

Mouse Pathology Core

Manual Differential Cell Counting for Blood and Bone Marrow

Training in manual differential cell counting can be obtained by contacting the Core.

Published recommendations are noted below (Kogan, et al. Blood, 2002, 100:238-245, Supplemental Materials)

Immature forms/blasts

We propose that the immature cells of non-lymphoid neoplasms be referred to as “immature forms/blasts” and that these cells be enumerated according to the following guidelines:

Size: medium to large

Cytoplasm: basophilic, with or without azurophilic granules

Nuclear to Cytoplasmic Ratio: high

Nuclei: predominantly round to oval, but modest peripheral indentation or a small central opening can be present

Chromatin: fine to moderately fine

Nucleoli: small but often present; can be indistinct or absent

Position of Nucleus: whereas lymphoblasts and erythroblasts typically have a central nucleus, myeloblasts often have a nucleus that is more peripherally located.

Notes:

1. Some of the immature cells present in leukemias can have rather condensed chromatin or be somewhat small. If these cells otherwise have features of immature forms/blasts they should be included in the differential count as such.
2. This proposal for terminology and definition of the immature cells of murine non-lymphoid hematopoietic neoplasms should be seen as one step in an ongoing process aimed at identifying features of disease that reflect underlying cellular biology and predict pathogenic behavior. Additional information may result in revisions to this recommendation.
3. “Young forms” has been used as a synonym for “immature forms/blasts.”

Mature Forms, Neutrophilic

Cells with pale blue or neutrophilic cytoplasm,

With ring form nuclei

With diameter of center of ring >50% of diameter of nucleus, or fully developed segmentation

With coarsely distributed chromatin

[Note, indented rather than ring form nuclei can be seen in murine neutrophils in some circumstances. When such cells are present they should be counted as “Mature Forms, Neutrophilic” when the nuclear indentation exceeds >50% of the diameter of the nucleus]

Mature Forms, Monocytic

Largest leukocytes

With round, oval or bean-shaped nucleus

Often with nongranular slightly basophilic cytoplasm, with or without vacuoles.

Forms that cannot be distinguished from Young Forms should be counted as Young Forms.

Some forms cannot be distinguished morphologically from large lymphocytes

Intermediate Forms (including neutrophilic and monocytic cells)

All other cells of the neutrophilic or monocytic series, that is, cells showing partial maturation of cytoplasm and nucleus in various combinations.

Eosinophilic Cells

Lymphocytes

Nucleated Red Blood Cells

Other Cells

Includes megakaryocytes, plasma cells, reticuloendothelial cells, mast cells, stromal cells, endothelial cells, and unidentified cells. In some settings, it is desirable to separately enumerate sub-types of Other Cells.

Note regarding ring-shaped nuclei. In contrast to humans, normal blood cells of mice can have ring-shaped nuclei. Ring forms are not limited to neutrophilic and eosinophilic granulocytes. Ring-shaped nuclei can also be seen in monocytic lineage cells and in cells that are relatively undifferentiated (Biermann H, et al. Murine leukocytes with ring-shaped nuclei include granulocytes, monocytes, and their precursors. J of Leuk Biol. 1999;65:217-231).

Reticulocyte Count for Mouse (Manual)

Stain Solution

- 0.5% New Methylene Blue-N (Sigma)
- 1.4% Potassium Oxalate
- 0.8% NaCl

Filter after making, and before each use; particles develop over time and if not filtered they can make for an awful stain.

Staining method

1. Start with 10 λ of stain in an Eppendorf tube
2. Add 10 λ of whole blood
3. Gently mix. Incubate at room temp for 20 minutes.
4. Make smear using 2.5 λ of blood/stain mix, a little more than for a standard stain. Be sure to pull it all the way to the end. Typically easiest to read near feathered edge. Read in same general location across mice.

Counting method

1. Count at least 500 cells to determine the percentage of cells that are retics.
2. Must have a CBC to get the Hct value.
3. Corrected Retic count = Retic count (%) x Hct / 45

J. Kang 9/28/05. Ref: Socolovsky et al Blood 98:3261-73,2001.

Provided by Jing Kang, Laboratory of Dr. Andrew Leavitt.