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Erythrogram

The erythrogram or erythron includes all tests that evaluate RBC, including the following:

- **Assessment of red blood cell numbers:** Hematocrit (**HCT**) or packed cell volume (PCV), **hemoglobin** concentration, **RBC count**. The cell counts (hematocrit or packed cell volume, red blood cell count and hemoglobin) are usually interpreted similarly, although in human medicine, hemoglobin measurement as the preferred measurement of red blood cell mass.
- **RBC indices:** Mean cell/corpuscular volume (**MCV**), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (**MCHC**), RBC distribution width (**RDW**). These are interpreted along with the changes in RBC number. If the patient is anemic, these indices can be useful guides as to the cause of the anemia.
- **Regeneration:** Reticulocyte counts (**percentage** and **absolute**). These are only routinely performed in dogs and cats. Regeneration is **assessed** in other species by serially quantifying associated RBC changes during blood smear.

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examination (number of macrocytes in horses, camelids and ruminants), degree of **polychromasia** (camelids, ruminants).

- **RBC morphologic features:** These can give clues as to underlying disease pathogenesis or can identify the cause of the anemia, including parasites.

HCT/PCV

Although used synonymously, these terms actually represent different ways of measuring the proportion of blood composed of red blood cells. Both are expressed as % of the blood (SI units are L/L).

Hematocrit (HCT)

The hematocrit is actually a calculated value obtained from modern automated hematology analyzers. It is the product of the mean cell volume (MCV) and the red blood cell (RBC) count, both of which are directly measured by the analyzer (see below). Therefore, if there are any inaccuracies in measurement of the MCV or RBC count, the HCT will reflect those inaccuracies.

$$HCT (\%) = (MCV \times RBC) \div 10$$

Packed cell volume (PCV)

PCV is a directly measured value obtained from centrifuging blood in a microhematocrit tube in a microhematocrit centrifuge. The PCV is measured as the height of the red cell column in a microhematocrit tube after centrifugation. It is the quickest and most readily available measure of the red cell component of blood. Unlike the HCT, this measurement is affected by plasma trapping and how the red blood cells pack within the column. Red blood cell packing is species-dependent – it takes longer for ruminant RBCs to pack compared to dogs, cats and horses. Therefore, the microhematocrit tubes are spun for 10 minutes in ruminants versus 3 minutes in other species.

- Examination of the “crit tube” can also provide subjective information about the color and clarity of the plasma (icterus, hemolysis, lipemia), and the size of the “buffy coat” (which contains WBC and platelets).
- Additionally, one can score and break the tube as desired to remove the plasma for refractometric protein estimation to extrude the buffy coat for smear-making. The “buffy c

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preparation of nucleated cells, which can be useful if looking for low-incidence cell-types of potential significance (e.g., mast cells).

Hemoglobin

Hemoglobin concentration (Hgb) is reported as grams of hemoglobin per deciliter of blood (g/dL) and is measured after lysing the red blood cells *in vitro*. Since red blood cells are approximately 33% hemoglobin, the hemoglobin concentration of whole blood normally is about one third of the HCT (i.e., the mean corpuscular hemoglobin concentration or MCHC is 33%). This is species dependent, because camelids have more hemoglobin in their red blood cells (MCHC of 45%).

RBC count

The red blood cell count on the routine CBC is the concentration of red cells, expressed in millions/ μ L of whole blood. While RBC counts can be performed by manual techniques, these are time-consuming and inaccurate. Automated counts are most commonly performed using “impedance” or electronic counters, which we use for performing cell counts in body fluids. RBCs can also be counted by laser light scattering (flow cytometry). This is the technique used by the hematology analyzer at Cornell University.

MCV

The mean cell volume (MCV) indicates the volume of the “average” red blood cell in a sample. It is expressed in femtoliters (fL; 10^{-15} liters). Traditionally, MCV was a calculated parameter, based on the hematocrit or PCV and the RBC count derived by using the following formula:

$$MCV \text{ (fL)} = (HCT/PCV \div RBC) \times 10$$

Present-day automated hematology analyzers provide a more accurate, **direct** measure of MCV, based on the actual volume of the cell as it passes through a laser (newer laser-based hematologic analyzers) or an electronic beam (“impedance” methods). The amount of laser light scattered in a forward direction or the amplitude of pulses created in the electronic field as the cells through the detector is equivalent to the cell volume, which is averaged based on the number of cells analyzed by the instrument. With impedance-based methods, instruments also

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“channelize” the scatter or impulses, segregating them into channels representing relative ranges of cell size. With laser-based methods with the ADVIA series of analyzers, ®, a proprietary algorithm based on Mie light scattering properties can segregate the RBCs into different categories based on MCV (macrocytic, normocytic, microcytic) and hemoglobin concentration (hypochromic, normochromic, hyperchromic), although this has been optimized for human red blood cells and not the various RBCs we see in different animal species.

MCH

The MCH or mean cell hemoglobin represents the absolute amount of hemoglobin in the average red blood cell in a sample. Its units are picograms (pg) per cell. The MCH is calculated from the hemoglobin concentration (Hgb) and the RBC count using the following equation:

$$MCH (pg) = (Hgb \times 10) \div RBC$$

This value usually tracks with the mean cell volume as it is dependent on the volume of red blood cells and is generally a less useful measure than MCV or MCHC, which we use to characterize an anemia (e.g. microcytic hypochromic). For example, a low MCH could be due to smaller than normal cells with normal Hb concentration or normal sized cells with lower than normal Hb concentration. It is better to know the values for cell volume (MCV) and Hb concentration (Hgb) directly. Laser-based hematology analyzers also provide results for the hemoglobin content within intact red blood cells, i.e. this is measured directly by high angle light scatter of the laser versus being a calculated value. This is called the corpuscular hemoglobin or CH and is usually equivalent to the MCH unless there is something interfering with the measurement of hemoglobin by the analyzer, e.g. lipemia. Lipemia falsely increases the hemoglobin concentration, resulting in false increases in MCH and MCHC (see below). The optically measured CH and CHCM are more accurate in this setting. When the MCH and MCHC are inaccurate, such as with lipemia, the CH and CHCM will be reported instead of the MCH and MCHC, otherwise these optically measured values are not reported on a routine hemogram. For more information on this method of measurement, refer to the [hemoglobin](#) or [MCHC](#) page.

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MCHC

MCHC is the mean cell hemoglobin concentration, expressed in g/dL. It can be calculated from the hemoglobin concentration (Hgb) and the HCT or PCV using the following formula:

$$MCHC \text{ (g/dL)} = (Hgb \div PCV/HCT) \times 100$$

The normal value for MCHC is about 33%. Red cell populations with values below the reference interval can be termed “hypochromic”. This can occur in a strongly regenerative anemia, where an increased population of reticulocytes with low Hb content “pull” the average value down (an increased MCV would be expected under this scenario). Low MCHC can also occur in iron deficiency anemia, where microcytic, hypochromic red cells are produced as a result of the lack of iron to support hemoglobin synthesis. Values for MCHC significantly *above* the reference interval are not physiologically possible due to limitations on the solubility of Hb. Sample-related problems of analysis, however, can result in spurious high values. Lipemia or other causes of turbidity in the lysate can cause falsely high hemoglobin concentrations, which raises the apparent MCHC. Under these settings, we report out the mean corpuscular hemoglobin concentration (CHCM) measured optically by our machine instead. Red blood cells from animals of the *Camillidae* family (camels, llamas, alpacas), however, truly do have higher MCHC (around 40-45 fl) compared to those of common domestic animals. This is possible due to higher solubility of the Hb molecule in these species.

RDW

The red cell distribution width (RDW) is an index of the variation in red blood cell volume within the red cell population. It is a parameter provided by automated hematology analyzers and is the electronic equivalent of anisocytosis or variation in red blood cell size that is judged by smear examination (although the latter shows diameter of flattened cells versus volume of cells in solution as detected within a hematologic analyzer). Mathematically, it is the coefficient of variation of the RBC volume, i.e.,

$$RDW \text{ (\%)} = (Standard \ deviation \div mean) \text{ cell volume} \times 100$$

A high RDW indicates that the red blood cells are more variable in volume than normal. This may be due to the presence of smaller or larger red blood cells or a combination of either

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scenario. For example, increased numbers of immature red blood cells during a regenerative response to an anemia will increase the RDW, because immature anucleate red blood cells are larger than normal. Conversely, the presence of increased numbers of smaller cells (e.g. in iron deficiency anemia) will also increase the RDW. The cause for a high RDW may be revealed by examination of a blood smear to identify the presence of small or large red blood cells. Note that low numbers of smaller or larger red blood cells may increase the RDW before an increase or decrease in mean cell volume (MCV) can be seen on the hemogram. An RDW within reference intervals provides little information on variation in red blood cell size. An RDW below the reference interval is not a clinically relevant finding.

Reticulocytes

Reticulocytes are young, anucleate erythrocytes, which contain more RNA than mature RBC (these contain very little RNA). It is important to identify these immature RBC, because their presence in blood in sufficient numbers indicates the bone marrow is responding to an anemia and indicates the anemia is not due to defective bone marrow production, but due to loss (hemorrhage) or destruction of RBC (hemolysis). These immature RBC can be quantified by detecting their RNA which can be precipitated by supravital dyes (e.g. new methylene blue) or stained with DNA/RNA dyes, e.g. thiazole orange. The reticulocytes are quantified as a percentage of total RBC (reticulocyte percentage) or as an absolute reticulocyte count (product of the percentage and RBC count). If they contain a lot of RNA, the blue of the RNA can offset the red of hemoglobin in these cells, giving them a purple tinge, when stained with routine hematologic stains (e.g. Wright's stain or Diff-quick). They are then called polychromatophils and the degree of polychromasia can be used as a quick estimate of the bone marrow's response to an anemia in most species. Quantification of reticulocytes (% , absolute) is only done in dogs and cats. More information on reticulocytes can be found under [assessment of regeneration](#).

nRBC

Nucleated RBCs are also immature RBC that can be seen in blood in various situations, including bone marrow injury, a regenerative response or in some healthy animals. nRBCs are counted (regardless of technique, i.e. manual or automated) as WBC, because we are counting nuclei and not cells. For this reason, the

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obtained “WBC” count (from an analyzer or a hemocytometer) is actually a nucleated count which includes WBC and nRBC. The obtained nucleated count must be corrected for the number of nRBC in the circulation. To correct the “WBC” or nucleated count, the number of nRBCs per 100 leukocytes is recorded during the differential leukocyte count when performing a blood smear examination. Then a correction is made as follows:

$$\text{Corrected WBC (thou/}\mu\text{L)} = \text{obtained nucleated cell count} \times (100 \div [nRBC + 100])$$

RBC morphology

Red blood cells are assessed for morphologic changes and presence of erythroparasites, which are semi quantified. These changes can yield clues on underlying diseases or be diagnostic in themselves and are listed below. Skill and experience are needed to correctly identify significant poikilocytosis and distinguish it from artifactual changes caused by poor sample handling and/or smear-making technique.

- **Shape (poikilocytes):** Acanthocytes, echinocytes, eccentrocytes, keratocytes, schistocytes, spherocytes, ovalocytes, target cells, stomatocytes. Semi-quantified as few, moderate or many. Some of these changes are more important than others, e.g acanthocytes and schistocytes indicate fragmentation injury, whereas keratocytes and eccentrocytes indicate oxidant injury (along with Heinz bodies). Large numbers of spherocytes are usually seen in immune-mediated hemolytic anemia. In contrast, echinocytes are frequently artifacts.
- **Size:** Macrocytes, microcytes. Semi-quantified as few, moderate or many. Anisocytosis (variation in size) is semi-quantified as mild, moderate or marked.
- **Color:** Hypochromasia (too little hemoglobin), polychromasia (immature cells containing RNA or reticulocytes). Semi-quantified as mild, moderate or marked. Ghost cells can also be semi-quantified (few, moderate, many).
- **Inclusions:** Siderocytes (iron), basophilic stippling (RNA), Howell-Jolly bodies (retained nuclei), Heinz bodies (oxidized hemoglobin). Semi-quantified as few, moderate or many.
- **Patterns:** Agglutination (noted as present or absent), rouleaux formation. Rouleaux formation is semi-quantified

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as mild, moderate or marked.

- **Infectious agents:** e.g. *Babesia*, *Anaplasma*. Semi-quantified as few, moderate or many.