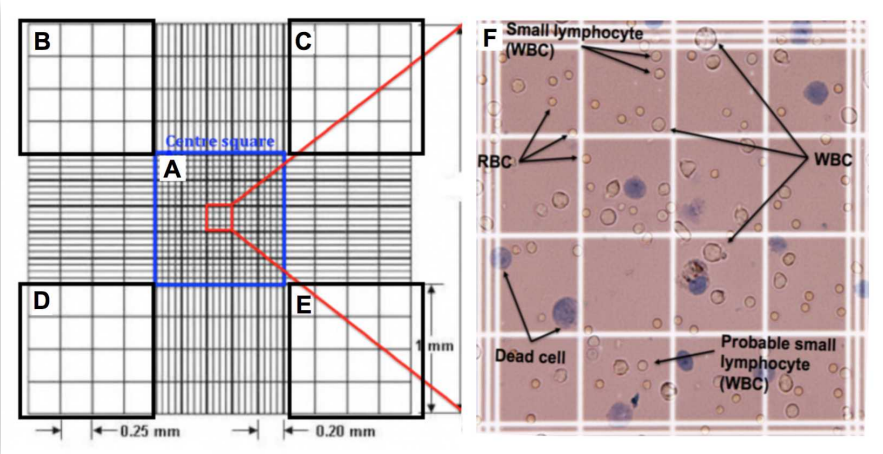

Figure 6

4.1 Count the total number of LIVE (clear) and DEAD (blue) leukocytes in the large centre square ("A", blue in Figure 6A) which consists of 5x5 smaller squares (edges marked by triple lines in panel F), each of which contains 4x4 smaller squares (edges marked by single lines in panel F).

4.2 Count the total number of erythrocytes in the same area, if desired.

4.3 If fewer than 100 total live leukocytes are counted in the centre square, then count the cells in the other large squares progressively, making sure each square is completely counted (B, C, D, E) until at least 100 live leukocytes have been counted in total.

If too few cells are present with this 1/50 dilution, then this process can be repeated at a higher concentration.

Calculate concentration 1m

5 Calculate cells/sample using the following formula:

$$\frac{Cells}{sample} = \frac{Live\ cells\ counted}{Large\ squares\ counted}\ x\ 10^4\ x\ dil.factor\ x\ vol.(mL)\ \text{of sample}$$

Calculation

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6 **Determine required cell numbers and distribute into plate**

- Calculate volume containing the required cell numbers: $\mathbf{V_{req} = (C_{req} / C_{sample}) \times V_{sample}}$
- **Vol** (required, mL) = (**Cells** required / **Cells** in sample, total) x **Vol** (sample total, mL)

Example: if we want $3x10^6$ WBCs from a bone marrow sample with $1.4x10^7$ WBCs in 1 mL of FACS buffer

- $V_{req} = (C_{req} / C_{sample}) \times V_{sample}$
- $V_{req} = (3x10^6 \text{cells} / 1.4x10^7 \text{cells}) \times 1 \text{ mL}$
- $V_{req} = 0.214$ mL = 214 µL (i.*e. 214 µL of sample contains* $3x10^6$ *WBCs)*

7 Using a pipette, transfer the necessary volume for a desired number of cells into relevant wells of a U- or V-bottom 96 well plate. For flow cytometry: typically stain 1 × 10^6 cells per sample.

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