Interpretation of Graphical Reports from the Advia 2120, Sysmex XT-2000iV and Cell-Dyn 3500

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The 2 hour presentation shall show various cases in which the hematology instrument graphics are important in the diagnosis of a disease or in detection of laboratory errors. It is important to evaluate scatterplots for poor separation between cell populations and to understand the instrument alarms. The illustrations are from normal and sick animals and will help participants understand graphic from instruments they may not have. The order of examples in this document will not follow the lecturers.

Advia 2120

Figure 1 Composite of common Advia dot plots and histograms from a normal dog.
Advia 2120 identifies leukocyte subpopulations by size (y-axis) and peroxidase staining (x-axis). Equine WBC are identified well on Advia. In canine samples the monocyte cluster (green ring in Fig 2a) often extends into the lymphocyte area (dark blue dots) and even light blue "LUC -large unstained cells". Canine LUC are usually monocytes, immature lymphocytes, basophils or blast cells (Fig 2a-c). Perform a manual differential count with samples with increased number of LUC. Canine eosinophils vary in characteristics and are may be undetected by Advia. Canine and feline basophils are not detected by Advia.

Figure 2a-b. a) Advia Peroxidase dot plots with canine blood. Red dots are neutrophils, orange are eosinophils, green are monocytes and blue are classified as "lymphocytes". Note in this case the base of the lymphocyte cluster has no clear separation from the black dots which indicate debris, NRBC or large platelets. b) Neutrophils are larger and had less peroxidase staining and are partly misclassified as monocytes. c) Canine sample with acute lymphoblast leukemia (large peroxidase negative cells starting in the lymphocyte area).

Figure 3a-c. a) Equine Advia Baso dot plot is a long oval cluster. Red dots which are granulocytes and blue dots which are mainly mononuclear cells. b) Advia Baso dot plot from a rabbit with 13% basophils (orange dots in baso box). c) Acute lymphoblast leukemia in a dog. Some immature lymphocytes were misclassified as basophils by Advia.
Figure 4. Advia RBC graphics from a dog with IMHA. See Fig 1 for how normal sized RBC appear in the various graphics. Green ovals show the macrocytic hypochromic immature RBC. Black ovals show autoagglutination.

Figure 5. Dog with severe neutropenia and relatively many NRBC which are in the "granulocyte" area of the Baso dot plot and under and among lymphocytes in the Perox dot plot (green circles). Large active monocytes are seen in the LUC area.
Figure 6a-b  a) Normal Advia canine platelet dot plot. Platelets (blue dots) are identified by size (larger is somewhat up along the y axis) and optical density (more dense is somewhat to the right along the x axis). Platelets up to 30 fl are spread through the dot plot. Platelets 30-60 fl in size are along the top of the dot plot. b) Advia Platelet scatter dot plot from a dog with IMHA and RBC ghost cells forming a separate long upright oval, misclassified as platelets.

Figure 7. Advia Platelet histograms from a canine sample with RBC ghost cells. The black ovals show where the RBC ghost cells appear.
Sysmex XT-2000iV

Figure 8. Sysmex graphics from a normal canine blood.

Sysmex uses a fluorescence flow cytometry method with red semiconductor laser for a 4 part differential leukocyte count (neutrophils, lymphocytes, monocytes and eosinophils). Leukocytes are stained with polymethine stain, a fluorescent dye that is assumed to bind to nucleic acids and cytoplasmic organelles, such as endoplasmic reticula. Side fluorescence light (SFC) and laser side scatter light (SSC) show separate cell clusters for the differential count. Monocytes and lymphoid cells with high content of RNA in the cytoplasm are high up on the Y-axis (fluorescence side light). Cells with high complexity, for example eosinophils, are more to the right (laser side scatter). An adaptive cluster analysis system is used for identification of the different cell populations. All or parts of the differential count are not released (grey) when the instrument is not able to identify separate cell populations.
Equine leukocyte clusters are usually well separated and the differential leukocyte count works well except in occasional cases with left shift. Canine and feline neutrophils are located just beneath lymphocytes. This is problematic when immature or toxic neutrophils with higher RNA content move upwards and merge with the lymphocytes (Fig 9 a, b). Monocytes with high content of RNA in the cytoplasm show high fluorescence intensity. Sysmex also classify some cells with high nucleic acid content, above and beside monocytes, as lymphocytes (Fig 9c). Monocytes do not show up as a well gathered cell population, so especially in canine samples the border to lymphocytes often are indistinct. Eosinophils are well separated and identified in dogs, horses and most cats. Basophils were not identified by Sysmex in canine and feline samples. In most cases when the instrument did not release a complete differential count there were good reasons for that and a manual count was needed (Fig 9a). However it is important to evaluate the scatterplots, because some few samples are released with severe errors (Fig 9b)

Figure 9a-e a) Sysmex Diff dot plot from a cat in which the neutrophil and lymphocytes counts were not separated so not reported (appear as grey clusters). b) A feline sample with incorrect identification of lymphocytes and neutrophils. c) A canine sample with "wrapping" of large RNA rich lymphocytes (pink) around the edge of the monocyte cluster (green).

With Sysmex it is possible to manually reanalyze samples as shown in figure 9d and e. Sysmex Diff dot plot from the same dog in which the neutrophil (blue) and eosinophil counts (orange) were manual reanalyzed to obtain results.
Figure 10. a) Canine Sysmex RET dot plot with normal reticulocytosis. b) Canine Sysmex RET dot plot with iron deficiency anemia showing microcytic reticulocytes (purple and orange dots lower along the y-axis) merge with large platelets (blue green dots at bottom) indicating inadequate separation and incorrect cell identification by the instrument.

Figure 11. a) Normal Sysmex PLT-O dot plot. b) Very large platelets of a Cavalier King Charles spaniel were reanalyzed to allow determination of how many of the dog's platelets were normal sized (blue dots) or large (yellow dots).

Sysmex uses both impedance and optical platelet count for erythrocyte and thrombocyte counts. PLT-I is used for PCT and the calculation of MPV. For the optical platelet counts, cells were stained with a fluorescent stain (polymethine), which stains nucleic acids. As with other impedance instruments Sysmex impedance platelet count is not correct if large platelets are present. The optical platelet count works well for many species. Sysmex optical platelet count include more large platelets and agree better with manual feline platelet counts than Sysmex's impedance platelet counts. PLT-O count was considerably higher than PLT-I for samples from cats (about 60% higher). All feline samples had platelet distribution alarm and no results were presented for MPV and PCT. Platelets aggregation in feline samples will however still cause erroneously low results independent of which method that are used.
Cell-Dyn 3500

The Cell-Dyn analyzer identifies leukocyte subpopulations in unstained cell preparation using optical measurements of laser light scatter at 4 different angles at 0°, 10°, 90° and 90° depolarized light scatter. Multidimensional analysis of the light scatter identifies the cells as neutrophils, lymphocytes, monocytes and eosinophils. It is very important to evaluate and adjust the settings in a veterinary lab. Many Abbott representatives do not know how to optimize the settings for samples from animals.

Fresh equine leukocyte subpopulations are in most cases well separated and the differential leukocyte count works generally well. This is also true for fresh canine and feline samples without pronounced pathological changes. Ruminants, rats and rabbits with many small lymphocytes often have an error with loss of lymphocytes and poor lymph/mono separation. In all cases with NRBC, RRBC and KWOC-flag the WIC-count should be used together with a manual differential count. Canine eosinophils are often false low, because in some dogs they are similar to neutrophils in 90° depolarized light scatter (Fig 12d). It is important to set the gain for 90° depolarized to maximum and minimize the “baso-box” for canine and feline samples. Cell-Dyn does not identify canine or feline basophils.

Figure 12. a) Cell-Dyn WBC dot plot (0°/10°) of a normal dog. Orange dots are neutrophils, green = eosinophils, purple = monocytes and blue = lymphocytes. b) Cell-Dyn WBC dot plot from a cat with 4+ toxic neutrophils which move upward. The orange neutrophils have moved to the top of the plot. c) Canine acute lymphoblast leukemia. d) Problems with eosinophils (90°depl/90°) in a canine case. e) Canine basophils show up besides neutrophils (arrow).
Cell-Dyn uses only impedance methods for both erythrocyte and platelet enumeration. Impedance RBC works quite well for most species in Cell-Dyn. However, small erythrocytes seen in goats, sheep, calves and animals with iron deficiency anemia may cause poor separation between erythrocytes and platelets. False low values for feline platelet are very common. The histogram has to be scrutinized to judge if platelet count could be trusted, this is important for all species. If no MPV is released this could be an indication of problems with the platelet count. MPV results from CD are not accurate.

Figure 13. a) Cell-Dyn Erythrocyte histogram from a dog with normal erythrocytes. b) Histogram from a dog with a regenerative anemia with macrocytic hypochromic erythrocytes. c) Histogram from a dog with iron deficiency anemia with microcytic hypochromic erythrocytes.

References


