FUNdamentals of Hematology:
What, When and Why?

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Introduction

Hematology is the study of numbers and morphology of blood cells and encompasses some of the most important and commonly performed tests in veterinary diagnostics.[1,2] The complete blood count (CBC) is the main diagnostic test for hematology and defines the number of red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) in a sample. The CBC also provides information about some specific physical parameters of the RBC population.[2]

RBCs, WBCs and PLTs each perform vital physiological functions, so determining their numbers and physical characteristics can provide important health information.[1]

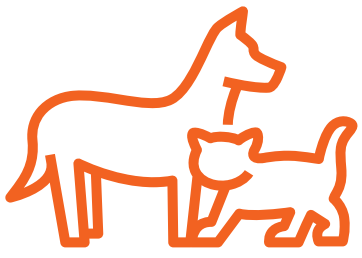
Hematology testing consists of 2 components:
quantitative and qualitative evaluation of the blood.[2]

The quantitative evaluation of the blood includes a CBC, which is performed on an automated analyzer or through manual techniques; however, it is typically and preferably performed on an automated analyzer.[2-4] The CBC is a diagnostic tool that classifies, enumerates and differentiates the different types of cells present in the peripheral blood.[2] This quantitative evaluation of the blood provides different cell population counts and their associated indices, as well as graphic representations when performed on an automated analyzer.[2] Typical CBC parameters provided in a report are shown in **Table 1** below.

Table 1. CBC Parameters

RBC Parameters[2]	WBC Parameters[1]	PLT Parameters[2,5]
RBC Count	WBC Count	PLT Count
Mean Cell Volume (MCV)	Lymphocyte (LYM) Count and %	Platelet Crit (PCT)
Hematocrit (HCT)	Monocyte (MON) Count and %	Mean Platelet Volume (MPV)
Hemoglobin (Hgb)	Granulocyte Count and %[a]	
Mean Corpuscular Hemoglobin Concentration (MCHC)	Neutrophil (NEU) Count and %[b]	
RBC Distribution Width (RDW)	Eosinophil (EOS) Count and %[b]	
Reticulocytes*	Basophil (BAS) Count and %[b]	

*Not reported on all automated analyzers.
[a]Reported on a 3-part WBC differential.
[b]Reported on a 5-part WBC differential.



- Evaluating RBC parameters serves to detect the presence of anemia and erythrocytosis, and in some situations, evidence of an etiology may be detected[1,5]
- Evaluating WBC parameters serves to assess whether there is evidence of stress, excitement, inflammation/infection, hypersensitivity, or neoplasia[1,5]
- Evaluating the PLT parameters serves to determine if the PLT count is lower than normal (thrombocytopenia) or higher than normal (thrombocytosis). This is often a sign of an underlying medical condition, and it is sometimes a side effect from medication, giving the clinician an indication that additional tests to diagnose the cause may be necessary[1,5]

A CBC can be complemented with the manual measurement of the packed RBC volume, which is a comparable result to the HCT calculated from the automated CBC results.[2] Ideally, a CBC should be part of a minimum database, along with a serum/plasma chemistry analysis and a complete urinalysis, so that results can be interpreted together to deliver a comprehensive laboratory diagnosis.[5] See **Figure 1** for an example of an automated CBC report.

The other component of a hematological assessment is the qualitative evaluation of the blood, also known as a blood smear or blood film evaluation.[2] Blood smears are made using ethylenediamine tetraacetic acid (EDTA)-anticoagulated blood and stained using Romanowsky-type stains, following the manufacturer's protocol.[2,3] The stained blood smear is examined for morphological characteristics of the blood cells.[2]

A blood smear should be examined with every CBC[2]

The blood smear is performed to confirm the WBC differential count, provide an estimated PLT count, and to look for changes in cellular morphology of RBCs, WBCs and PLTs. Microscopic evaluation of a well-prepared blood smear is a vital diagnostic procedure to not only verify the automated analyzer's results, but also to identify very important diagnostic information that automated analyzers cannot evaluate.

Figure 1. Automated CBC Report

zoetis

Clinic Name : Zoetis Demo Clinic
Requesting Doctor : N/A

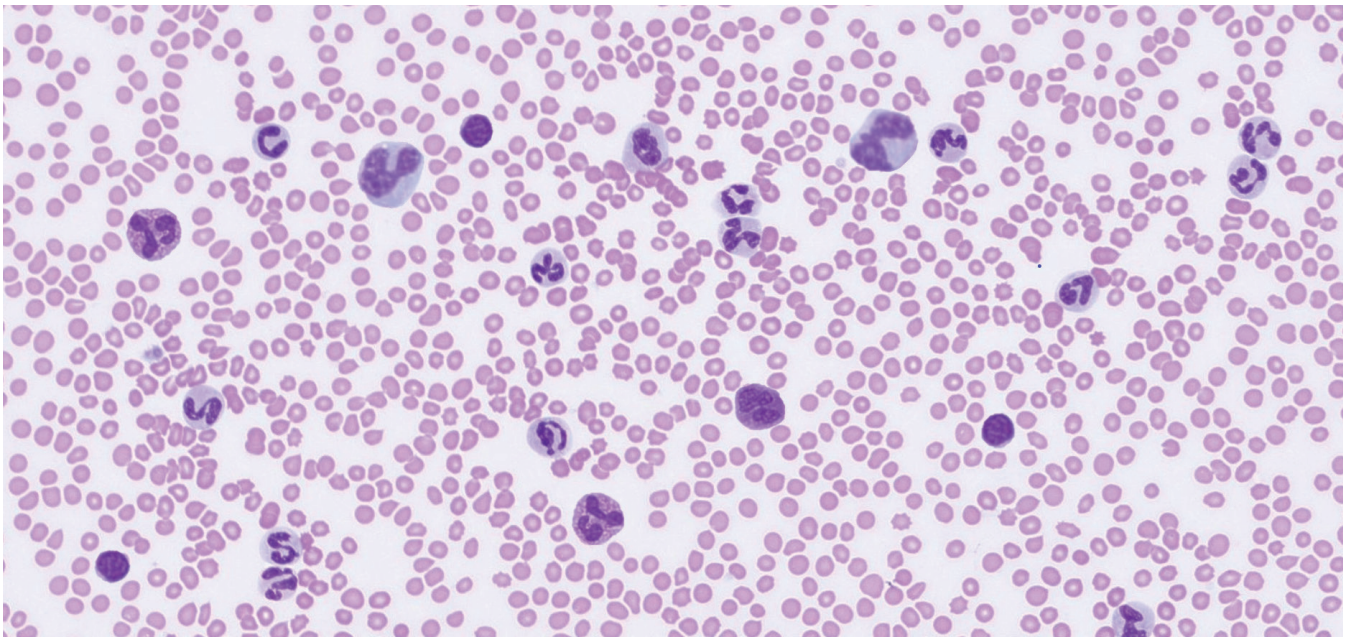
Owner Name : Matt White
Patient Name : Zelda
Patient ID : 1000675

Species : N/A
Breed : Domestic Long Hair

Hematology

Test	Ref Range	Units	Graph	04 NOV 2021 05:35 PM	07 DEC 2021 12:15 PM	24 JAN 2022 11:28 AM	23 FEB 2022 10:10 AM
WBC	6 - 17	10^9/l		6.77	7.72	11.79	8.25
LYM	1 - 4.8	10^9/l		4.61	2.52	3.93	2.75
MON	0.2 - 1.5	10^9/l		0.88	0.37	0.72	1.27
NEU	3 - 12	10^9/l		3.77	3.98	11.02	8.16
EOS	0 - 0.8	10^9/l		0.15	0.80	0.23	0.60
BAS	0 - 0.4	10^9/l		0.27	0.09	0.14	0.02
LYM%	0 - 100	%		89.0	51.3	80.8	15.8
MON%	0 - 100	%		34.9	83.9	91.9	100.0
NEU%	0 - 100	%		6.4	61.3	7.0	20.4
EOS%	0 - 100	%		2.0	29.6	94.9	89.0
BAS%	0 - 100	%		45.8	63.8	52.6	12.5
RBC	5.5 - 8.5	10^9/l		5.69	7.07	5.76	8.49
HGB	12 - 18	g/dl		13.4	15.0	13.2	12.3
HCT	37 - 55	%		54.47	54.51	48.94	52.67
MCV	60 - 77	fl		75	65	75	61
MCH	19.5 - 24.5	pg		23.8	23.4	21.2	19.5
MCHC	31 - 39	g/dl		33.1	35.2	31.5	38.4
RDWc	14 - 20	%		17.2	18.6	14.1	17.6
RDWs		fl		0.0	0.0	0.0	0.0
PLT	200 - 500	10^9/l		428	467	219	249
MPV	3.9 - 11.1	fl		7.6	5.9	5.6	6.7
PCT		%		0.00	0.00	0.00	0.00
PDWc		%		0.0	0.0	0.0	0.0
PDWs		fl		0.0	0.0	0.0	0.0

Figure 2. Example of a Blood Smear



High-resolution image from VETSCAN IMAGYST™.

When to Perform a CBC

As stated, a CBC is an important and powerful diagnostic tool, as well as a component of a minimum database for each patient. It is a routine blood test used in all stages of health and illness. It can, and should, be utilized as part of[5]:

- Preventative health screenings
- Identifying the presence and cause of a patient's condition, in conjunction with other tests, to formulate a list of differential diagnoses
- Monitoring responsiveness to therapy
- Gauging the severity of an illness

To gain the full benefit of the CBC, it must be used in conjunction with a detailed history and physical examination, as well as with additional components of the minimum database, including a chemistry panel and complete urinalysis.[5]

Automated WBC Differential

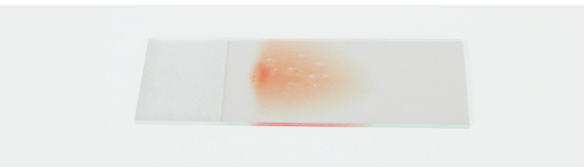
A CBC also includes a differential WBC count, which is a breakdown of the amount of each subpopulation of the WBCs present in the total WBC population. Since each WBC component has a very specific function, the differential count may be used to identify abnormal levels of specific WBC subpopulations and may offer diagnostic information about specific underlying health conditions.[6]

Automated analyzers are generally classified as either 3-part or 5-part differential analyzers:

- The 3-part differential analyzer classifies the cells into 3 groups: a small WBC group (lymphocytes), a medium-size WBC group (monocytes, eosinophils and basophils), and a large WBC group (neutrophils)[7]
- The 5-part differential provides a more complete differential panel and classifies the WBCs into 5 groups: neutrophils, lymphocytes, monocytes, eosinophils and basophils[6]

The ability of 5-part differential analyzers to enumerate the less abundant cell types, namely monocytes, eosinophils and basophils, separately rather than as a mixed cell population, is a significant enhancement. In cases of ill patients, a 5-part differential is more informative in helping identify the cause of illness. In this case, a 3-part differential is not ideal, and accuracy is negatively affected with pathological samples.[6]

The blood smear is a crucial part of every automated CBC, but the advantage of 5-part over 3-part systems is the potential of reducing the manual differential count review and directing the reviewer to look for specific pathologies by assessing cellular morphology as the qualitative evaluation of the blood.[2,6,8]



Why Perform a Blood Smear Evaluation?

A blood smear evaluation is an essential step in an overall patient health assessment, as it provides the qualitative evaluation of the blood cells that cannot always be detected with an automated cell count.[2,9]

A blood smear evaluation complements both point-of-care and reference laboratory automated hematology counts and should be performed regularly with every CBC. It is especially integral for animals who are sick and those with hematologic abnormalities.[2] A blood smear enables the veterinarian to confirm results, assure quality, and may provide additional insights to guide diagnosis and treatment.[9-12] It provides the ability to confirm automated CBC results by verifying RBC, WBC and PLT counts.[4] In addition, a blood smear evaluation detects cell morphology that is not reported by automated CBC analyzers.[2]

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Ideally, a blood smear evaluation should **always** be done as part of every CBC, but it is **vital** that it be performed in the following clinical instances:[2]

- Anemia (low RBC count)
- Thrombocytopenia (low PLT count)
- Neutrophilia or neutropenia (verify count and examine cells)
- Lymphocytosis
- Severe illness like sepsis
- Suspicion of parasites
- When certain warning flags are present on the automated CBC report

In 2 surveys at Michigan State University, the frequency (%) of anemia was shown to be 23% to 29% and 10% to 20% in dogs and cats, respectively. In the same surveys, 5% to 15% of dogs and 7% to 60% of cats were thrombocytopenic. These are examples of cases in which a blood smear should always be performed to confirm anemia and/or low PLT counts. In addition, for anemic patients, cell morphology can be as equally important for patient diagnosis as confirming the number and presence of reticulocytes.[10]

Alternatively, a blood smear evaluation **should not** be utilized as a replacement for a CBC, as automated analyzers are more precise and accurate than manual counting of cells, if properly maintained.[4] Some examples of the morphological changes that can be identified on a blood smear evaluation are shown in **Table 2**.[2-4]

Table 2. Examples of Morphological Changes

RBCs	WBCs	PLTs
Polychromasia	Left Shift (increased neutrophil band cells)	Macroplatelets
Anisocytosis	Toxic Changes	
Spherocytes	Reactive Lymphocytes	
Heinz Bodies	Blast Cells	PLT Clumping
Fragmented RBCs		
Nucleated RBCs	Mast Cells	
RBC Parasites		

Blood smears inform clinical decisions[2]

The practice of a blood smear evaluation is routinely performed in reference laboratories to supplement high-quality, "gold standard" automated CBC analyzers. In individual veterinary practices, "gold standard" CBC analyzers are impractical due to cost, maintenance and space, making the blood smear evaluation even more critical in these situations. Failure to perform a blood smear evaluation can result in potential errors in clinical decisions.

In some instances, blood smears are not performed in veterinary practices due to challenges with interpretation and availability of expert evaluation. Blood smear evaluation success is dependent on a number of different components, which include:[3]

- Quality of the blood smear preparation
- Stain maintenance
- Ability to evaluate correct areas of a blood smear
- Ability to differentiate artifacts from morphologic abnormalities
- User experience with interpreting blood smears

Evaluation of blood smears will continue to play a critical role in confirming the presence of abnormal cell populations that the automated CBC analysis identified as suspect and flagged for the operator's attention. When there is a lack of confidence in dealing with these challenges, blood smear analysis should be referred to an expert.[3]

With utilization of the VETSCAN IMAGYST™ AI Blood Smear, challenges regarding workflow, time and interpretation can be alleviated for the veterinary practitioner.

References: 1. Kahn CM, Line S, Aiello SE. Diagnostic procedures for the private practice laboratory. In: Kahn CM, Line S, Aiello SE, eds. *The Merck Veterinary Manual.* 10th ed. Merck & Co., Inc.; 2010:1487-1492. **2.** Villiers E. Introduction to haematology. In: Villiers E, Ristic J, eds. *BSAVA Manual of Canine and Feline Clinical Pathology*. 3rd ed. British Small Animal Veterinary Association; 2016:27-37. **3.** Weiser G. Laboratory technology for veterinary medicine. In: Thrall MA, Weiser G, Allison RW, Campbell TW, eds. *Veterinary Hematology and Clinical Chemistry*. 2nd ed. John Wiley & Sons, Inc.; 2012:3-33. **4.** Harvey JW. Hematology procedures. In: Harvey JW, ed. *Veterinary Hematology: A Diagnostic Guide and Color Atlas*. Elsevier Inc. 2012:11-32. **5.** Barger AM. The complete blood cell count: a powerful diagnostic tool. *Vet Clin North Am Small Anim Pract*. 2003;33(6):1207-1222. doi:10.1016/s0195-5616(03)00100-1. **6.** Münster M. Overview of the benefits of switching from a 3-part differential to a 5-part differential haematology analyser. March 2012. Accessed January 5, 2022. https://www.sysmex-europe.com/fileadmin/media/f100/SEED/Sysmex_SEED_Switching_from_a_3-part_differential_to_a_5-part_differential_haematology_analyser.pdf. **7.** Bürgi W, Marti HR. Automated blood count analysis by trimodal size distribution of leukocytes with the SYSMEX E-5000. *J Clin Chem Clin Biochem*. 1989;27(6):365-368. doi:10.1515/cclm.1989.27.6.365. **8.** Houwen B. The differential cell count. *Laboratory Hematology*. Carden Jennings Publishing Co., Ltd. 2001;7:89-100. **9.** Zabolotzky SM, Walker DB. Peripheral blood smears. In: Cowell R, Valenciano Amy, eds. *Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat*. 5th ed. Elsevier Inc.; 2020:438-467. **10.** Weiss DJ, Tvedten H. The complete blood count, bone marrow examination, and blood banking: general comments and selected techniques. In: Willard MD, Tvedten H, eds. *Small Animal Clinical Diagnosis by Laboratory Methods*. 5th ed. Elsevier Inc.; 2012:12-37. **11.** Stirn M, Moritz A, Bauer N. Rate of manual leukocyte differentials in dog, cat and horse blood samples using ADVIA 120 cytograms. *BMC Vet Res*. 2014;10:125. doi:10.1186/1746-6148-10-125. **12.** Sharkey L, Heinrich D. In-clinic hematology: the blood film review. *Today's Veterinary Practice.* Published 2015. Accessed January 5, 2022. https://todaysveterinarypractice.com/in-clinic-hematology-the-blood-film-review/.

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