Analysis of Blood with a Light Microscope

by Dr. Robert Berdan

April 27th, 2021

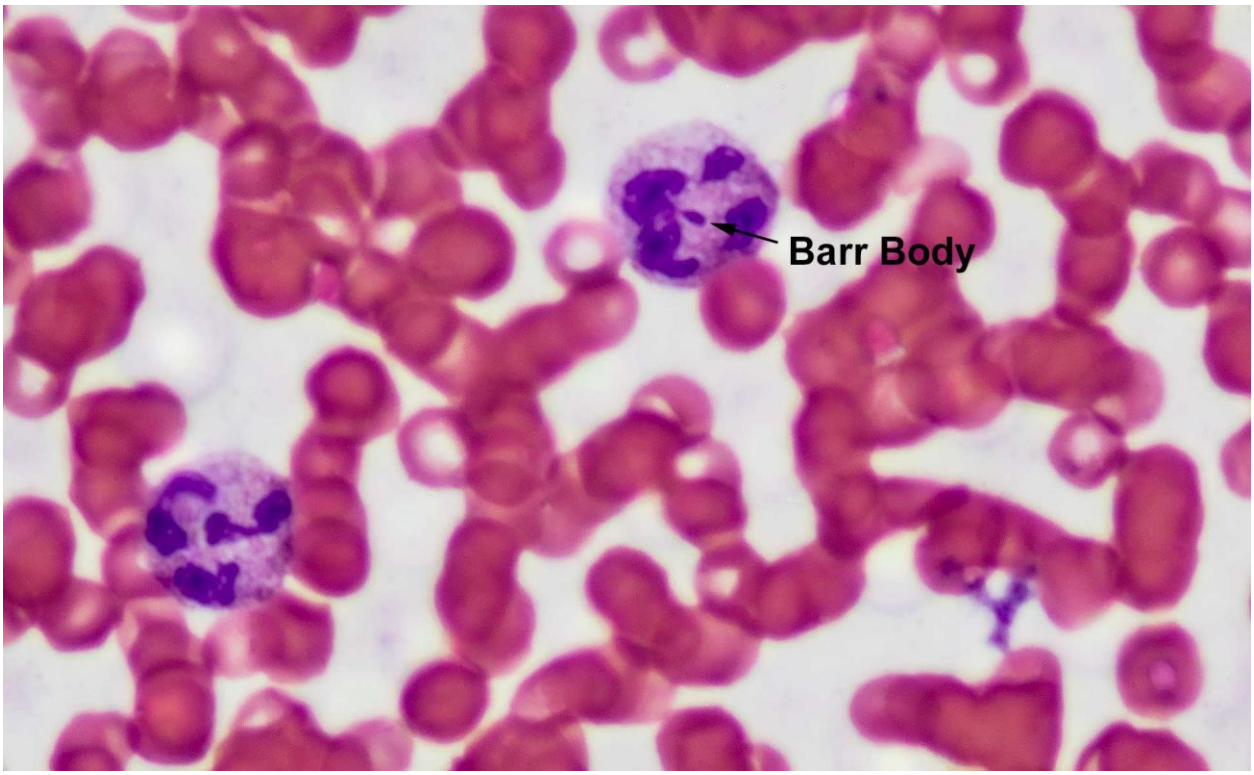

Fig. 1. Above is a picture of a human blood smear consisting of two white blood cells surrounded by red blood cells and a few platelets after staining with Wright's stain. 1000X oil immersion objective bright field microscopy. The arrow points to a Barr body which is a small drumstick found attached to the nucleus that represents an "extra" inactivated X chromosome indicating this blood sample is from a female.

Introduction

Blood analysis is an important diagnostic tool and **can** be even be used to determine the sex of an individual. One theory suggests that the reason we may be attracted to the colour red more than any other is because it might alert us to injury i.e. we are bleeding. When we cut ourselves we bleed and if the cut is small it usually clots within minutes due to blood platelets. The most numerous blood cells are red blood cells (erythrocytes) that carry oxygen from our lungs to the various tissues in our body. Plasma is a clear-yellow fluid that also contains several different types of white blood cells and each type of cell plays a specific role in our immune defense. Blood plasma also contains nutrients, amino acids, fatty acids, sugars and hormones and may contain pathogens.

If you are unwell your doctor may send you to get your blood tested if it is suspected that you might have an infection, disease or other ailment. What the blood is tested for depends on what you doctor suspects might be wrong. Testing could include chemical analysis, or analysis of the different blood cell types or both. In Canada laboratory personnel that do the testing require several years of training. If your doctor suspects cancer or some other ailment the blood samples will also be inspected by a histopathologist whose training requires a bachelor's degree, a degree from Medical school and 3-7 years internship and residency program. I mention this because there is another group that examines live blood with a light microscope and their training program lasts only a few weeks. This Live blood analysis group looks at your blood using dark field microscopy. This group cannot diagnose illnesses but will show you your live blood with a microscope and suggest changes in your diet or adding supplements to improve your health. They should not and cannot diagnose illness – see Live Blood Analysis (Wikipedia). For the most part analysis of blood is quantitative, the cells are stained (.e.g. Wright's stain) and the number of platelets, and different types of white blood cells are counted manually or automatically with a flow cytometer. In addition the shape and size of red blood cells are analyzed. In some instances bacteria, fungi and parasites can be detected in blood smears. Proper blood analysis involves a team of trained experts, though anyone with a light microscope can examine blood of humans or animals.

Blood analysis for both humans and animals is usually conducted by staining the cells and observing them by bright field microscopy. Other microscopy techniques like phase contrast; differential inference contrast (DIC), dark field microscopy, and immunofluorescence can also be used. In this introductory article I show different white blood cells examined by a variety of microscopy techniques.

Staining Blood

Bright field microscopy is used to view blood cells after making a blood smear as shown in Figure 2 and after staining with Wright's stain (there are other stains that can be used as well). Place a small blood spot on one side of a glass microscope slide after pricking a finger, use a second slide to contact the blood spot which spreads across the top slide edge by capillary action. Then pull the top slide at about a 30 degree angle to form a thin blood smear. Allow the blood to dry for a few minutes and then stain the cells. For staining I put the slides in Petri dishes and flood the surface of the blood smear with Wrights stain for 2- 5 minutes, then add buffer solution for 2-5 minutes, rinse the slide in buffer and dry the slide in air. I cover the slide with Permount™ (Fisher Chemical) and a 50 mm long coverslip and view the slide using bright field microscopy. It takes about 15 minutes to make a prepared blood smear. With practice good slides provide an even distribution of blood. I may heat the slide for a few moments on a hot plate set on low so the Permount™ spreads and the coverslip dries flat. To see details in the white blood cells and identify (platelets) also called thrombocytes use a 40X, 60X or 100X objectives. The 100X objective with oil immersion fluid is required to get clear views of the white cells and see details in the nucleus.

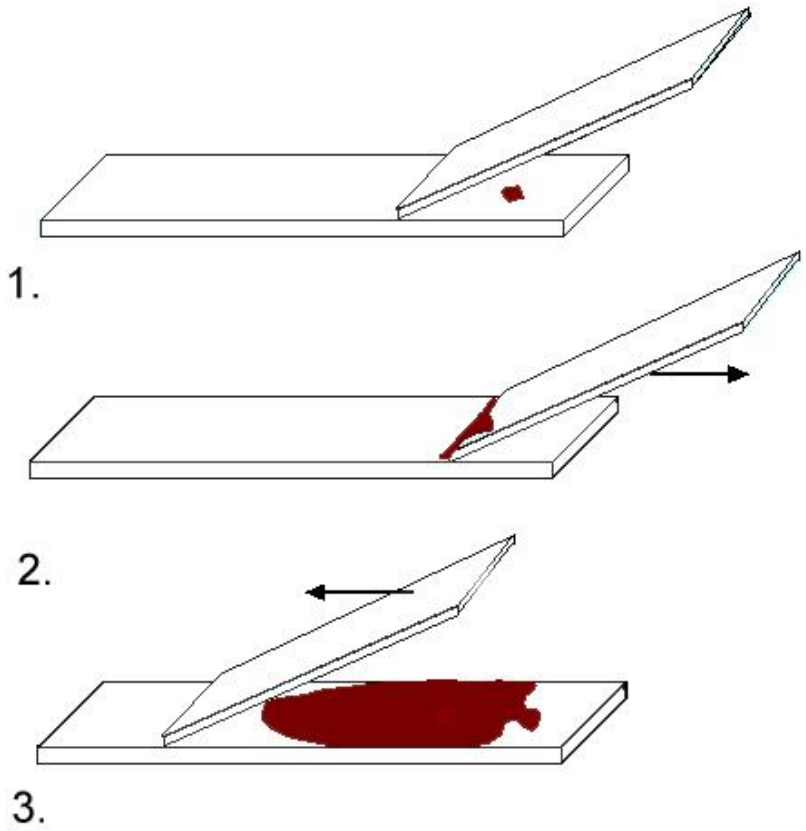

Fig. 2. Above shows the steps in how to make a blood smear. 1. Put a drop of blood on a microscope slide 2) Pull another slide to the right so it touches the blood drop and the blood moves by capillary action along the edge of the slide then 3) move the slide at approximately at a 30 degree angle away from the drop of blood to make a thin smear, dry the slide and stain the blood cells. Wrights stain from Benzmicroscope.com.

The red blood cells are about 7-10 microns in diameter; they are biconcave disk shaped and have no nuclei. You might see some immature red blood cells with some dark blue staining spots that represent ribosomes and messenger RNA. The shapes of the red blood cells may change slightly depending on the external solution, or whether the person has a disease e.g. sickle cell anemia. Red blood cells may stack in what is called a Rouleaux formation which can often occur at the edge of the blood smear, or it can be due to infection, multiple myeloma, inflammatory conditions, pH changes, diabetes and cancers. Fibrinogen may interact with sialic acid on the surface of the red blood cells to facilitate the formation of rouleaux (Wikipedia). Generally examine the slide in the central regions not at the edges. Also beware that viruses can not generally be detected by an ordinary light microscope, unless viruses are stained with fluorescent antibodies after which they may appear as bright coloured dots.

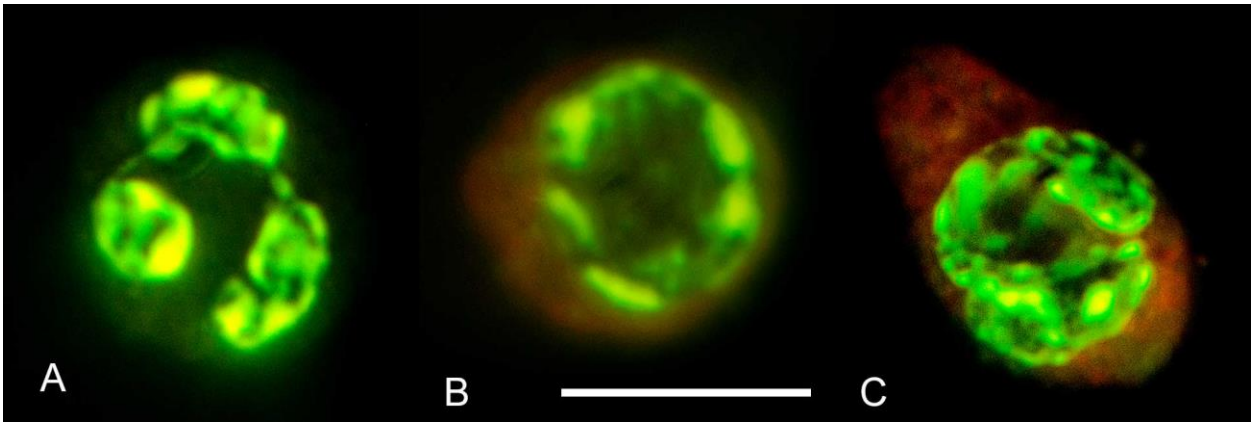

Fig. 3 Human white blood cells after staining with Acridine orange and viewed by fluorescence microscopy. A) Neutrophil B) Lymphocyte c) Eosinophil? Acridine orange stains DNA green, and mRNA red. Scale bar = 10 microns (0.010 mm). 1000X

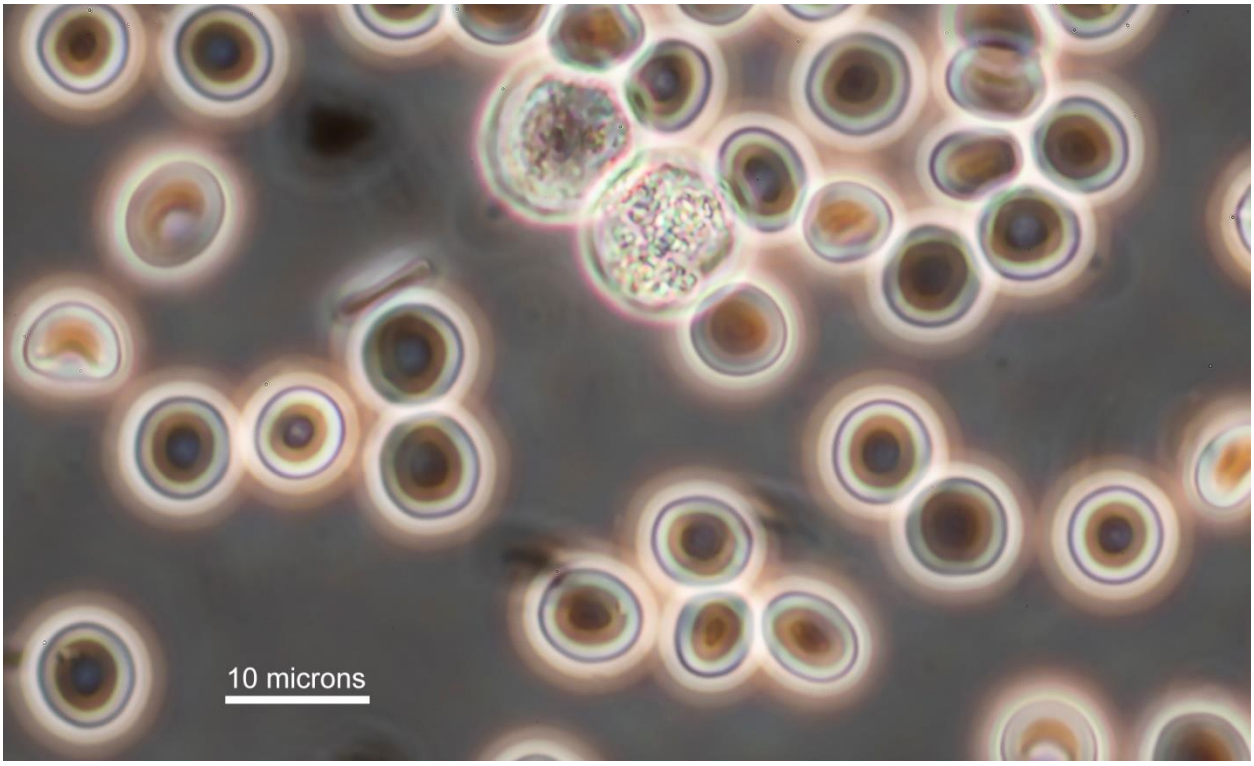

Fig. 4 Live blood sample viewed by Phase contrast microscopy 1000X. The majority of the cells in this picture are red blood cells. Near the top of the picture are two white blood cells next to each other, but without staining they can't be definitively identified. With phase contrast the biconcave shape of the red cells creates the appearance of concentric rings in the red blood cells (erythrocytes). Phase contrast microscopy is useful for detecting some bacteria or other pathogens.

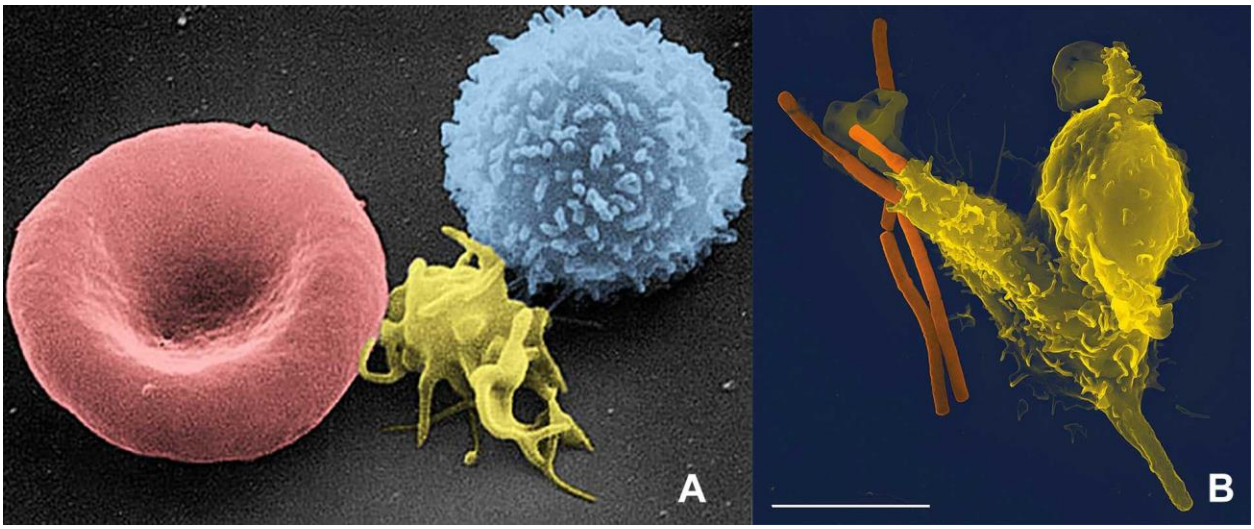

Fig. 5 A scanning electron microscope (SEM) image of a normal red blood cell (left), a platelet (middle), and (right) a white blood cell courtesy National Cancer Institute https://en.wikipedia.org/wiki/Blood#/media/File:Red_White_Blood_cells.jpg **B** Neutrophil engulfing bacteria *Bacillus anthracis*. Photo by Volker Brinkmann - (November 2005) *PLoS Pathogens* **1** (3): Cover page. DOI:10.1371. Scale bar = 5 microns. Images are false coloured as SEM photos are generally black and white.

White Blood cells

In a Wright's stained blood smear white blood cells have distinct shaped nuclei and can be easily distinguished. White blood cells play important roles in the body's immune response. Changes in the number of specific white blood cells can indicate an allergy, or disease. Individuals infected with COVID-19 virus often have decreased numbers of Lymphocytes (T, B, NK cells) and the overall white cell counts are elevated, especially the neutrophils (Han, H. et. al. 2020). COVID-19 patients also exhibited elevated monocytes and coagulation abnormalities which can cause blockage of blood vessels (L. Thomas, 2021 and M. Kubankova et al. 2021).

The diagnosis of any kind of disease should include a clinical history, physical examination and laboratory testing. The presence of bacteria in blood (septicemia) or parasites is serious and demands immediate attention by a doctor. Also see "The study of blood and its contents" a web site with more pictures of blood cells and various ailments (J. Sprute).

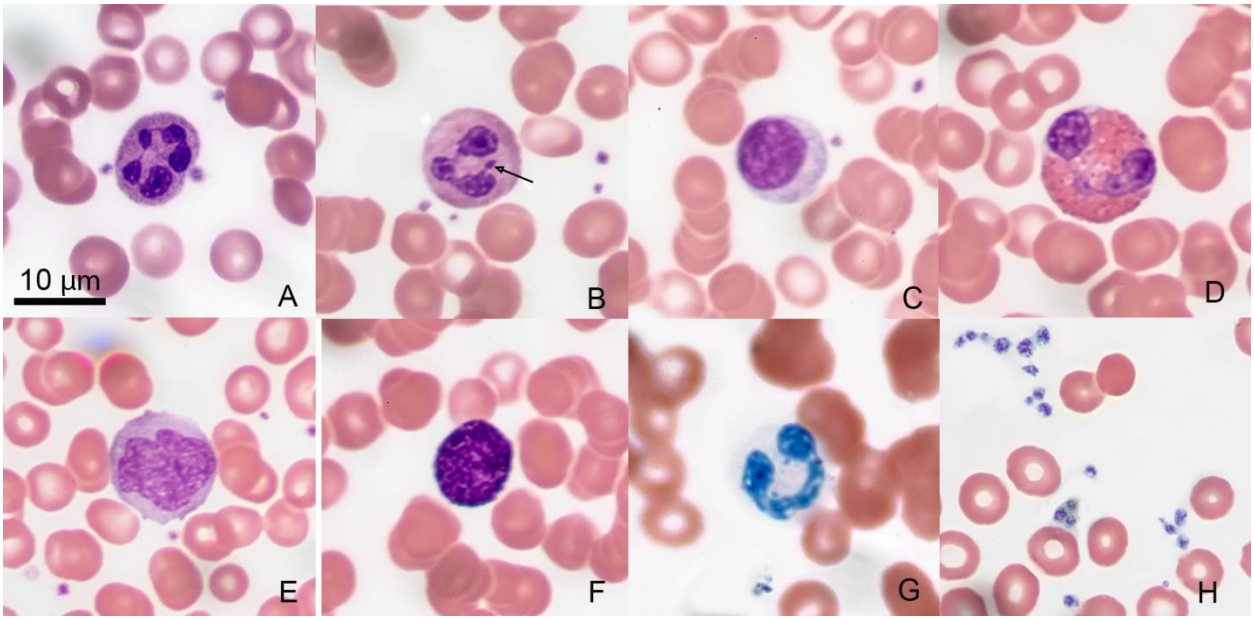

Fig. 6 Different white blood cells stained with Wright's stain – A Neutrophil, B Neutrophil with a Barr body C lymphocyte D Eosinophil E Monocyte F Basophil G Stab cell – young Neutrophil H platelets (thrombocytes). Bright field microscopy 1000X.

Macrophages and Monocytes

Monocytes are the largest white blood cell and can differentiate into macrophages and dendritic cells. They are amoeboid in appearance and lack granules in their cytoplasm. These large cells can be about 20 microns in diameter with a large and/or bean shaped nucleus. The nucleus is not divided into lobes and the cells are present from 2-10% of the white blood cells. Monocytes are attracted to damaged tissue and are induced to become macrophages which engulf tissue and debris. These cells can persist for several months. In the lungs of a person exposed to asbestos macrophages try to engulf the asbestos particles which may damage the macrophage's nucleus and in turn some of these cells can become cancerous.

Neutrophils

Neutrophils are the most abundant type of white cells consisting of 40-70% of white cell population. These short lived cells (live 5-35 hours) are easy to identify due to their segmented nuclei in stained blood preparations. Neutrophils can have from 2-5 nuclei, however they can exhibit hyper segmentation (6 or more nuclear segments) if there is a Vitamin B deficiency. Neutrophils with a single nuclear band are called stab cells and they are immature cells. When the cells are activated they change from a circular to more amoeboid shape and engulf bacteria and other pathogens. White blood cells from females may exhibit a small drum stick shape structure attached by a fine thread to the nucleus in up to 17% of cells (W.M. Davidson and D.R. Smith (1954). These are inactive X chromosomes and females have two X chromosomes one is inactivated. These inactive X chromosomes are called Barr bodies

named after the discovery by Dr. Murray Bar and his graduate student E. G. Bertram in 1949 at the University of Western in London, Ontario.

Eosinophils

Eosinophils are white blood cells 12-17 microns in size that play a role in allergic responses and asthma. They consist of usually less than 7% of white blood cells. Their cytoplasmic granules stain with acidophilic dyes like eosin that appear pink. The eosinophils stay in circulation for about 8-12 hours. Eosinophils move to inflammatory sites where they are activated and release their toxic granules. Some eosinophils fight viral infections and worm colonization. They have a band shaped nucleus surrounded by pink granules.

Basophils

Basophils are a type of white blood cell with large granules that obscure the nucleus when stained. When unstained their nucleus has two lobes. They make up 0.5 to 1% of white blood cells and play a role in the immune response and inflammation. They are involved in asthma, atopic dermatitis (eczema) and hay fever. They are called Basophils because they stain with basic dyes. These cells contain histamine which is a vasodilator and heparin that prevents clotting.

Platelets (Thrombocytes)

Platelets are cytoplasmic fragments derived from multinucleated giant cells called Megakaryocytes of bone marrow. In blood stained with a basophilic stain they appear as blue clusters (see below). They appear as oblate spheroids 2-3 microns in diameter and range from 200,000 to 450,000 per microliter. They are only found in mammals, in birds and amphibians they circulate as intact mononuclear cells. They can also be activated by abnormalities in blood vessel walls resulting in inappropriate clots (thrombosis) obstructing blood flow. They can be measured and counted with a microscope and hemocytometer slide.

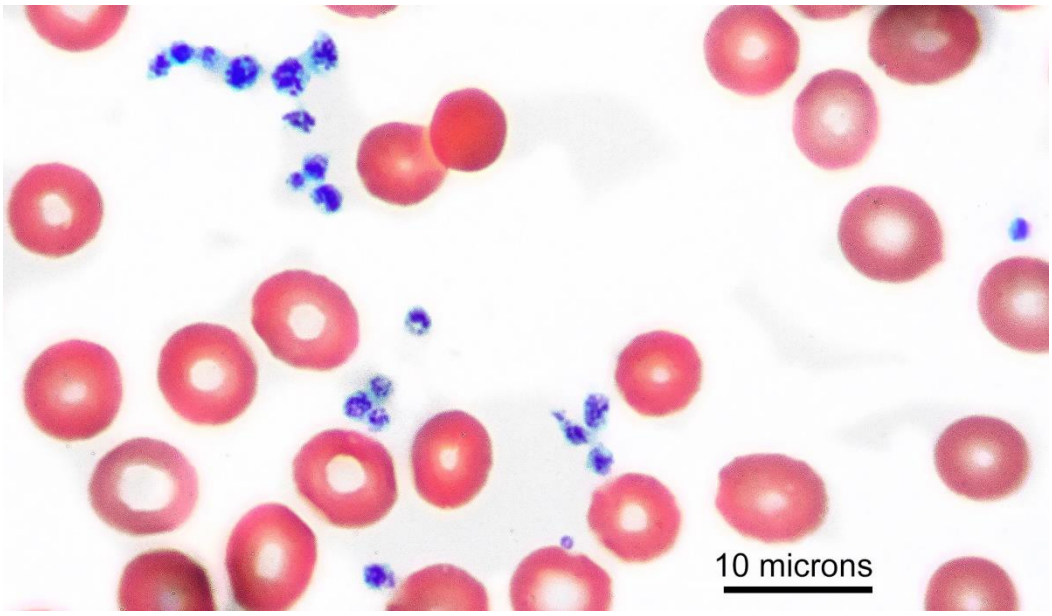

Fig. 7. Erythrocytes (red blood cells) and platelets (blue) – Blood smear Wright's stain 1000X Oil immersion bright field microscopy.

Barr Bodies or Sex Chromatin

M.L. Barr and E.G. Bertam (1949) were the first to notice dark chromatin staining structures in some cat nuclei and noted they were only found in female cats. Later on W.M. Davidson and Dr. Smith (1954) noticed drum stick-like structures attached to the nuclear lobes of some female neutrophils. Today we know that these structures are inactive X chromosomes. Females have 2 X chromosomes and one inactive X chromosome can be detected by staining. Several studies have confirmed they are present in a percentage of neutrophils. Barr bodies can also be visible in some men with Kleinfelter's syndrome with a 47XXY chromosomal karyotype. Barr bodies can be also seen in cheek epithelia cells and some hair cells after staining. The Barr bodies are about 1 micron in size.

Lyme disease

Lyme disease is caused by a bacteria spread by deer ticks to humans and if not diagnosed can cause serious symptoms. The bacteria can sometimes be detected in blood smears as shown below.

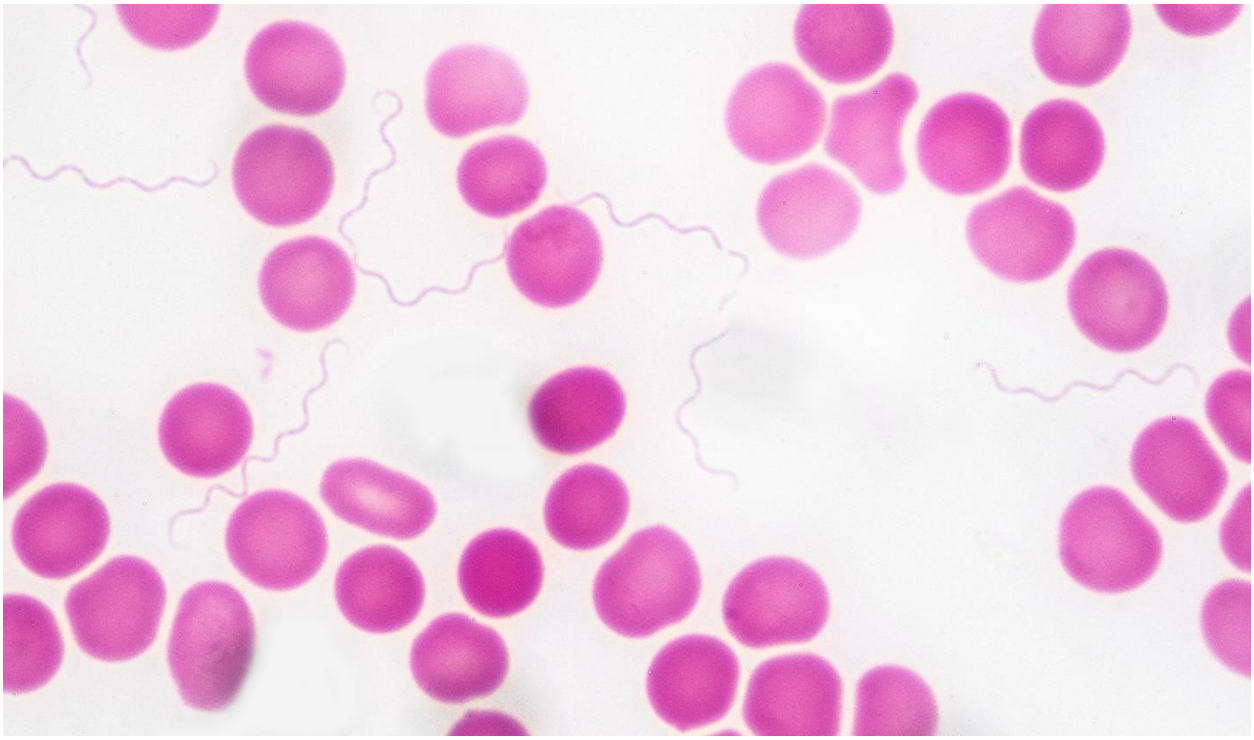

Fig 8 Above is a photo of a blood smear showing *Borrelia burgdorferi*, a spirocheate bacteria (appear as wavy lines) 1000X Oil immersion with bright field microscopy. This bacteria from deer ticks causes Lyme disease.

Lyme disease is caused by bacteria *Borrelia burgdorferi* (C. Kurokawa, 2020). They are difficult to detect immunologically or in blood smears by light microscopy. Lyme disease is spread by ticks (*Ixodes scapularis* – a deer tick) and anyone hiking outdoors should be aware of the ticks. I have lead photography tours in the Canadian Rockies and mention them to my groups especially in spring. For photographers simply putting their camera bag on the ground can attract ticks where they attach themselves and later crawl up into the persons hair, then when asleep they crawl onto your body suck your blood and infect you. You likely won't feel the tick bite, but the ticks engorge themselves with blood and can infect you. When I was bitten by a tick my doctor put me immediately on the antibiotic Erythromycin for 10 days and I was OK (I don't know if the tick was infected). Some of my friends have not fared as well.

First thing after a day hiking in regions where the ticks are common e.g. around deer, big horn sheep, small mammals etc, you should take a shower and wash your hair to get rid of any ticks before going to bed. If you can't shower check your hair and body for ticks before going to sleep. Some folks exhibit an expanding red rash about a week after being bitten. If you get a rash visit your doctor as soon as possible and alert the doctor where you were hiking. The rash is not itchy or painful and only develops in about 75% of people. Early symptoms of Lyme disease may include fever, headache and fatigue. The disease is not transmittable between people, animals or via food. If you exhibit tick bite symptoms and have been walking in an area where ticks are found let your doctor know. Immunological tests can be

negative so get a 2nd test a few weeks later or take the prescribed antibiotics. In 10-15% of untreated people Lyme disease can cause neurological conditions which can occur 1-12 weeks after a tick bite.

Live Blood Cell Analysis

Live Blood Cell Analysis uses dark field light microscopy to observe a fresh blood sample. This analysis is not accepted in standard laboratory practice but is considered a form of alternative medicine. Live Blood Analysis is not regulated and anyone with a light microscope with dark field accessories can become a practitioner. Training involves a four week course, though some practitioners may have additional training in nutrition. Live blood cell practitioners cannot legally diagnose or treat medical illness. They offer live views of your blood on a computer monitor and may suggest nutritional supplements. Some practitioners have been incriminated and charged for making incorrect claims (Wikipedia). As a scientist I can appreciate the attraction of seeing your own blood, though caution anyone to beware of Live Blood Cell Analysis claims and see your doctor if you have any concerns or wish to verify any diagnoses you may be given on the basis of live blood analysis.

If considering live blood analysis do some Internet research (e.g. Read letter by Tiffany Clouston who teaches Blood Technologists in Canada), Wikipedia and other credible medical web sites - https://www.csmls.org/Advocacy/Public-Awareness/Smoke-and-Mirrors-An-Inside-Look-at-a-Live-Blood).

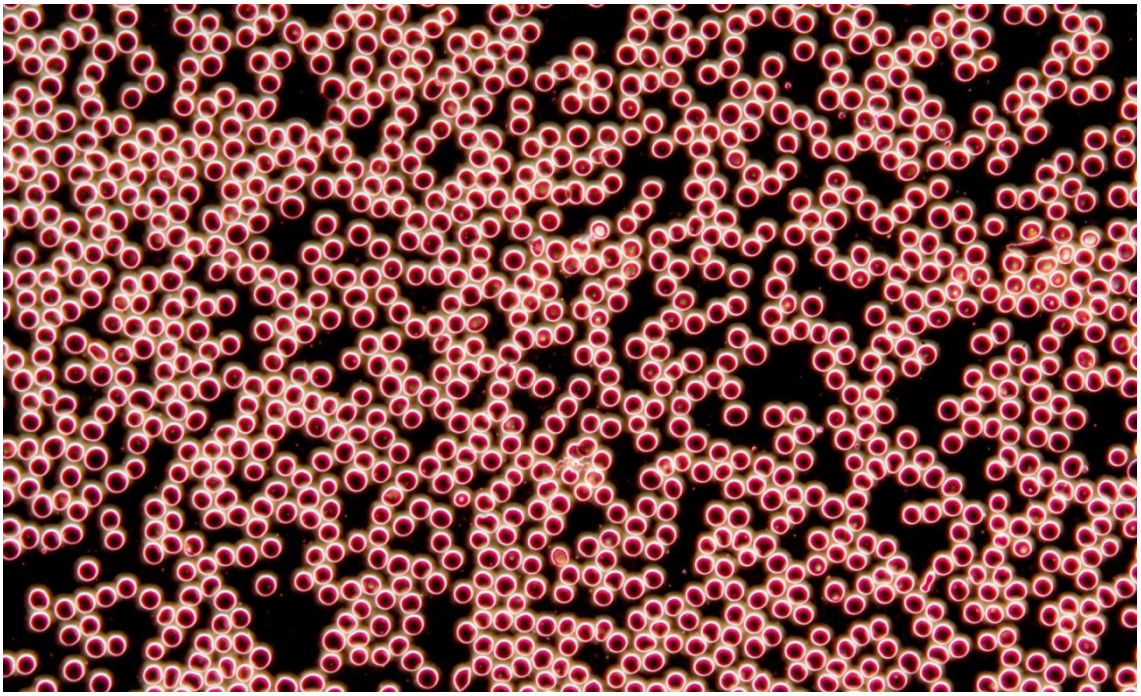

Fig. 9. Above photo shows live blood cells by Dark field microscopy 100X. Most of the cells shown are red blood cells. High magnification dark field microscopy can detect bacteria, some pathogens and alterations in blood cells.

Note that whereas many standard light microscopes can produce dark field at lower magnification

(10X, 20X and 40X objectives) using special opaque disks, or the "wrong" phase condenser disk, at higher magnification (60X or 100X objectives) dark field microscopy requires a special dark field condenser with a numerical aperture greater than that of a high power objective and uses oil immersion fluid between the condenser and bottom of the glass slide in addition to the coverslip and objective (Bagnell 1964). A Dark field 100X objective may also have an iris diaphragm built in to fine tune dark field light (C. R. Bagnell, 2012).

If you have a serious illness that requires blood analysis I urge you to visit your medical doctor and a get traditional blood analysis. The costs of traditional blood tests are paid for in Canada, while those of Live Blood Analysis are not. If you want to see your blood and get nutritional advice then live blood analysis is an option. An alternative option if you want to observe blood is to consider owning your own microscope.

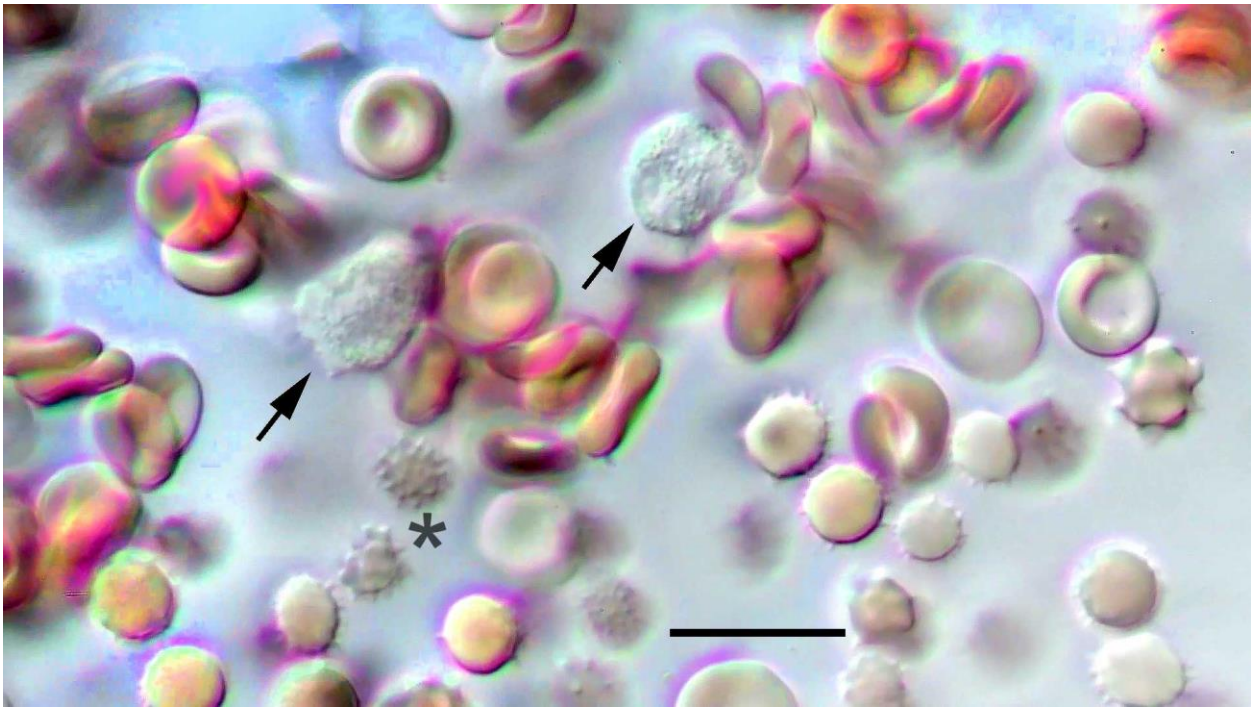

Fig. 10 Live blood cells using Differential Interference Contrast microscopy 1000X. The arrows point to two white blood cells and the asterisk (*) shows some crenated red blood cells which can occur in live blood samples or blood cells bathed in a hypertonic solution. Scale bar = 10 microns.

Animal Blood Cell Analysis

Veterinarians conduct animal blood analysis for a variety of reasons. Blood from other mammals appears microscopically similar to human blood. Blood cells from birds, reptiles, fish etc., look different being oval and have red blood cells with nuclei. Being able to analyze and distinguish animal blood from human blood is important in Forensic science. One research study used morphometric analysis (measured the diameter and circumference) of red blood cells to distinguish blood from cattle, sheep, goats, horses and dogs using both a light microscope and software (N. Adili et al. 2016). There are

Canadian animal blood banks for injured animals (http://www.canadiananimalbloodbank.ca/). A new technique to differentiate human and animal blood uses total reflection Fourier transform-infrared spectroscopy (E. Mistek-Morabito and I. K. Lednev (2020). Pet owners can have their animal's blood and urine tested by a Veterinarian.

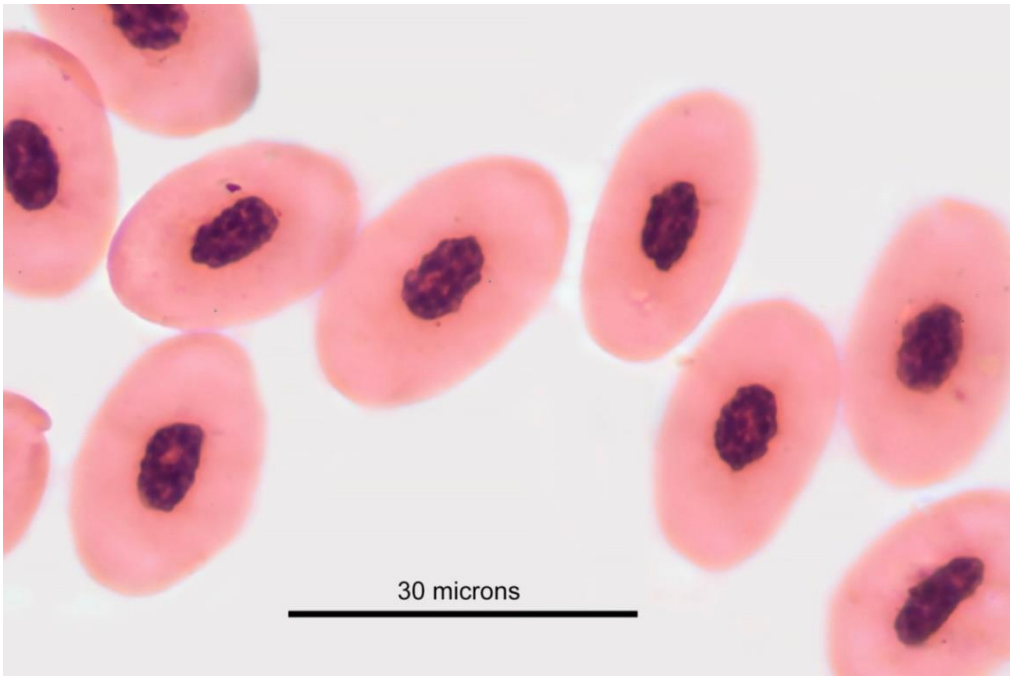

Fig. 11. Frog red blood cells from a prepared microscope slide. The red blood cells have a nucleus - 1000X

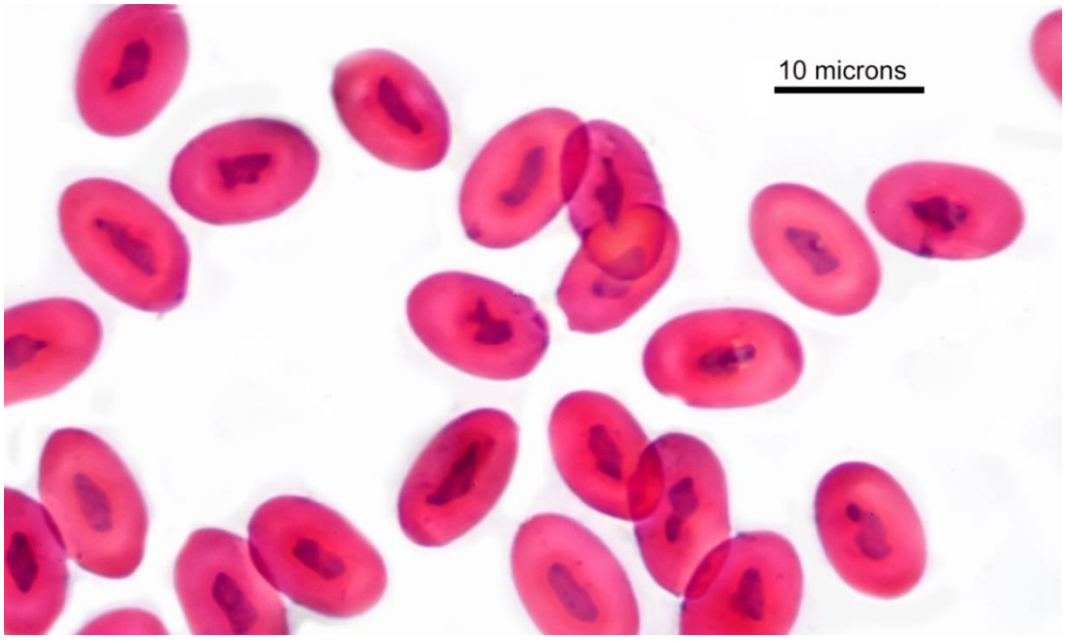

Fig. 12. Fish red blood cells have nuclei - photograph of cells on a prepared slide 1000X.

Summary & Conclusions

Blood cells from mammals appears similar microscopically to cells in humans, whereas red blood cells from birds, frogs and fish have nuclei. In humans blood contains several different types of white blood cells each with specialized functions and the number of specific white cells is diagnostic as is the shape and number of the red blood cells. Blood analysis by microscopy can be used to detect parasites, bacteria, fungi and other pathogens. Live blood analysis utilizes dark field microscopy to investigate blood, is controversial and practitioners are not qualified to make medical diagnoses. Anyone with a microscope can view blood cells, but caution is required in handling other people's blood because it might carry infectious agents (HIV human immunodeficiency virus, COVID-19, Lyme disease etc.). One should wear rubber gloves when handling human blood. Motic offers a wide variety of light microscopes suitable for blood analysis and a sales person can advise you on which microscope is most suitable for your needs and budget if considering purchasing a microscope..

Acknowledgements

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https://benzmicroscope.com/products/slide-stain-kits-wrights-blood-stain-2pk

J. Sprute Red Blood Cell Abnormalities in Morphology
https://hematologylearning.weebly.com/red-blood-cell-inclusions-and-abnormalities.html

Understanding your Pet's Diagnostic Testing – Blood & Urine
https://vetclinicmission.com/wp-content/uploads/2017/12/Understanding-Your-Pets-Diagnostic-Testing.pdf

YouTube Blood Analysis Videos

Red Blood cells viewed under the microscope in hypo and hypertonic conditions
https://www.youtube.com/watch?v=A8cI6FkcG4c

Wright and Geimsa stain for blood
https://www.youtube.com/watch?v=9xBcm-1NMq

What blood cells look like in a Microscope
https://www.youtube.com/watch?v=uxzDrvIoh4Q

Live Blood Analysis
https://www.youtube.com/watch?v=JpWRgNJQeAo

Wright's Blood Stain
https://benzmicroscope.com/products/slide-stain-kits-wrights-blood-stain-2pk

All About Blood Tests
https://www.healthline.com/health/blood-tests

Digital Chip Analyzes Blood
https://www.futurity.org/digital-chip-analyzes-blood-from-tiny-drop/

Looking at blood under the microscope
https://www.youtube.com/watch?v=_Hs-kUBbakU

Wrights Stain purchased from Benzmicroscope.com
https://benzmicroscope.com/products/slide-stain-kits-wrights-blood-stain-2pk