
pSMILE
SAFETY·QUALITY·RESULTS
Blood Cell ID
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and
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Objectives

- Learn how to make blood smears
- Demonstrate the ability to examine a stained smear under the microscope and accurately perform a manual differential, following the proper procedural steps.
- Identify and differentiate all major blood cell lines, including both mature and immature forms.

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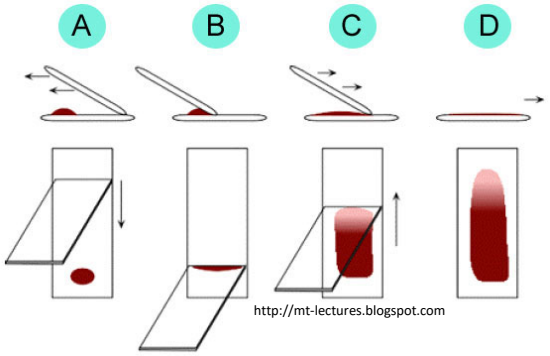
Slide Making (Push Smear)



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Requirements for Proper Smear Preparation:

- Clean glass slides
- Label frosted side up- label according to SOP
- Small blood drop
- Proper placement of drop
- Smooth spreading of drop
- Peripheral blood smear made from EDTA anticoagulated blood.
- Ideally made within 3 hours

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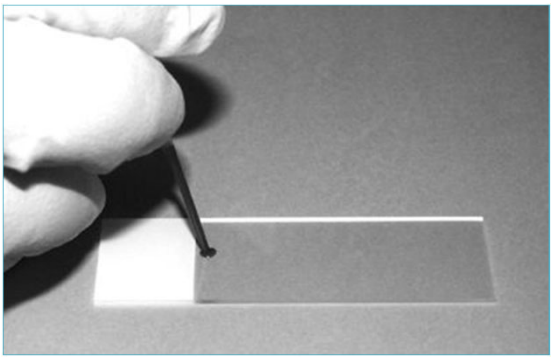
Step 1:

Place a small drop of mixed venous blood on a glass microscope slide.



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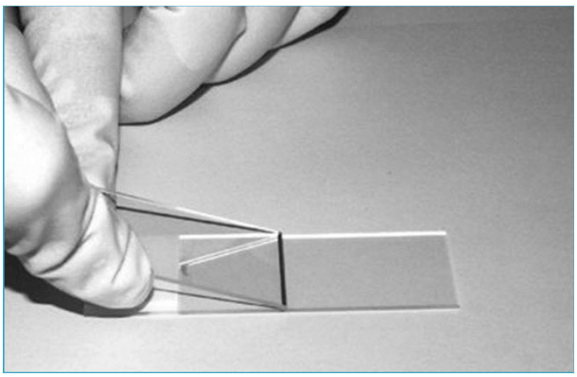
Step 2:
A spreader slide is positioned at angle and slowly drawn toward the drop of blood.



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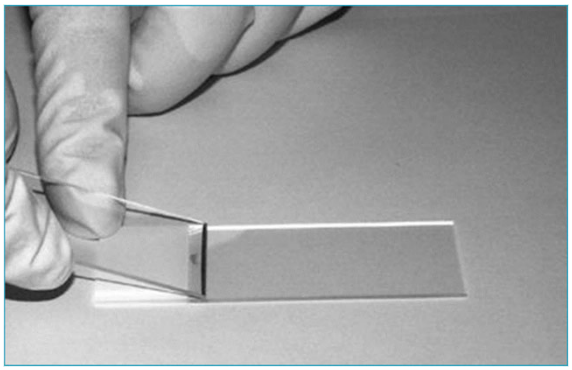
Step 3:
The spreader slide has been brought in contact with the drop of blood and is being drawn away…



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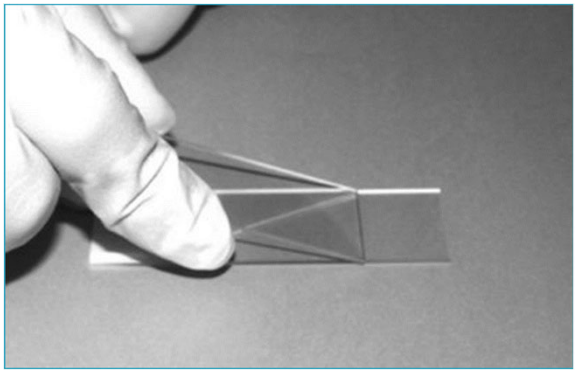
Step 4: Then slow push forward, toward the end of the slide



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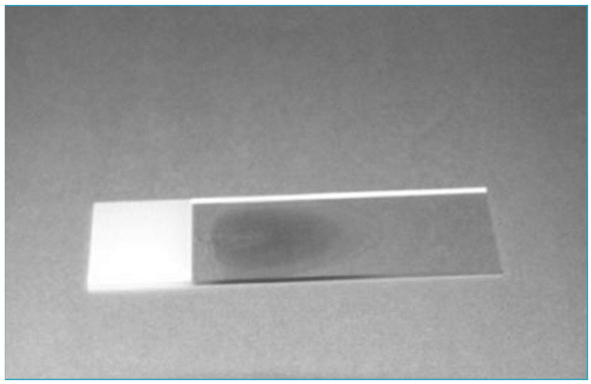
Step 5: Allow slide to dry 5-10 minutes



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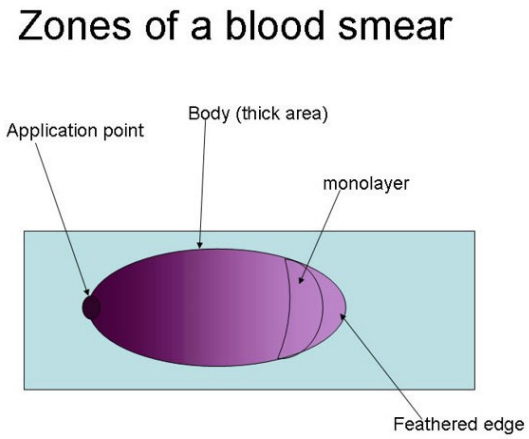
Characteristics of a good smear:

- Good smear is tongue shaped with a smooth tail
- Does not cover the entire area of the slide
- Has both thick and thin areas with gradual transition
- Does not contain lines or holes



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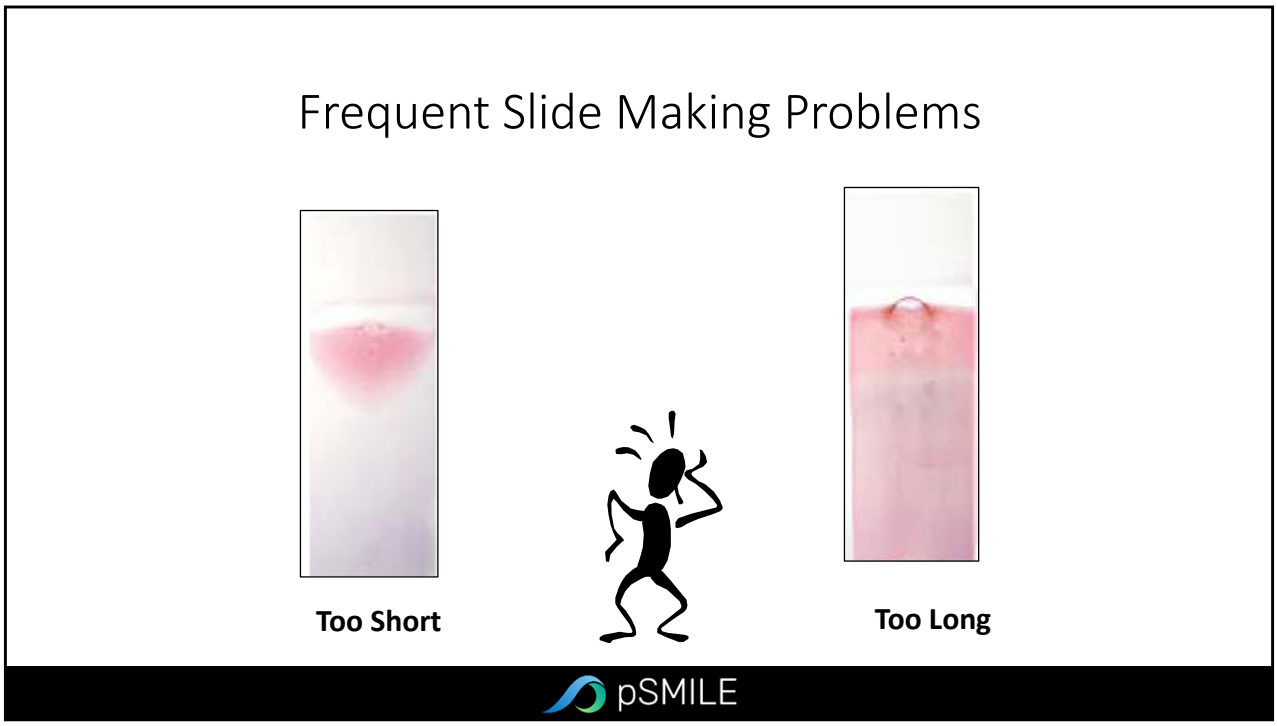
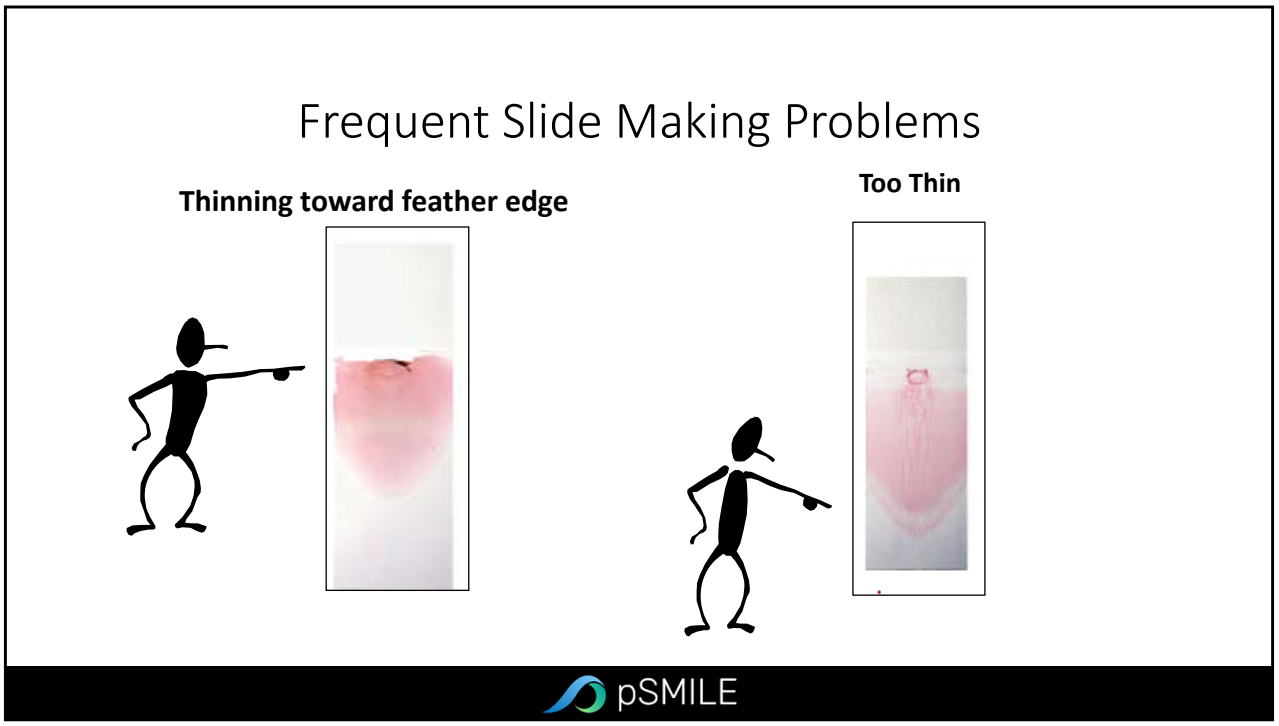


A fresh, well-made, peripheral blood film is crucial for accurate cell morphology assessment.

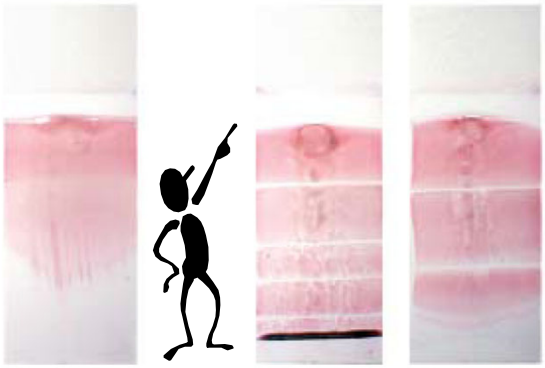
Too short –Not enough blood to perform and accurate differential.
Too long – Initial Drop of blood too large which may cause some cells to not be uniformly represented.



Extremely thin films (caused by too small a drop, too slow spreading or too low a spreader angle), may result in RBCs that appear as spherocytes and increased WBCs, such as monocytes and neutrophils, in the tails. An incorrect differential will result.

Frequent Slide Making Problems

Skipping and Variations in Thickness



Streaking

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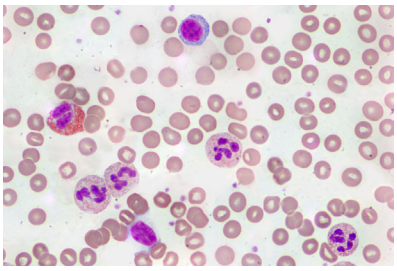
Blood films with excessive tails or gritty feathered ends indicate a spreader edge that is rough or dirty, or an
accumulation of leukocytes due to either slow spreading or a very high leukocyte count. In these smears cell distribution will be uneven.

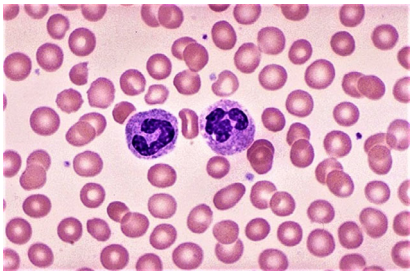
The message: Always scan the film under low power to determine slide quality

Practice making slides

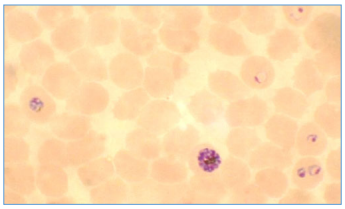
Types of Stains



Wright's Stain



Leishman Stain



Giemsa Stain

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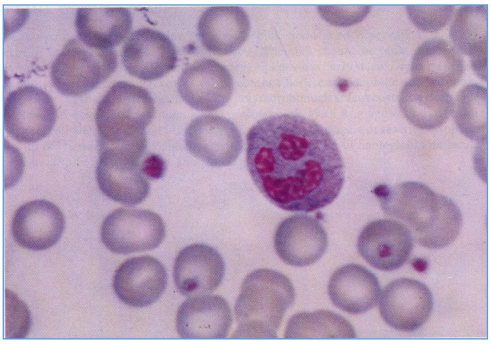
Some stains can look really beautiful, Giemsa for malaria.

Frequent Staining Problems



- Wright's Stain "Too Alkaline"

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Too Alkaline Stain:

1) thick blood smear
2) prolonged staining
3) insufficient washing
4) alkaline pH of stain components

Correction:
1) check pH
2) shorten stain time
3) prolong buffering time

Frequent Staining Problems

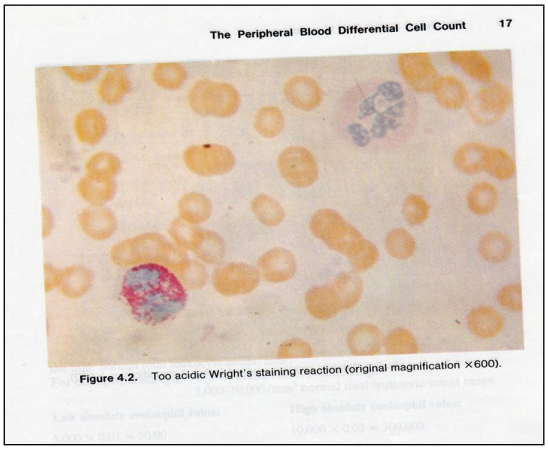

Figure 4.2. Too acidic Wright's staining reaction (original magnification ×600).

- Wright's Stain "Too Acidic"

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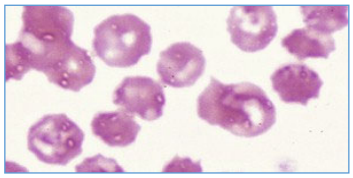
Too Acid Stain:

1) insufficient staining time
2) prolonged buffering or washing
3) old stain

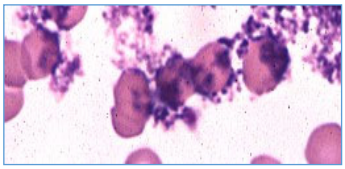
Correction:
1) lengthen staining time
2) check stain and buffer pH
3) shorten buffering or wash time

Frequent Staining Artifacts

Water Artifacts



Stain Precipitate



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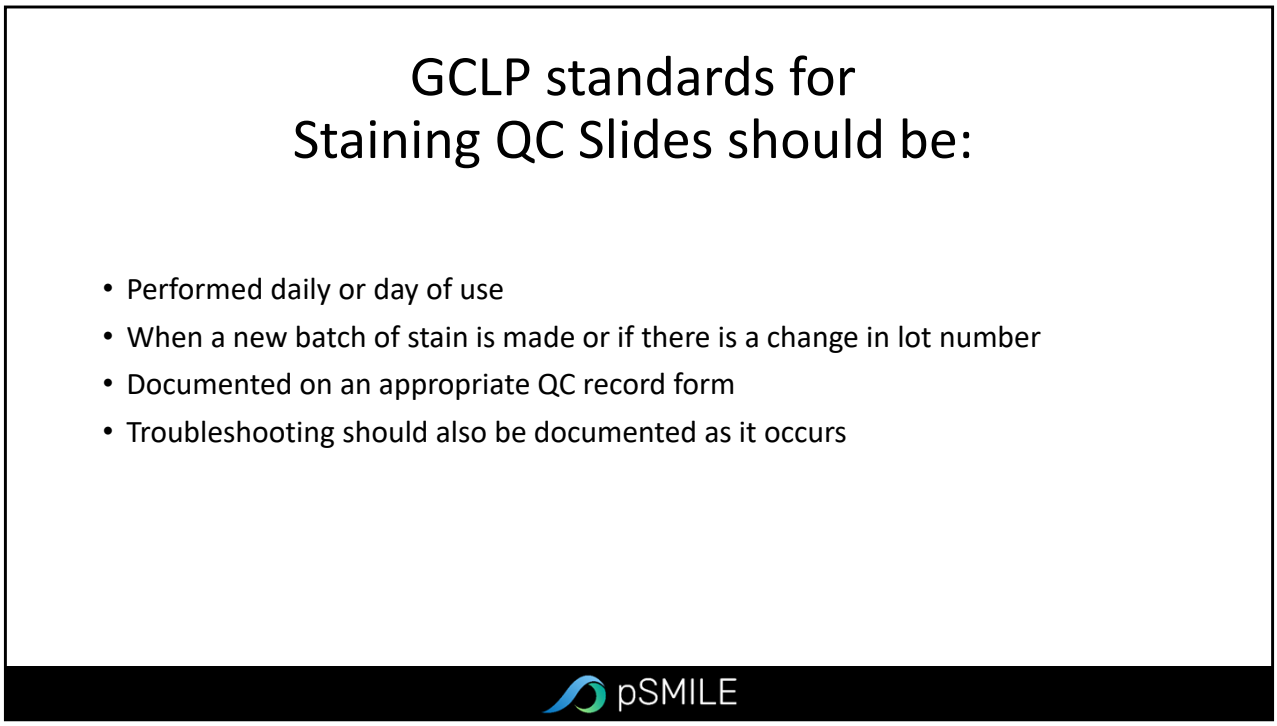
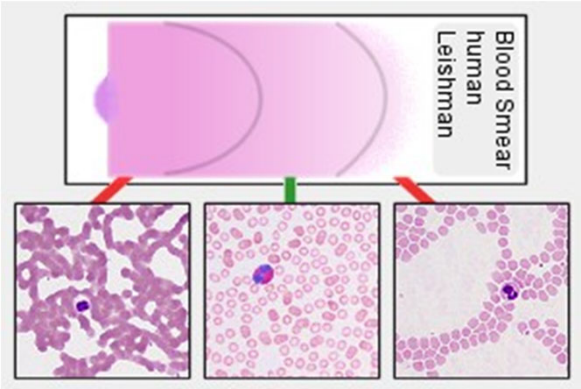


In the hand: an example of Slide staining QC scheme. Please note this is only an example and your laboratory must modify and implement a QC scheme base on your laboratory policies and needs.

Examination of Peripheral Blood Smear



http://www.lab.anhb.uwa.edu.au/mb140/corepages/blood/blood.htm

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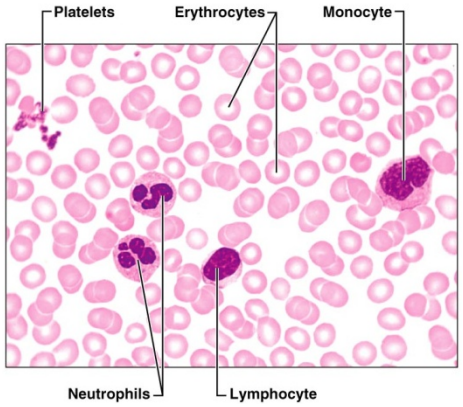
What to Look for & Report

- WBC estimates
- Platelet estimates
- Numeration of WBC inclusions, RBC morphology and inclusions
- Manual differential



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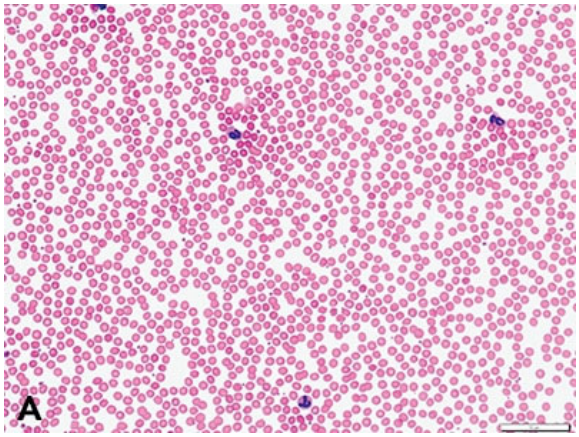
Low and High Power Magnification



A. Examination of Peripheral Smear at Low Power (10x objective)

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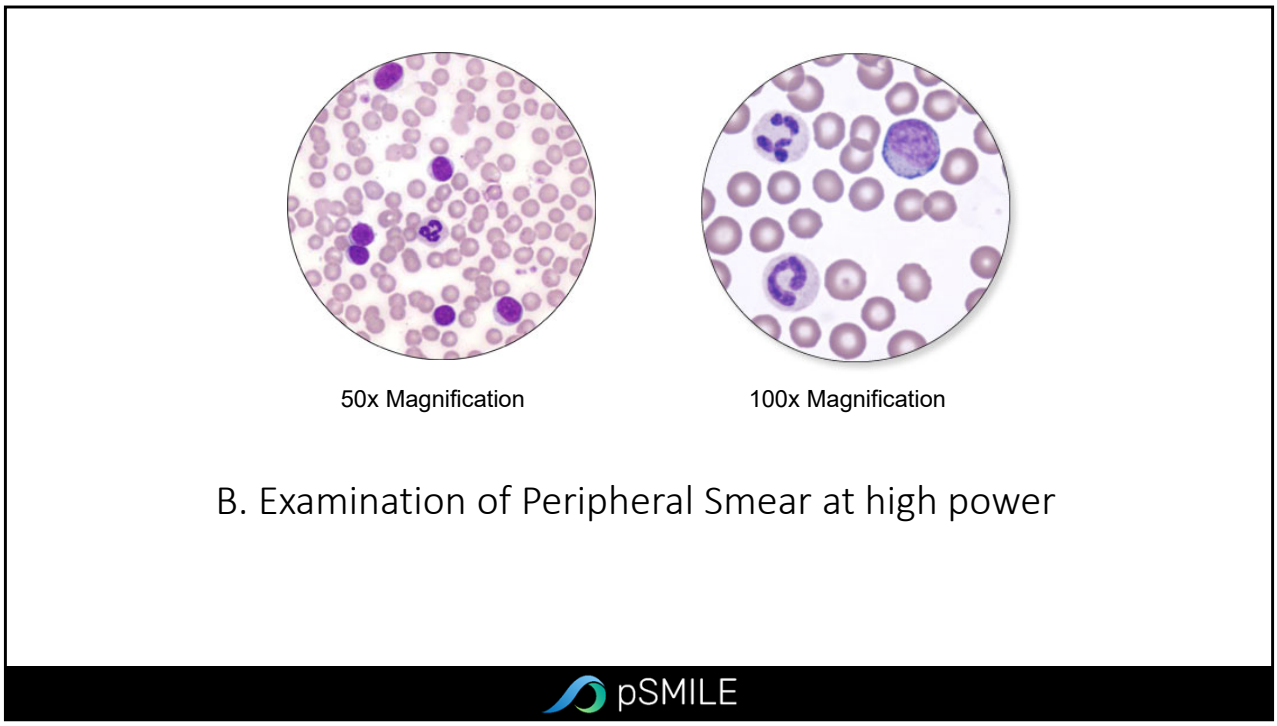
The "zone of morphology" (area of optimal thickness for light microscopic examination)

- Quality of the smear
- Rouleaux formation
- Platelet clumps
- WBC clumps and other abnormalities visible at low magnification



A differential count of at least 100 white blood cells is performed, and any abnormal morphology of RBCs, WBCs, and platelets observed during the differential count is recorded. Each morphologic abnormality observed should be quantitated ("graded") separately as to severity ("slight to marked" or "1+ to 4+").

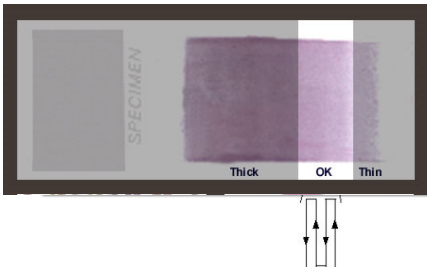
Perform differential count (50x or 100x)
WBC estimate (50x)
Platelet estimate and RBC Morphology (100x)

Record any abnormal morphology of RBCs and WBCs, and platelets that is observed

WBC Estimation

Performance of the leukocyte estimate using the high power (50x) oil immersion objective

WBC Estimate (count/mm^3) =
Average of WBC in 10 field counted X 3000



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Count the number of both intact and disrupted leukocytes in 10 microscopic fields where the erythrocytes are partially but not completely overlapped (zone of morphology). Divide the total number by 10 to establish the mean number of leukocytes per field. Multiply the mean by 3000 to determine the estimated WBC count/mm^3. These estimates should approximate that obtained by the cell analyzer, the automated leukocyte count/mm^3 is less than 25,000 the estimate number should agree within 20% of the automated count. If the estimate does not agree within 20%, repeat the WBC count on the instrument and/or prepare another smear to ensure that the correct sample was tested and/or used to prepare the smear. This method is less reliable when the automated leukocytes count/ mm^3 is greater than 25,000 and your laboratory has to establish policies to verify this high WBC count, these can include repeating the samples on the automated analyzer or performing a dilution.

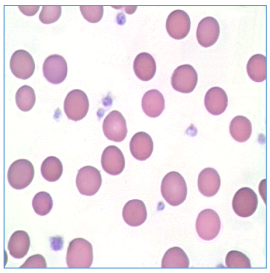
1. Count the number of WBCs in 10 field
2. Divide the total number by 10 to establish the mean
3. Multiply the mean by 3000 to determine estimated WBC count/mm^3.

Platelet Estimation

Performance of the platelet estimate using the high power (100x) oil immersion objective

Platelet Estimate =

Average of platelets in 10 field counted X 20,000



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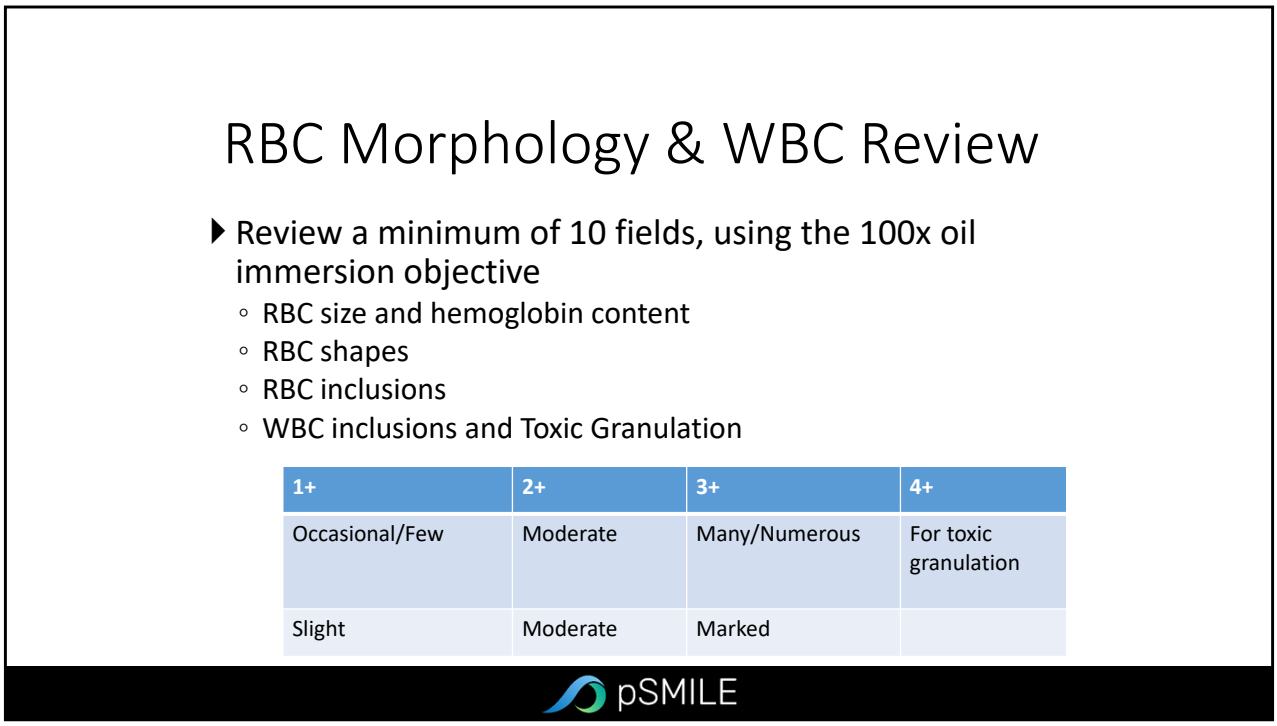
•If a significant numbers of giant platelets and/or platelet clumps are detected, a peripheral estimate of WBCs should be done to prevent reporting spuriously high white blood cell count.

•Do a manual platelet estimate count if a significant numbers of Microcytic red blood cells and/or small cell fragments are detected during the RBC morphology.

1. locate an area of approximately 150 red blood cells
2. Count all the platelets in that area
3. Repeat this until 10 field have been counted
4. Keep the total number of all 10 areas counted
5. Divide the total number by 10 to obtain the average platelet per field, then multiply by (x) 20,000 to obtain the platelet estimate

In the presence of significant platelet clumping this estimate is not accurate. The laboratory has to establish a policy as how to report the platelet count. Suggestions include; redrawing patient's sample in a blue top tube (sodium citrates which is the anticoagulant choice that prevents the in vitro platelet clumping), vortexing the sample (please see you hand out for a copy of this procedure) or only report-platelets appear decrease, adequate or increase and do not provide a platelet count.



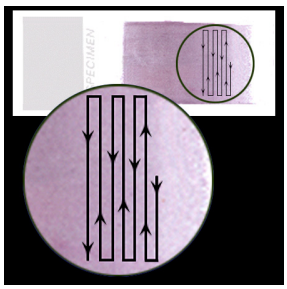
The RBC Morphology and WBC Review will be performed with every manual differential or as a separate examination. The test will be performed using the high power (100X) oil immersion objective. Begin the examination in an area where the red cells are touching but not overlapping. There are approximately 300 RBC's present in this area in patients with a normal hematocrit. Locate a mature (normal and not atypical) lymphocyte for comparative purposes and determination of Normocytic, Microcytic or macrocytic RBC.

Each morphologic abnormality observed should be quantitated ("graded") separately as to severity ("slight to marked" or "1+ to 3+"). 4+ is usually for toxic granulation. SOP for grading.

There is a SOP on your flash drive that give you an example how to set up for grading the different features

Differential

- Perform a 100 cell differential count using 50x or 100x objective
- 100x objective must be utilized to classify any immature cells



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Picture description: Scanning technique for peripheral blood differential count and morphologic evaluation. (a) Ten microscopic fields are examined in a vertical direction from bottom to top (or top to bottom). (b) The slide is horizontally moved to the next field (c) Ten microscopic fields are counted vertically. (d) The procedure is repeated until 100 leukocytes have been counted (for a 100-cell count). Or Use a differential cell counter, count and classify 100 white blood cells using the cross-sectional technique where the white cells are counted in consecutive fields as the blood film is moved from side to side as pictured

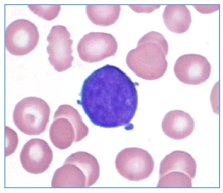
A differential count of at least 100 white blood cells is performed. There are situations when you want to use more or less cells based on your laboratory policy and also the WBC count.

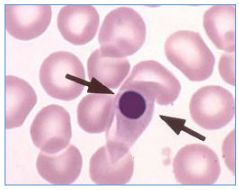
If the automated WBC count is less than 1.0 x 10^3/uL, a 50 cell count can be performed and converted to 100% by multiplying all values by 2. Add the following comment in result slip: 50 cells counted during the manual differential and converted to percentage.
If the automated WBC count is greater than 30.0 x 10^3/uL, a 200 cell count must be performed and converted to 100% by dividing all values by 2. Round accordingly and report only whole numbers. Add the following comment in the result slip: 200 cells counted during the manual differential and converted to percentage.

Differential- Correction

- Presence of NRBC or Megakaryocytes

$$\frac{\text{WBC X 100}}{\text{\# NRBCs and/or Megakaryocytes + 100}} = \text{Corrected WBC}$$





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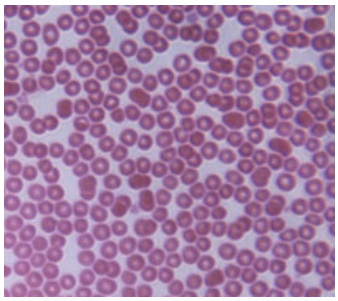
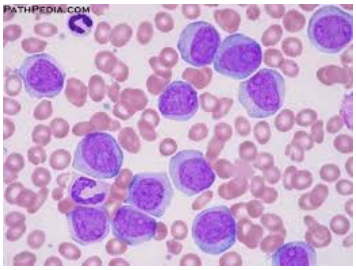
If nucleated red blood cells (NRBC) or Megakaryocytes nucleus are seen during the differential count, enumerate them separately from the white blood cell count.

Your laboratory has to establish a policy as when to use this formula for example: the WBC count requires correction if more than 10 NRBC or Megakaryocytes are counted during the differential count

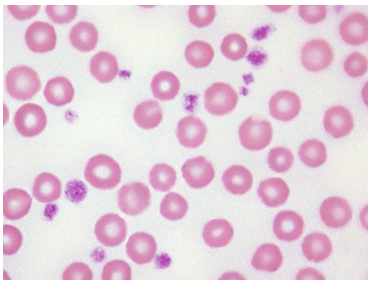
Blood Cell Overview



https://www.cdc.gov/parasites/blood.html



https://www.quora.com/What-are-the-main-functions-of-white-blood-cells



http://eclinpath.com/hematology/morphologic-features/platelets/normal-platelets/bovine-platelets/

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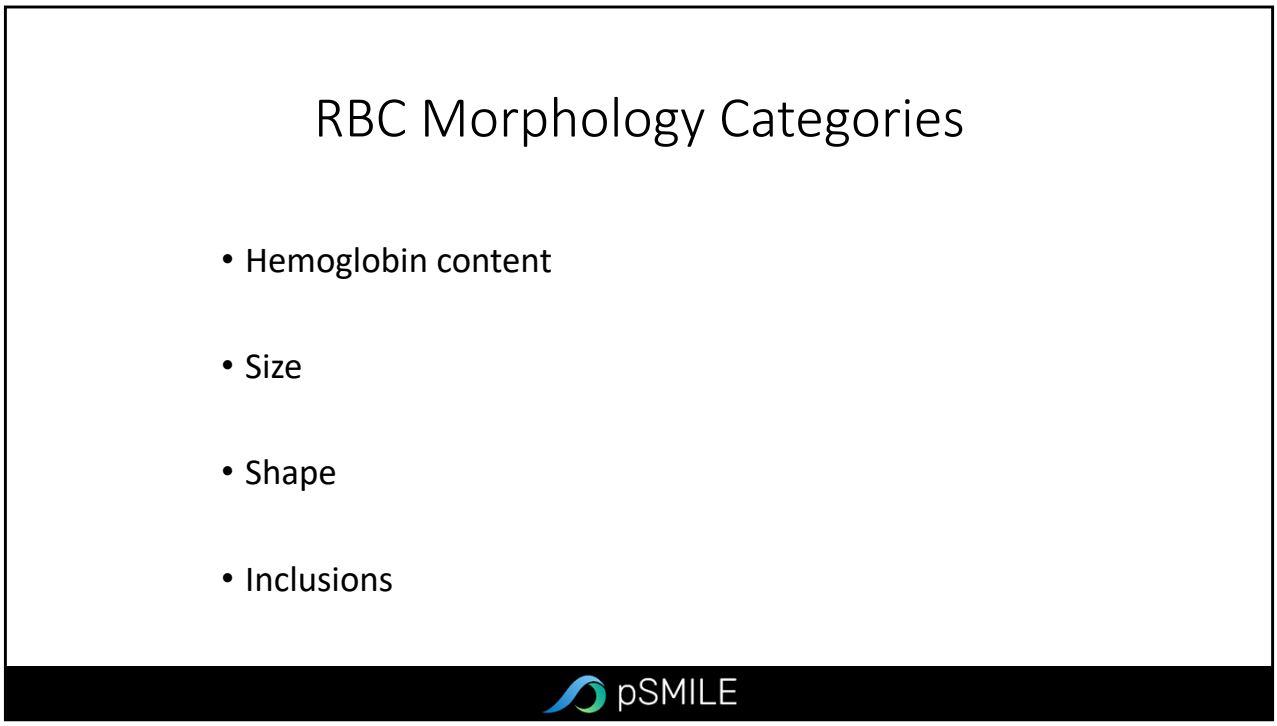


When performing RBC examination on the blood slide there are 4 categories you should evaluate and they are

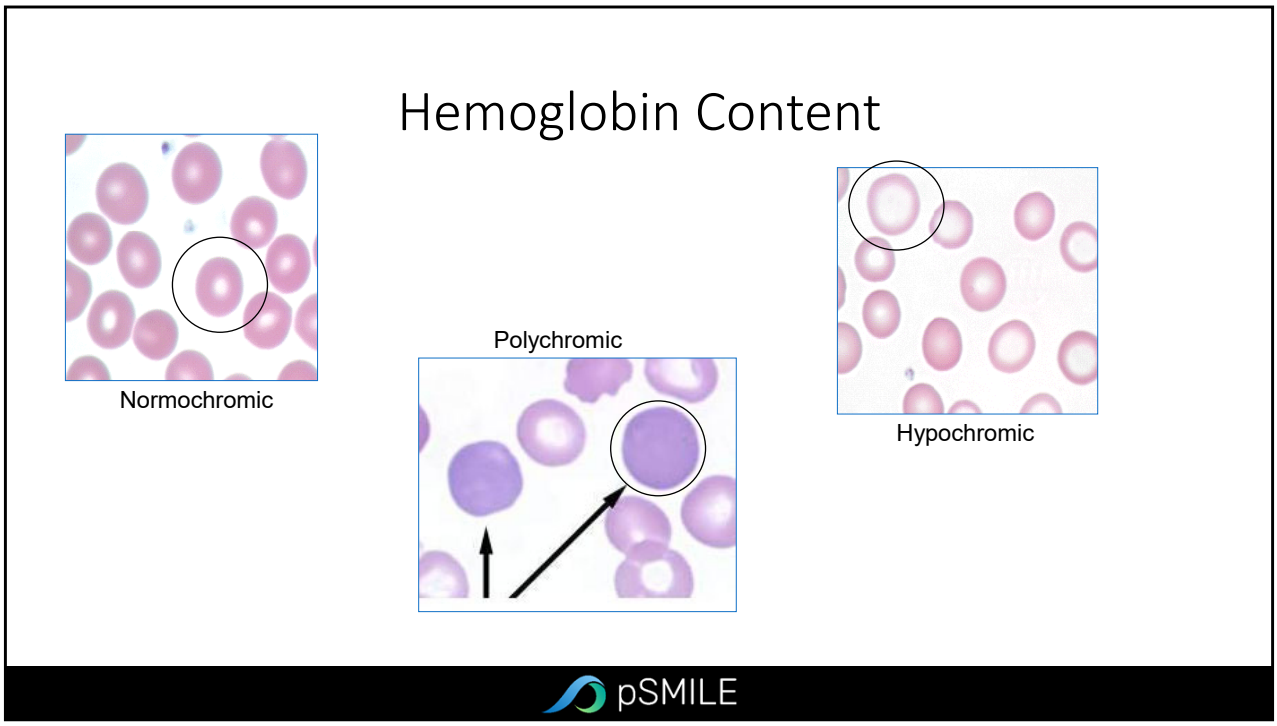
Hemoglobin content

Size

Shape

Inclusions.

We will look at the Hgb content first



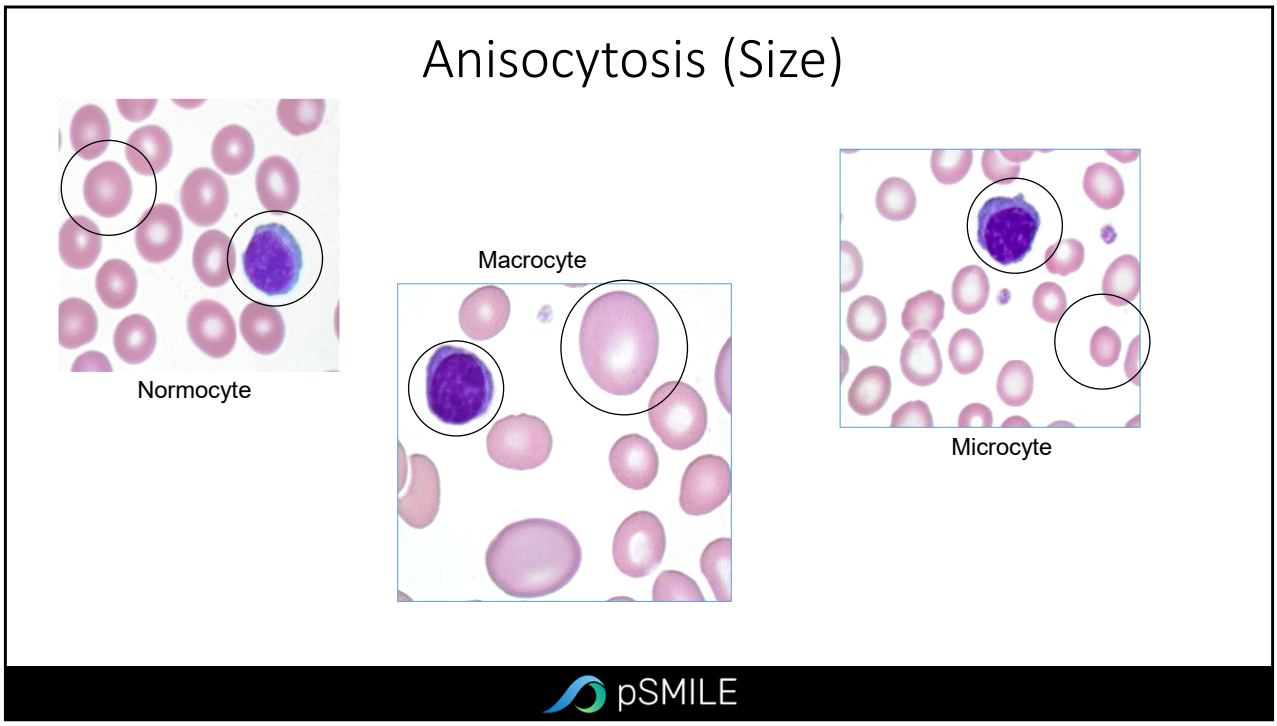
For hemoglobin content there are 3 possibilities and the way you differentiate them is the amount of central pallor in them.

If no more than 1/3 the diameter of the cell it is called a Normochromic RBC

If more than 1/3 the diameter of the cell it is called a Hypochromic RBC. Usually seen in iron deficiency anemia.

And finally in middle is a polychromic RBC which is slightly bluer in color and usually has no middle pallor

Can anyone tell me what test is performed to evaluate the % of polychromic RBC in the bloodstream?
Reticulocyte count



Now we evaluate the size of the RBC also divided into three possibilities

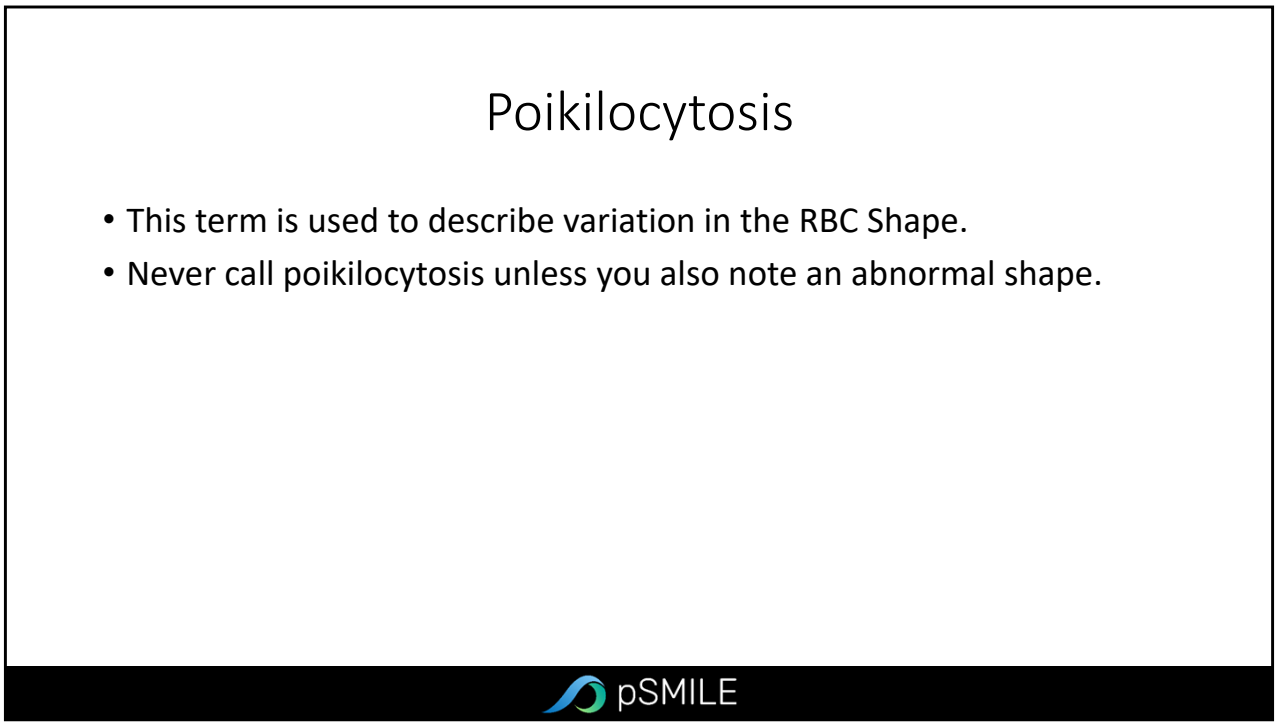
To tell the difference in size you compare the RBC to the nucleus of a lymphocyte.

If it is the same size it is a Normocyte.

If it is smaller it is a microcyte. They are usually seen in iron deficiency anemia and thalassemias.

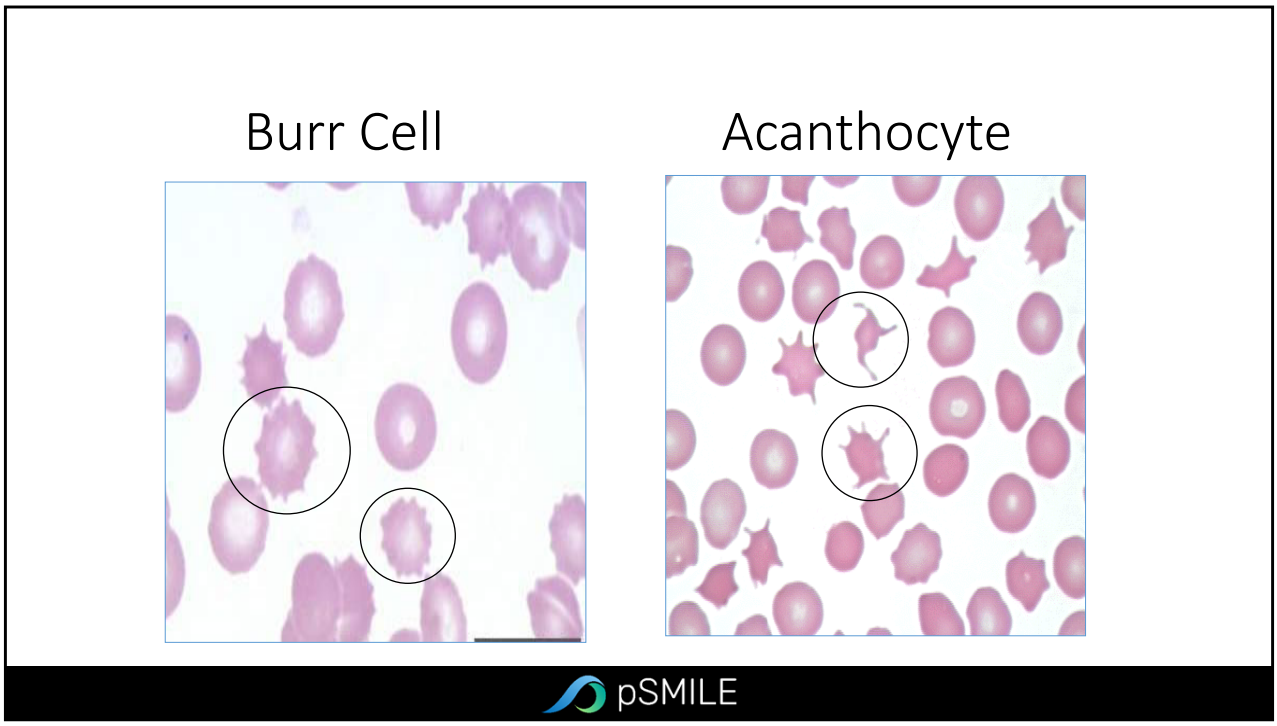
And if bigger it is a macrocyte .These type of RBC are usually seen in anemia due to Vitamin B12 or folate deficiencies.

So does anyone know the term use to describe variability of size of the RBC?
Anisocytosis
What is the parameter on CBC instruments that tell you Anisocytosis?
RDW



The next evaluation is the variation of shape of the RBCs which is called Poikilocytosis The most important thing to remember is if you call piokilocytosis **you must** also include the abnormal shape. You should never report just poikilocytosis. If you do it is telling the doctor “I see something and I am not telling.”

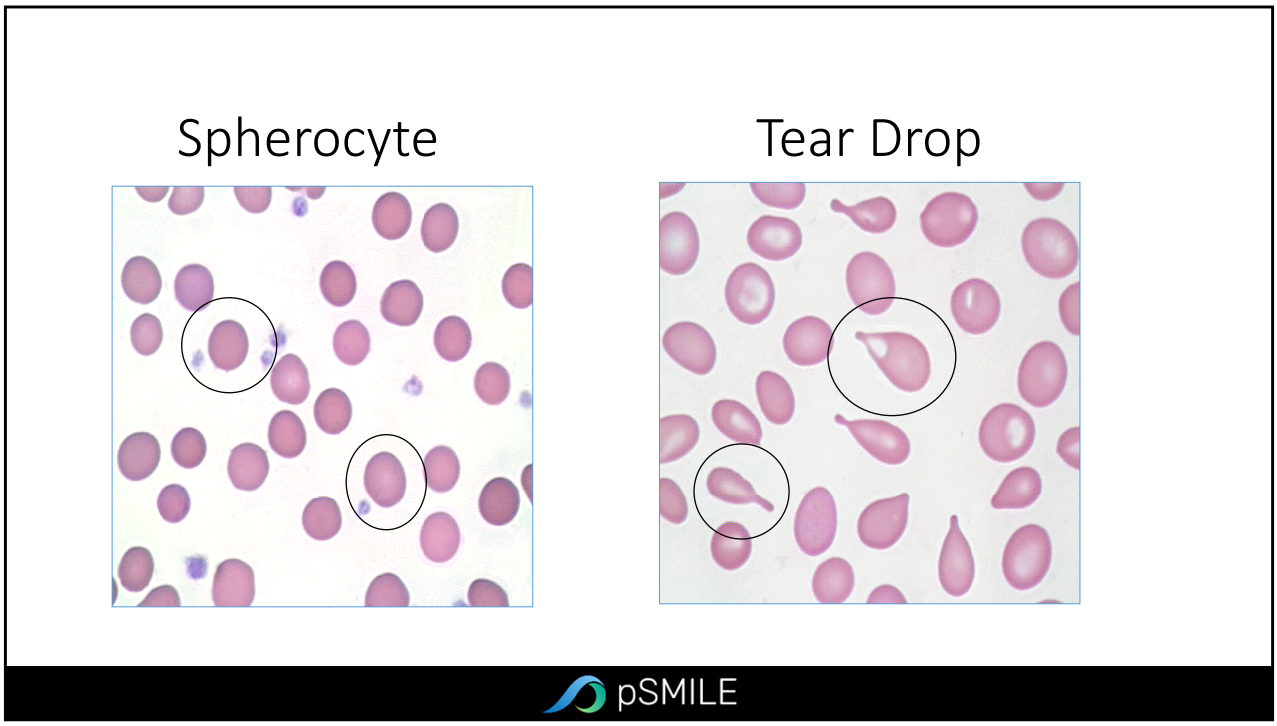
There are several shapes that can occur; we will now go over a few of the common ones seen.



On the right we have Acanthocytes.
The Greek prefix Acan means spike or horns. They have multiple spikes with no central pallor. Usually you do not see these in a normal smear. They are increased in patients without a spleen, advanced liver disease and abetalipoproteinemia. It is caused by a membrane abnormality.

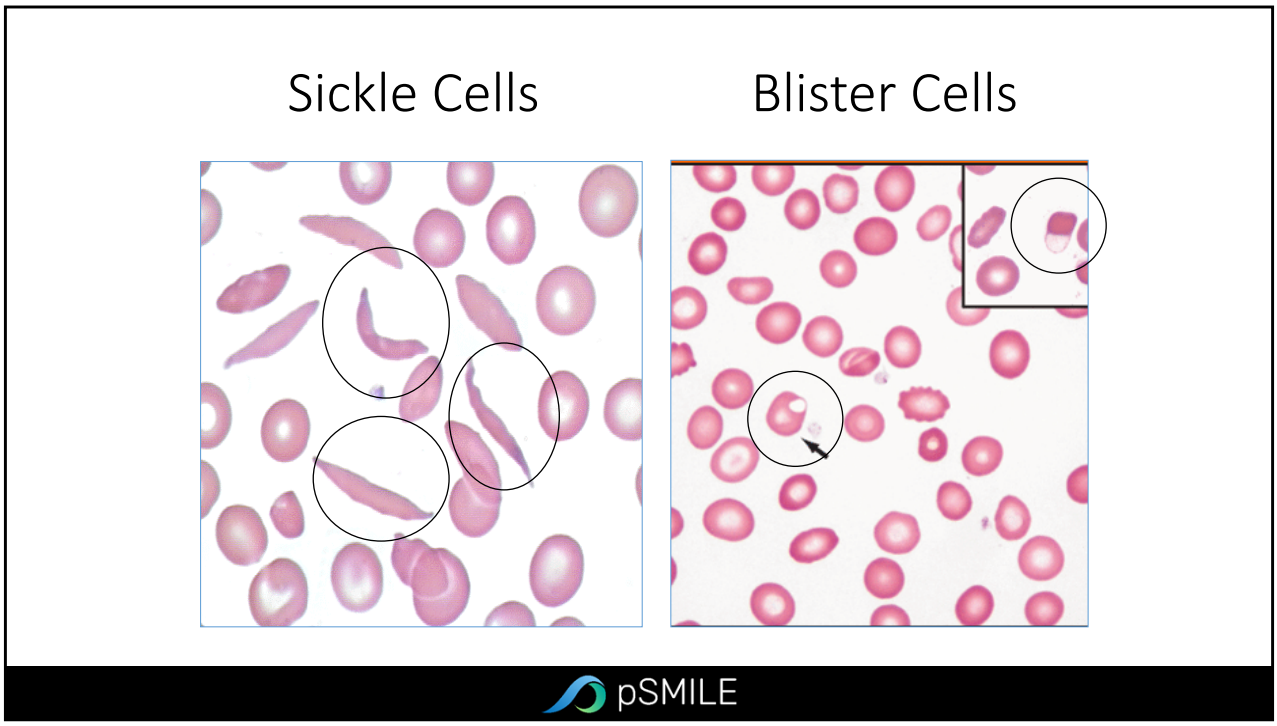
On the other side we have a cell that looks very similar- burrs cells- the difference is that they keep their middle pallor. Usually they are an artifact created during the making of the slide especially if the slide pH is not correct If it is not artifact then burr cells can be seen in patients with severe renal disease or burn patients.

To remember for your Cell ID Surveys when you are trying to decide whether to call it a burr or an acanthocyte - if it has a middle pallor it is a burr cell if none then it is a acanthocyte



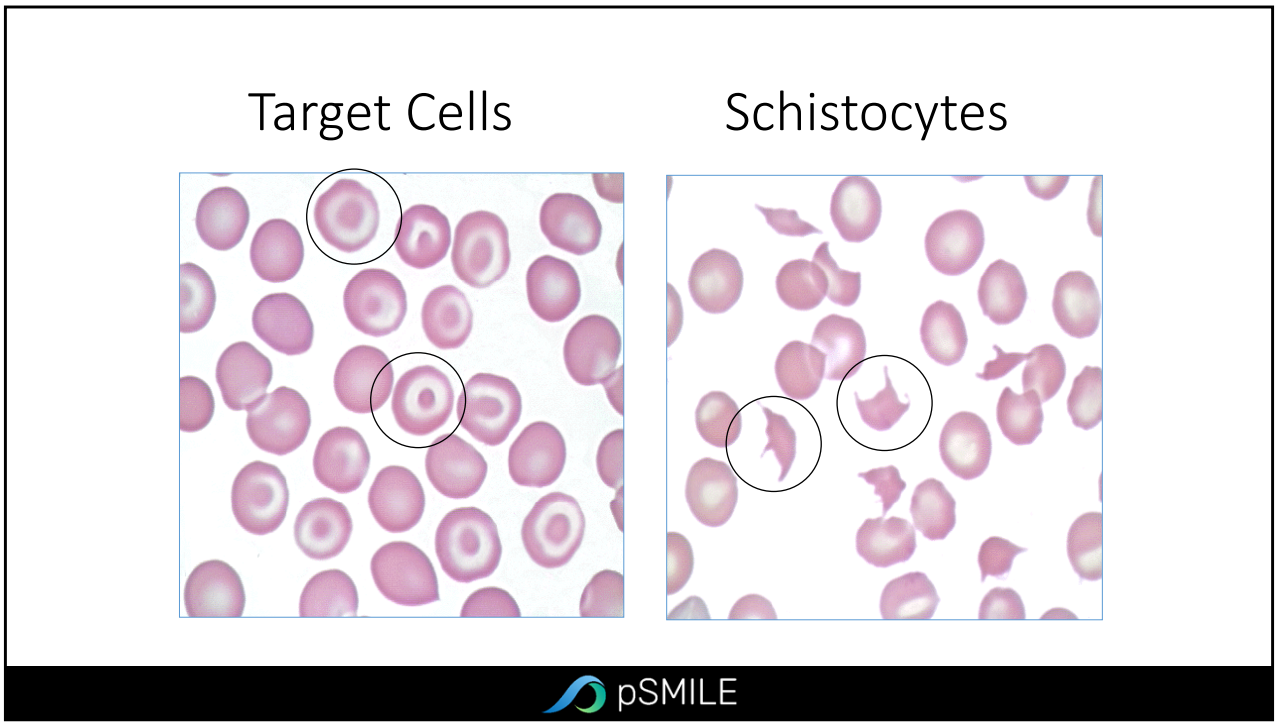
On the left we have Spherocytes
Spherocytes are usually round thicker than normal RBC without any middle pallor and usually smaller in size. They are formed when the membrane loses its biconcave shape and central pallor. The cells are usually removed in the spleen because they won't deform into smaller shape so they get caught in the spleen. Usually seen in Hereditary spherocytosis. This is a Autosomal Dominant Hereditary disorder seen mostly in patients with Northern European ancestry. They can also be seen at the edges but these are no true spherocytes.
Can anyone tell me what indices is increased with spherocytes? MCHC > 36.

For the tear drops they are formed either coming out of the bone marrow or going through the spleen. There are two processes that can cause them. First In certain conditions like Pernicious anemia or myelofibrosis when RBC come out of the BM or go through the spleen as a very slow pace the cell gets pulled our of shape until it finally pops off a small piece and the cell reform in the tear-shape..
The other way is if the RBC contains an inclusions such as Howell-Jolly bodies. As they try to maneuver though the small spaces either in the BM or spleen again they get stretched once again finally popping off the end piece reforming the tear shape.
They can also be artificially made if you push too hard making your slide. These are easy to detect because all the tails will be going in the same direction where in true tear drops they point in different directions.

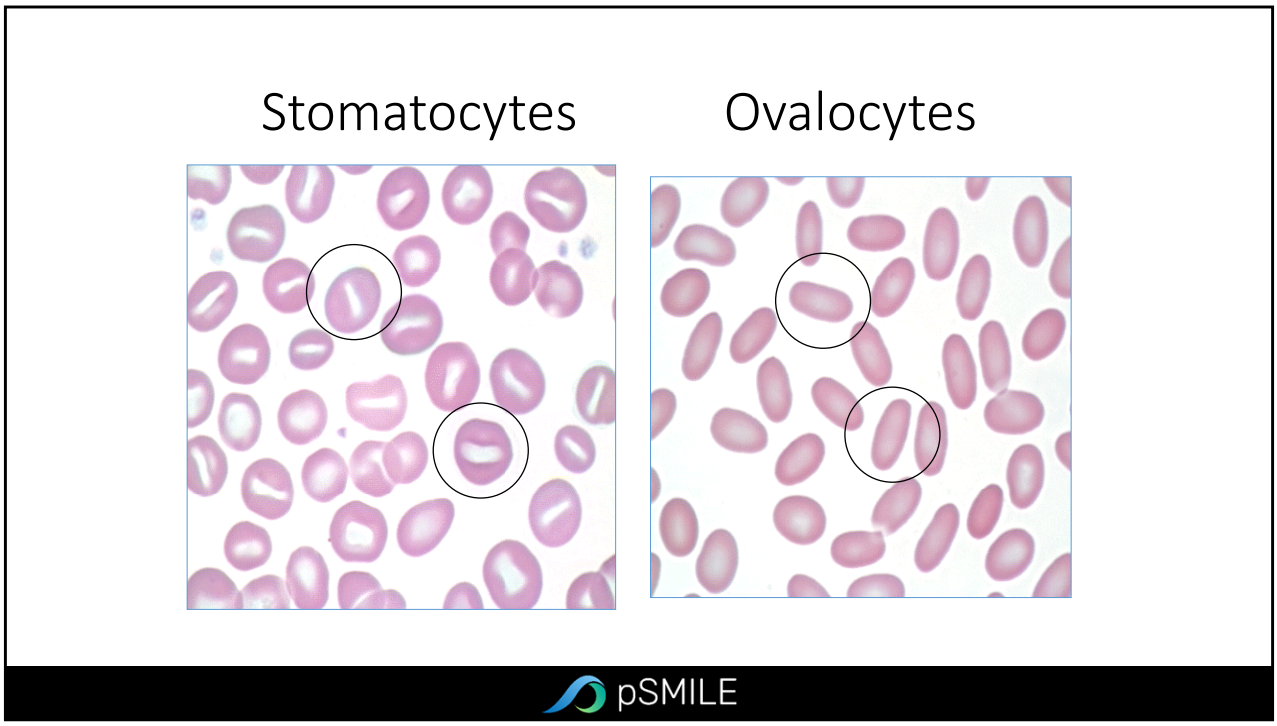


On the left we have Sickle Cells
Sickle cells occurrence are due to the substitution of valine for glutamic acid at the 6th position on the beta chain. Sickle RBC are more rigid due the substitution of these amino acids changing its electrical charge making it less soluble when it lacks oxygen. During low O2 concentration the RBC forms rigid filaments that eventual don't return to a normal shape as the energy is used up in the cell. True sickle forms are only seen in the anemia will the patient is SS. When the patient has the trait only one Sickle gene you get these cells called oats cells.
The one thing I like about hematology naming is that the cells are usually named for what they look like making it easier to remember.
On the other side are blister cells which appear to have a little blister on them. These can be seen in two different cases. One is microangiopathic hemolytic anemia and the other is seen in sickle cell anemia.

Studies show that patients with an increase in blister cells have a increase rick for pulmonary infarctions.



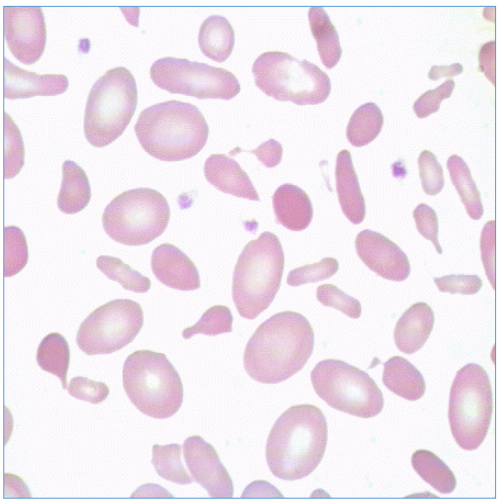
On the left are Target Cells
Another name you may have learned for target cells are Mexican hat cells. They can be found in a variety of hemoglobinopathies to post splenectomy. The are formed by an increased in the surface-to–volume ratio where the excess membrane “sinks” into the center of the cell giving it a target appearance
They can also be an artifact in making the slide. If you use a fan to blow over to dry your slides it could cause the cells to look like targets. They other way is if you wave your slide to dry it. If you wave it slowly these can be created so make sure you use a quick waving motion.
On the other side are schistocytes or fragmented cells. They can have a variety of shapes such as helmets, horned, triangular, but they all lack the central pallor of a normal RBC. They are usually associated with microangiopathic anemia, Disseminated Intravascular Coagulation (DIC), severe burns, Thrombotic Thrombocytompenic Purpura (TTP), Hemolytic uremic syndrome (HUS), to name a few conditions.
So in these conditions you get fibrin strands running across the blood vessels like a clothesline. The RBC travel through these vessels and get caught. The flow of blood finally causes the cells to be sliced into pieces. They reseal themselves thus forming the different shapes. Eventually the spleen will filter out these cells.



On the left are Stomatocytes that look very similar in appearance to a normal RBC the only difference in appearance is that the central pallor is more slit-like not round. Most commonly seen in hereditary stomatocytosis which is a membrane defect. This is also a autosomal dominant inheritance trait making up 10-50% of the RBC population. They are also seen if the blood has a high acidic pH. In the osmotic fragility test these cells will lyses quicker.

Then on other side we have the ovalocytes/ellptocytes. They usually have a rod shape with concentration of hemoglobin on the ends. In normal blood they make up less than 1% of the population. They can be seen in many diseases the most common is Heredity Elliptocytosis which is caused by an abnormality of the RBC skeletal membrane proteins. In this condition they will make up more than 25% of the cell population.
There is speculation that it may be beneficial to have this disorder in malaria prone locations because it seems to have a resistant to it.
They can also be seen in sickle cell anemia, severe iron deficiency anemia, thalassemias

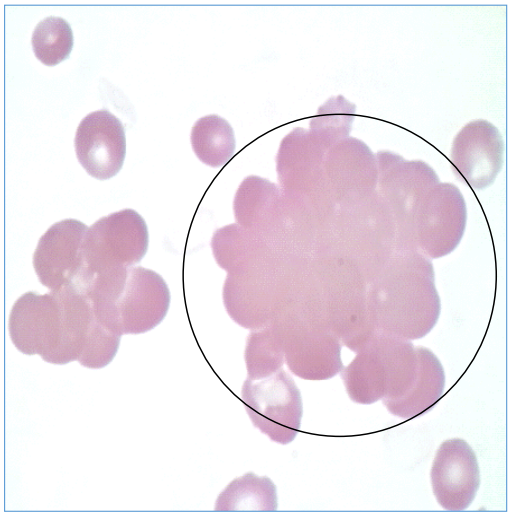
What do we have?



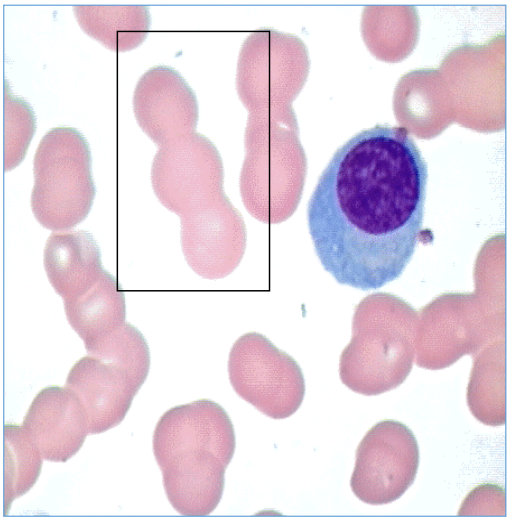
pSMILE

So let's see how many shapes you can find on this slide –
Elliptocytes/ovalocytes
Teardrops
Spherocytes
Schistocytes
Macrocytes Hypochromic
Anisocytosis – variability of the cells in size
Poikocytosis – variability of shape
So you see if you just called Poikocytosis and nothing else the doctor would not know anything.
This patient has Hereditary Pyropoikilocytosis which is a subtype of Hereditary Elliptocytosis -Congenital hemolytic anemia –It is both a defect and deficiency in the spectrin in the cell membrane.
The prefix Pyro in greek means fire
It is named this because in the osmotic fragility test these cells will lyse at the lower temperature than normal
You can also see a similar morphology in a patient that had severe thermal burns.
So not let's look at some inclusion that can be seen with RBC's

Cold Agglutination



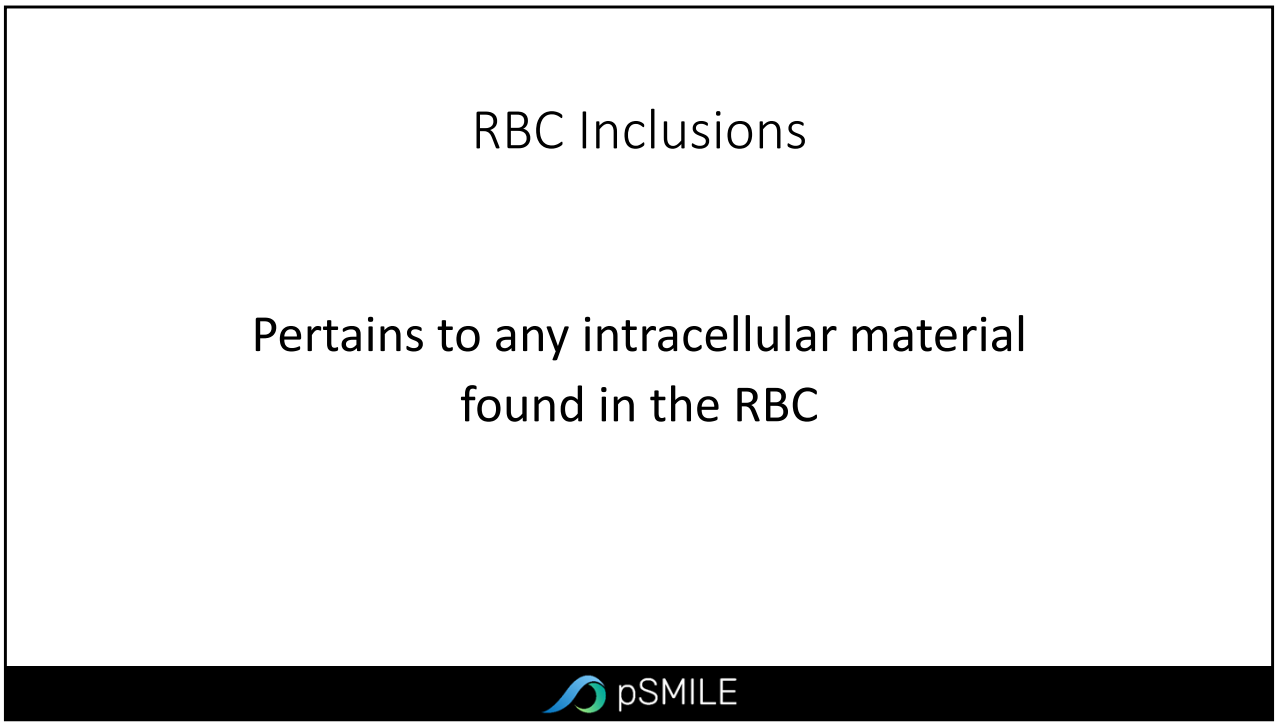
Rouleaux



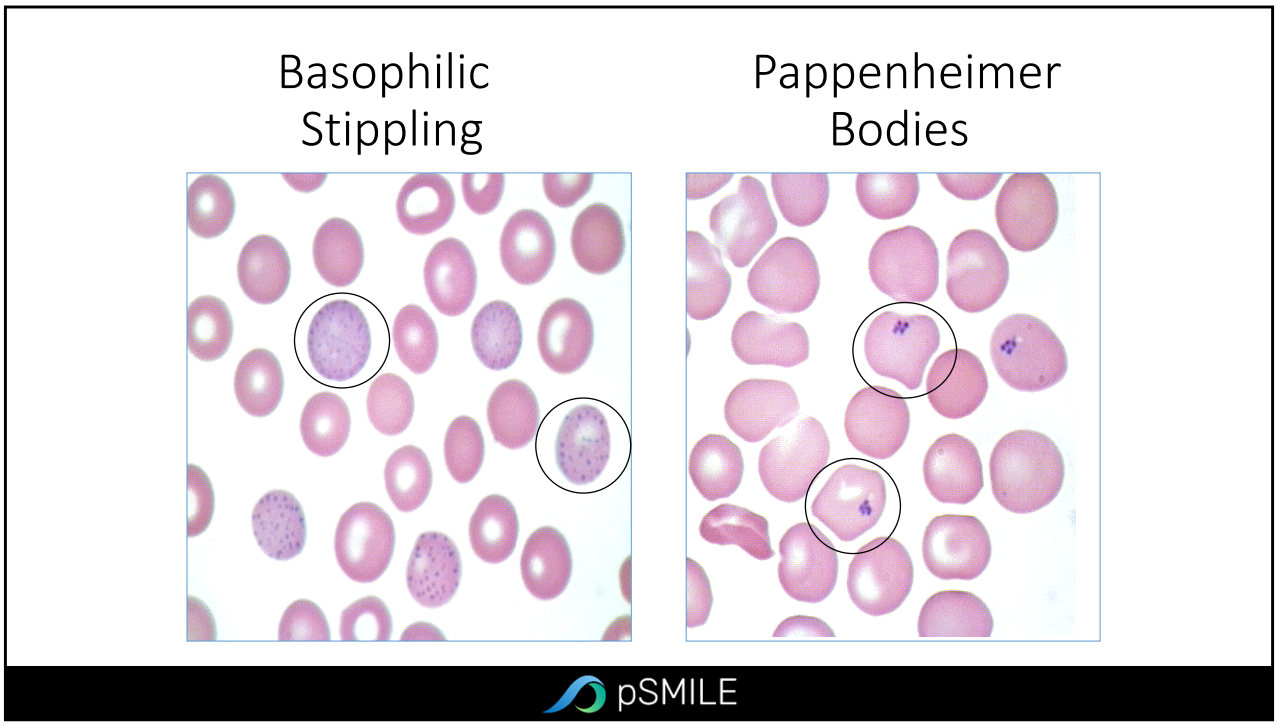
And I just wanted to show you these 2 slides that also give you some problems on Cell ID. Agglutination and rouleaux .

Rouleaux. Is described as stacks of coins. If you see this only in thick area of the slide it is an artifact If it is seen in the thin area then it is significant to note on your report. Most common disease state is multiple myeloma.
Agglutination is the clumping of RBC seen with cold agglutinins most commonly caused by an IgM antibody. It can occur after a viral or Mycoplasma infection, chronic idiopathic cases and underlying lymphoprolifereative disorders and PCH paroxysmal cold hemoglobinuria. For the agglutination that occur after a viral infection it is usually limiting that eventually goes away in a couple of months..

Breakout for unknown RBC grading.

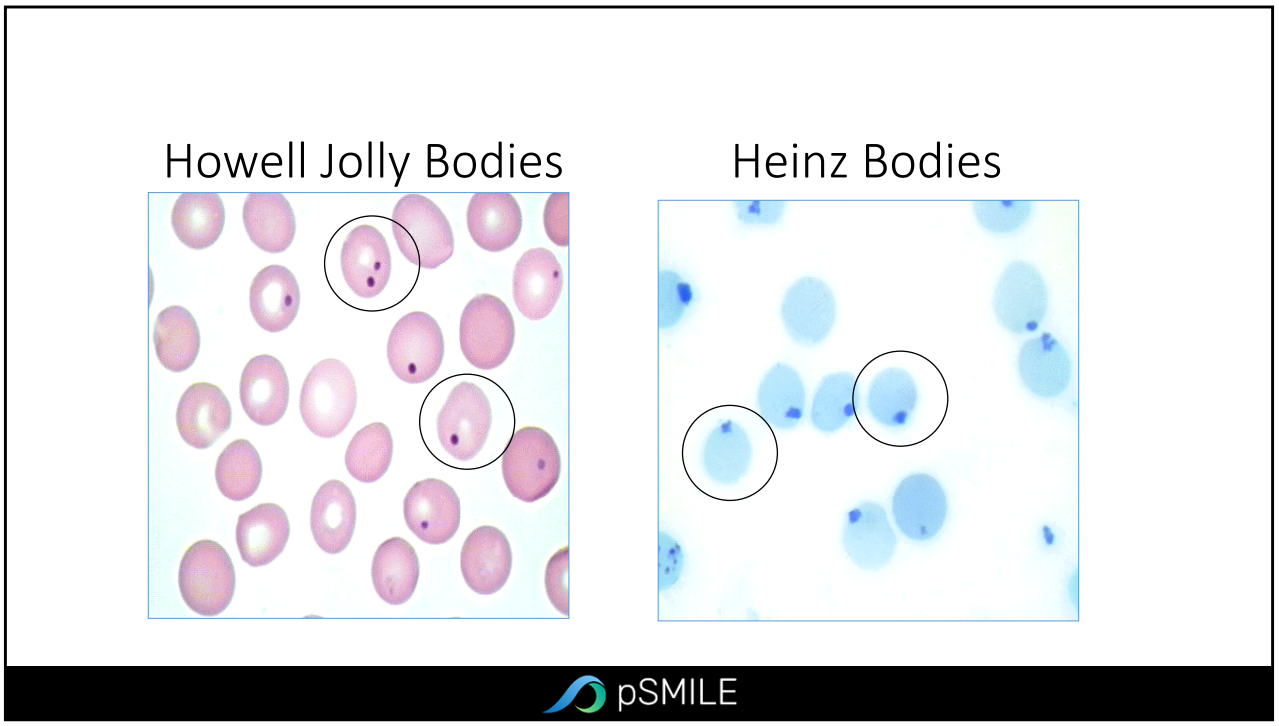


Inclusions pertains to any intracellular material found in the cell



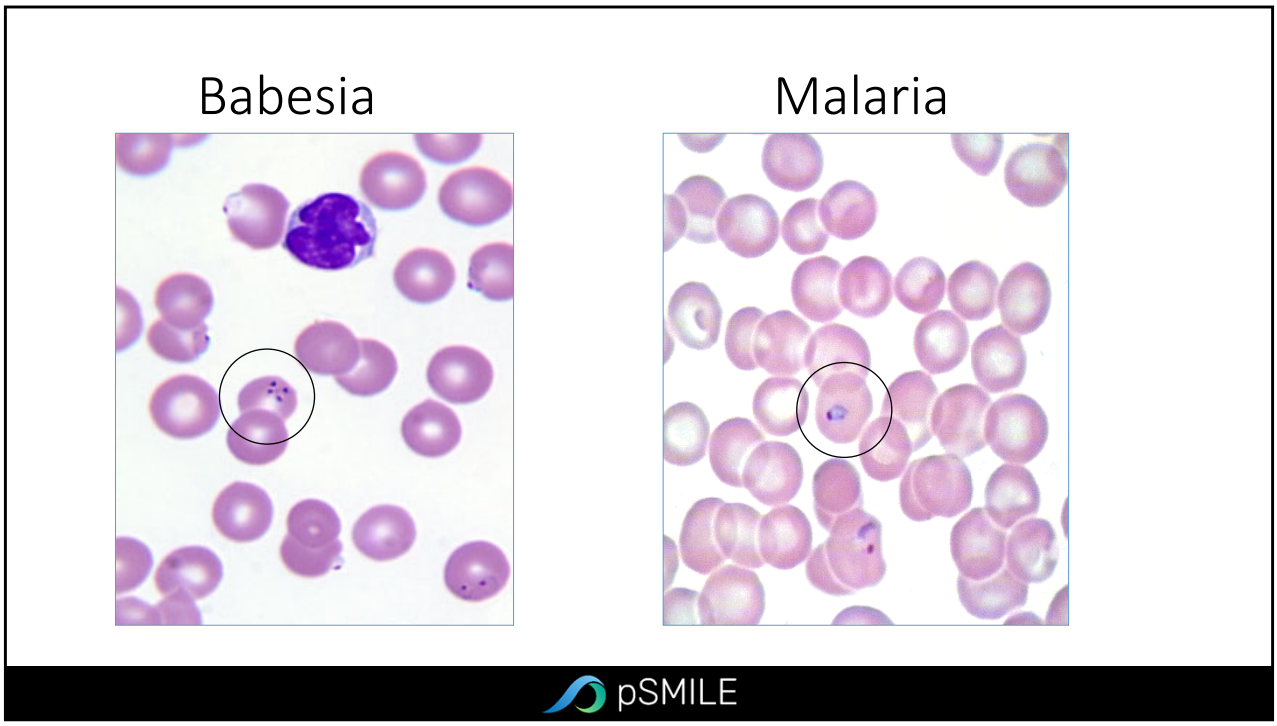
On the left is Basophilic stippling - Basophilic is due to staining aggregates of ribosomes containing RNA and may be seen in thalassemia, lead poisoning and sideroblastic anemias and sickle cell anemia. When it is coarse stippling it is never normal and should be noted.

On the side are Pappenheimer bodies which are composed of iron that are visible with either Wright stain or Iron stain such as Prussion Blue. They can be seen in a variety of disease states including sideroblastic anemia and following a splenectomy, thalassemias, and megablastic anemias.



These two slides look very similar.
On the left are Howell Jolly Bodies
Howell Jolly Bodies are nuclear fragments often seen in people without spleens – They are composed of DNA not Iron. They can be seen in severe hemolytic anemias and megablastic anemia.

On the other side are Heinz Bodies which are composed of denature hemoglobin and may be seen only using what special stains -supravital staining (new methylene blue or crystal violet). They are seen in patients with G6PD or unstable hemoglobins. They can also be seen if you use old blood more than 1 hour old. As earlier in the presentation it was important to make the slides as soon after collection as possible. So on a Cell ID survey to tell them apart if the stain used is Wright stain they are Howell jolly Bodies and if it is Supravital then they are Heinz bodies.



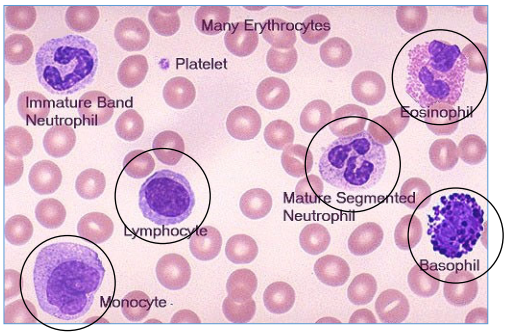
Here we have parasitic infection that can look very similar on surveys.
On the left is Babesia.
Babesia is transmitted by a tick and is seen in the US particularly in the New England and Wisconsin area. Usually it is a very mild disorder by it is worst in patients without spleens. This tetrad arrangements is a good way to differentiate it from Malaria.

Malaria seen on the other side is transmitted by mosquitoes. The RBC usually have a single ring form with a dot. In some varieties you may have more rings and also depends on the intensity of the infection.

On the Blood parasite and Cell Id surveys use the description of the patient to help figure out which it is. If the patient was only in the US then most likely the Babesia. If they have been in malaria infected areas then think malaria.

White Blood Cells

- There are five morphologic types of WBCs normally seen in the peripheral blood.
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - Basophils



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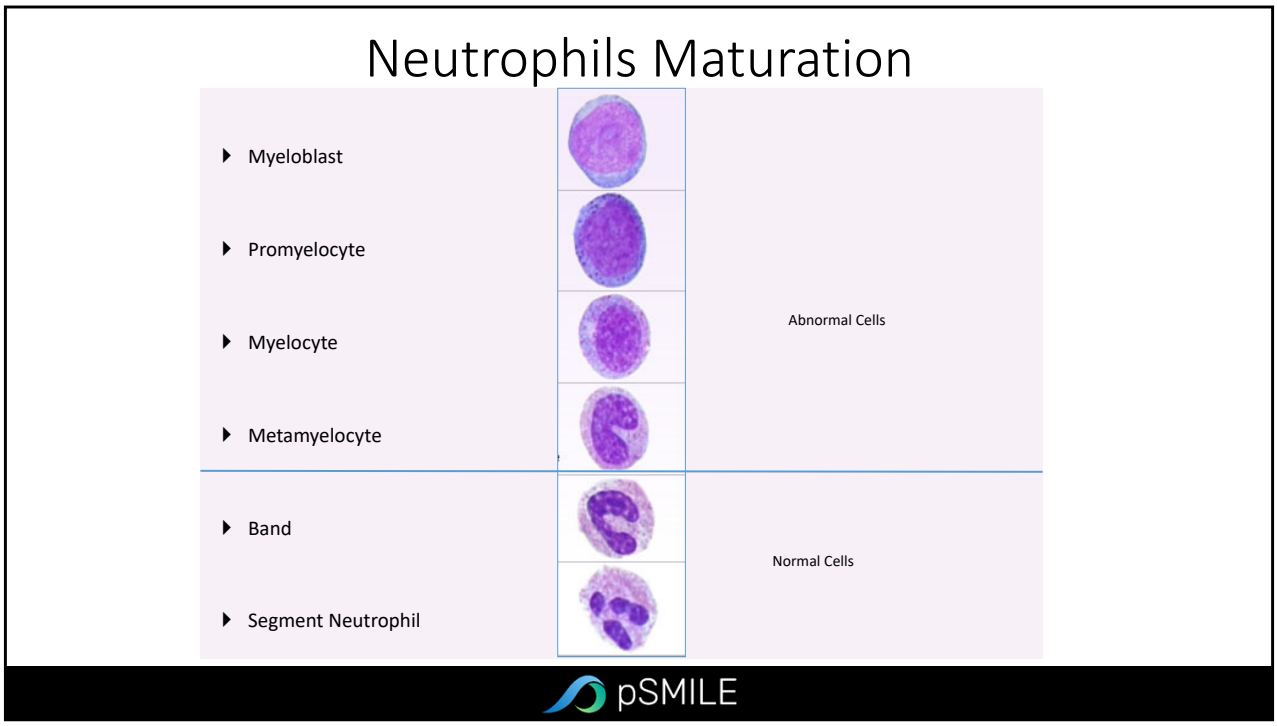
There are five different types of WBC seen normally in the blood and they are Neutrophil, Lymphocytes, Monocytes, Eosinophils and Basophils.

We will look at the granulocytes first



Here we have the maturation stages of the Neutrophil

First four stages are normally seen in the bone marrow. Stage one is the Myelobast. It is a large cell with a large and round nucleus with delicate chromatin and nucleoli present. You have scant blue cytoplasm around the nucleus with no granules.

Stage 2 is the Promelocyte – It is similar to the blast but the nucleus is starting to get slightly coarse and the primary granules appear in the cytoplasm.

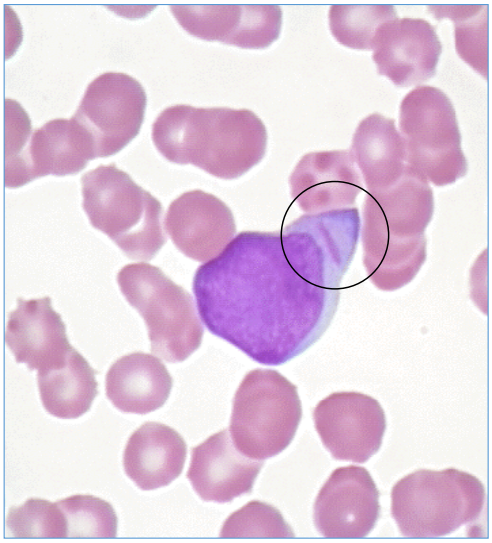
The third stage is the Myelocyte. The cell is decreasing in size and the nucleus in getting coarser without any nucleoli present. The cytoplasm is changing color it is more light blue to pink. At this stage the secondary granules appear depending on the cell line. For neutrophils they are fine blue pink in color.

Stage 4 is the Metamyelocyte. The nucleus is getting indented or kidney shaped. The chromatin of the nucleus is coarser and the cytoplasm is a light pink with fine pink-purple granules.

Stage 5 is the Banded neutrophil. The nucleus in indented more and the chromatin is getting coarser and darker. They cytoplasm is now light pink with fine pink-purple granules. These can be seen in low numbers in the peripheral blood

The most mature is the segmented neutrophil. It usually contains 2-5 lobes joined by thin filaments with very coarse chromatin. The cytoplasm is pale pink with fine pink-purple granules.

Auer Rods



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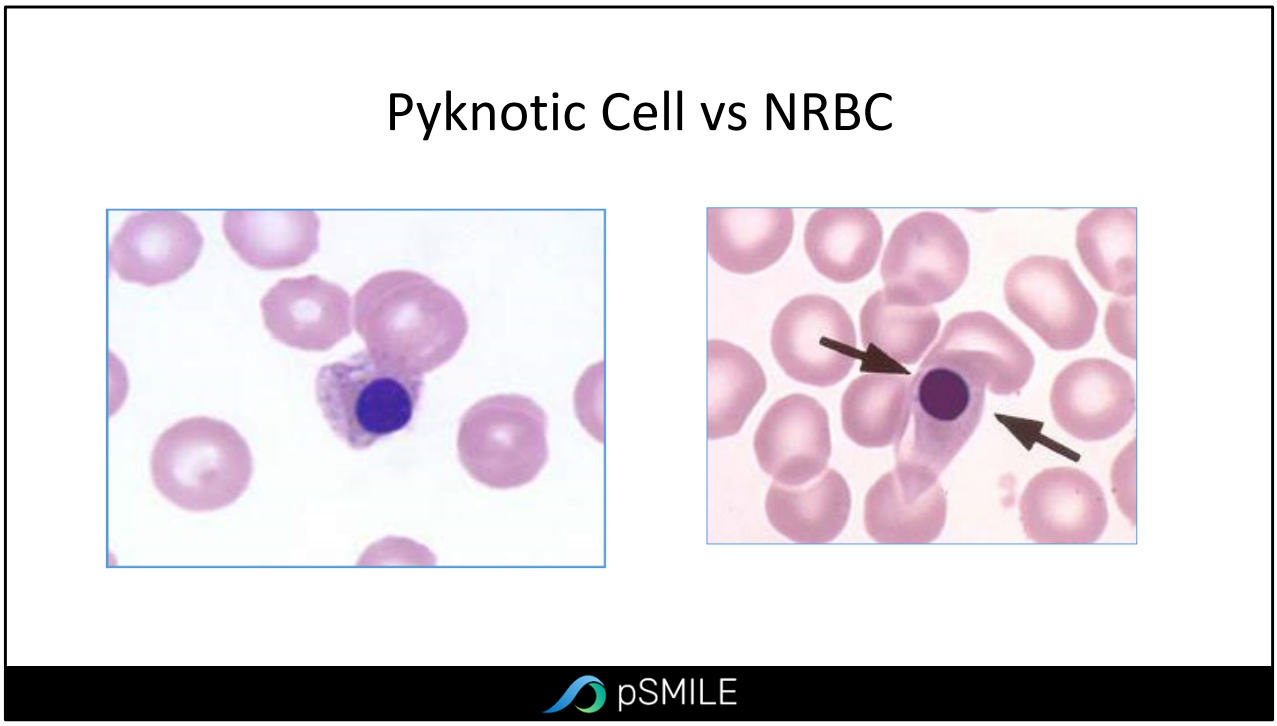
On the left is a Myeloblast with an Auer rod that can be seen signally or in clusters. The can be seen in myeloid leukemia blast. It is generally considered most diagnostic clue for acute non-lymphocytic leukemia- AML or AMML when you see these.

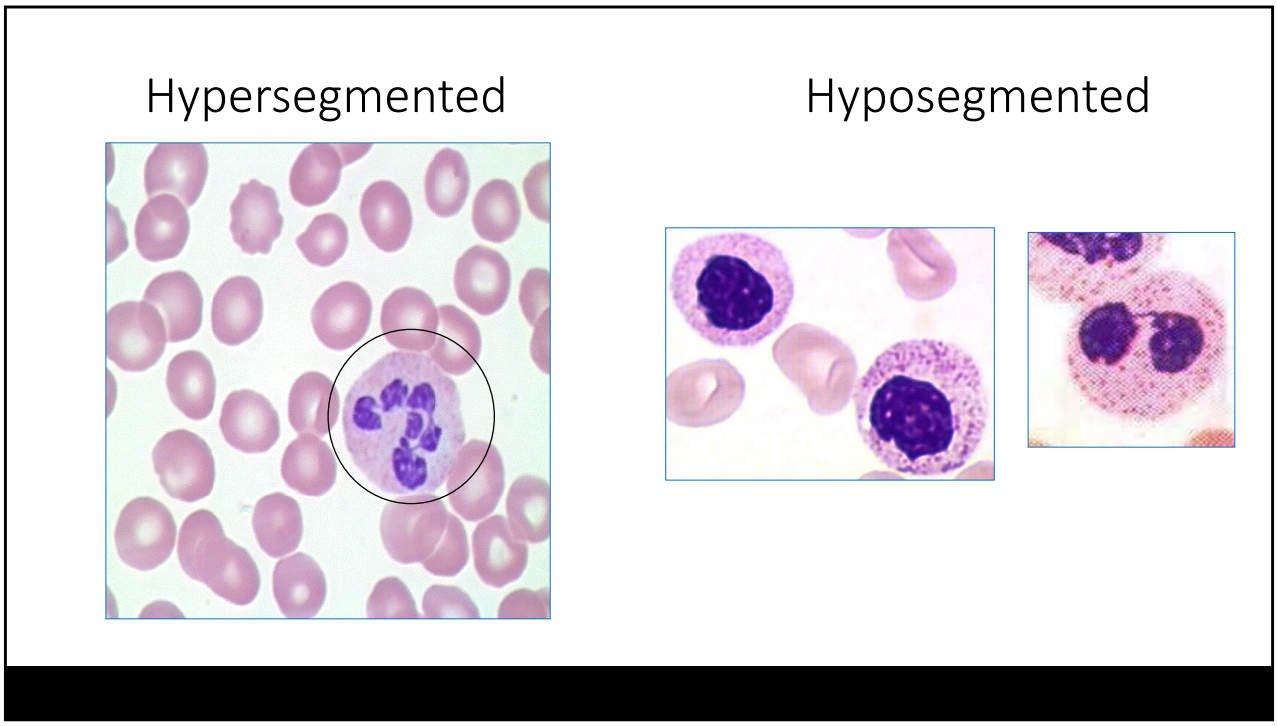
On the side on top is a NRBC . It has the condensed nucleus and pink cytoplasm.

Below we have a Necrotic or degenerated neutrophil also called a pyknotic cell. This is a neutrophil that has degenerated. The nucleus become dense and homogeneous it can have several lobes or be just one lobe as seen here which could easily be mistaken for a NRBC.

It is important to differentiate these two cells. The nucleated RBC cytoplasm will be pink in color with no granules while Pyknotic neutrophil will have granules.



Pyknotic cell chromatin condensation. Degenerative condition of a cell nucleus marked by clumping of the chromosomes, hyperchromatism and shrinking of the nucleus

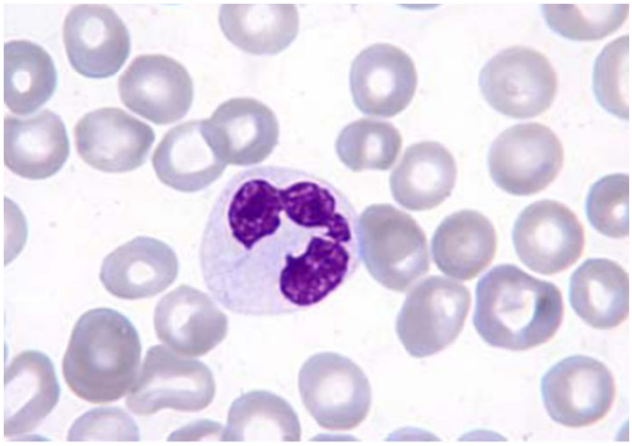


On the left we see have Hypersegmented Neutrophil that can be seen in B12 and folate deficiencies. This term is used when the cell contains 6 or more lobes. In these anemias they will be seen in greater than 5%.

On the right we have Hypo-segmented neutrophil
The upper slide is of a typical cell seen in Pelger-Huet Anomaly which is an inherited disorder. It usually has the classic bi-lobed nucleus connected by a fine filament making it look like a eyeglass or pince-nez

Beneath is we have the Pseudo-Pelgroid cells.
These cells are form due to abnormal maturation of both the nucleus and cytoplasm. It can cause the nucleus not to mature and divide into lobes but stay a single dense nucleus that could be mistaken for a myelocyte.
VERY IMPORTANT to differentiate that these are not myelocytes but pseudo pelgroid cells. If you call them myelocyte you could lead the doctor to think that they have an immature picture - possibly leukemia or a very bad bacterial infection. They should be given their own category and specified as Pseudo-Pelgroid cells.
These can cells be seen in myelodysplastic syndrome and patients receiving chemo.

Hypogranulation



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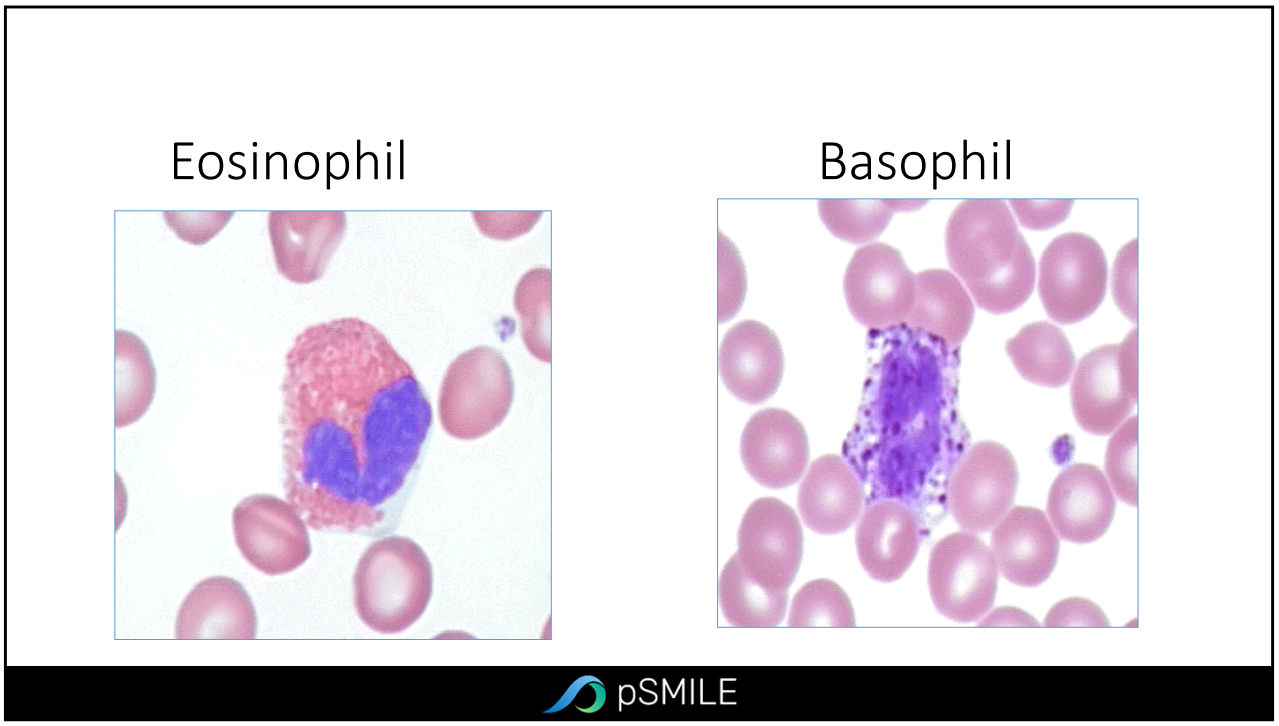
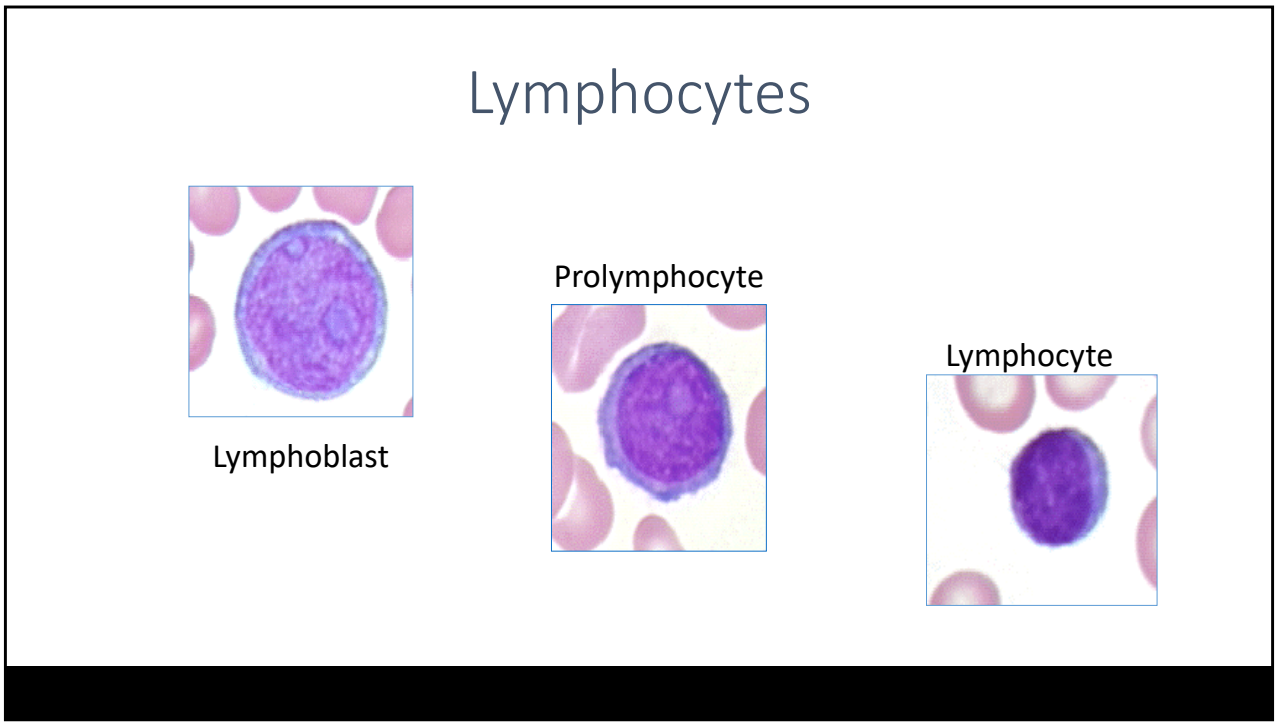
Here we have Hypogranulation witch the lack of granules.

This is usually seen in myeloprolifereative diseases but you can also see it in patients with HIV.



Two other granulocytes are the Eosinophils and Basophils . They start out the same but again at the myelocyte stage they differentiate into each cell line getting their distinguishing granules.
For eosinophil these are numerous, coarse, orange-red granules of uniform size. Mature Eosin usually have two round or ovoid nuclear lobes of equal size. They are normally seen in low numbers on the slide but increased in parasitic infections.

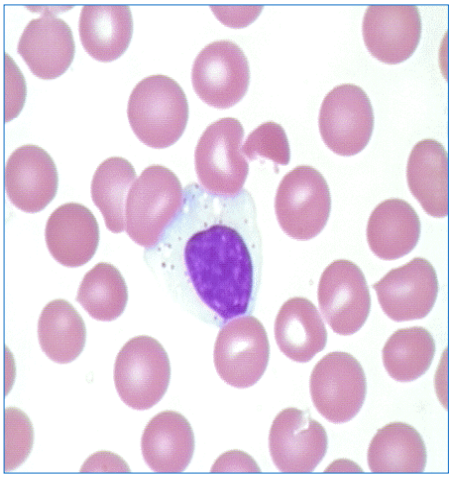
For the basophil they contain small to moderate number of coarse densely stained granules of different size and shapes. These granules are larger than those seen in the neutrophil. They are usual a blue-black color but can also stain purple to red. The granules usually can cover the nucleus. These also are seen in very low numbers in the blood but increased in certain leukemias such as CML and also slightly increased when Eos are increased.
Let's look at some inclusions and structural variations that should be noted on your manual differential or scan for these cell lines.



For the lymphocyte there was three maturations stages.

First we have the lymphoblast. There are actually 3 different types of lymphoblast. To differentiate them you need to do special testing such as flow to look for cell markers. They usually are a large cell with fine chromatin in the nucleus and several nucleoli. Like all blast there is scant amount of dark blue cytoplasm. They are usually only seen in the bone marrow but if in the blood most likely seen in young children with ALL.
Next is the Prolymphocyte. It is a little smaller, condenser nucleus with only one prominent nucleoli. Again not usually seen in peripheral blood but if seen most likely an older adult with CLL. They usually make up about 10% of the population.
Then the last stage is the mature lymphocyte. They can be seen both in the Bone marrow and the peripheral blood. They are small compact cells with a round or oval nucleus with very condensed chromatin and no nucleoli. There is small amount of dark blue cytoplasm.

Large Granular Lymphocyte



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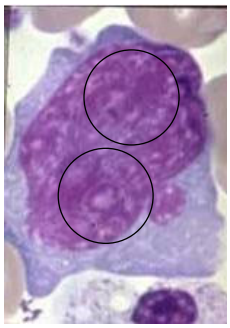
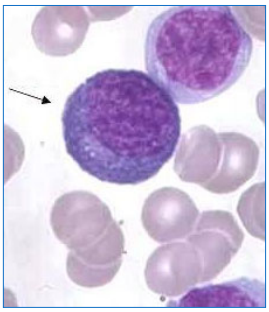
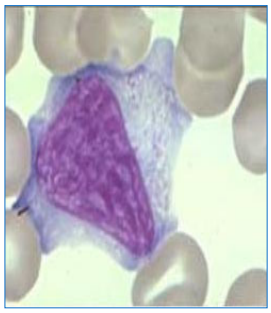
Some lymphocytes that have more abundant cytoplasm as seen here can contain a few, coarse, unevenly distributed, granules. These are referred to **as large granular lymphocytes**. They are seen in normal blood smears but may be increased in patients with reactive lymphocytes.

Reactive Lymphocytes



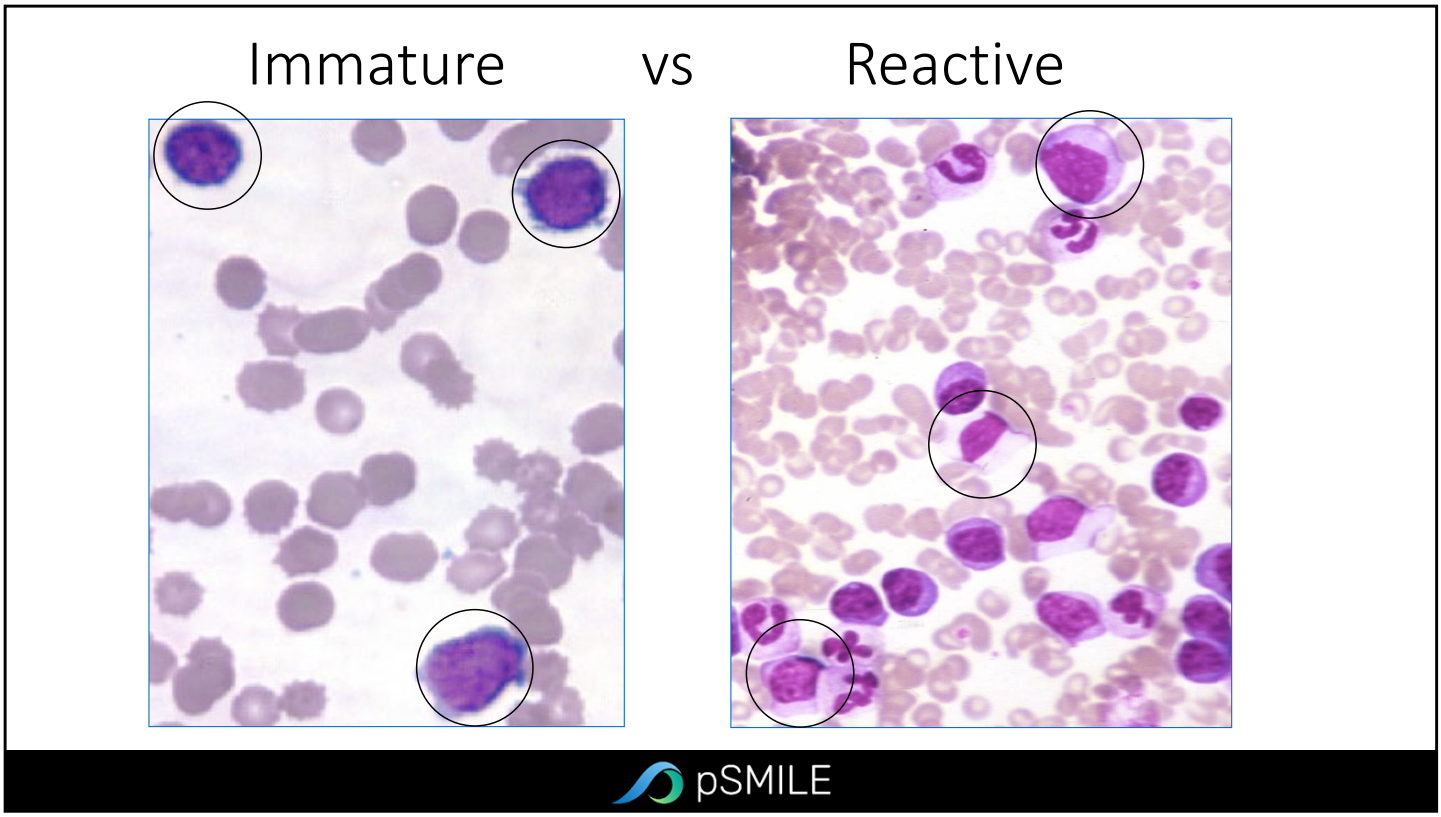




Here we have some examples of reactive lymphocytes. They can also be called atypical lymphs or abnormal lymphs. It all depends on your laboratory policy.

They may contain vacuoles and the nucleus are usually irregular with an open chromatin pattern and may contain nucleoli (Click). There are three classification of reactive lymphs – Downey Type I, II and III.

On the right (Click)we have the typical Type II with the abundant cytoplasm that mold around the red cells. This was called ballerina skirting in my training.



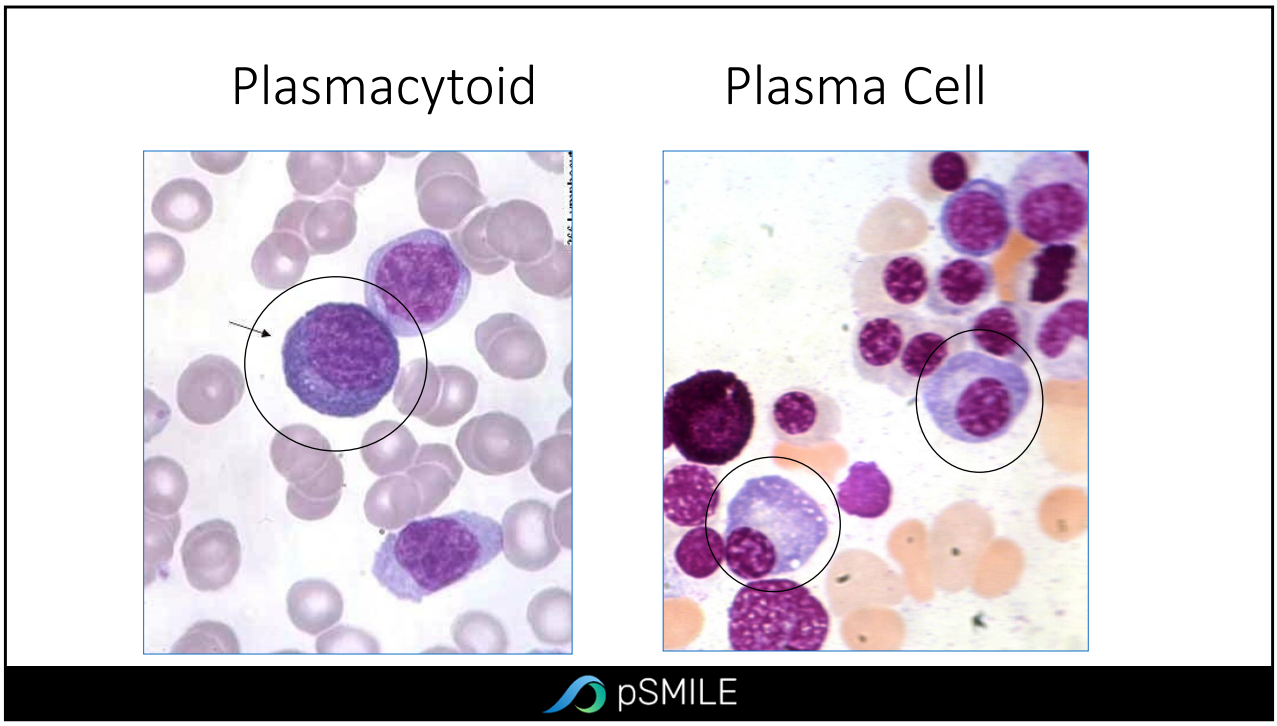
Sometimes it can be hard to tell if it is just a reactive lymphocyte population compared to a malignant – leukemia population.

Before you start counting your white cells as previous mentioned in the beginning of the talk you should scan of the slide to get a idea of the cells on it and make sure the cells are evenly distributed on the slide.

If you see a lot of variability in the lymphs as seen on the left where you have some with some with nucleoli(Click) in the nucleus. Some with abundant cytoplasm (Click) and skirting and others having eccentric nucleus (Click), than you can think - variability – reactive population.

Where on the right slide all the lymphs (Click) look alike. They have a couple of nucleoli in the nucleus and scant cytoplasm around them. Monotonous – monoclonal - malignant – leukemia.

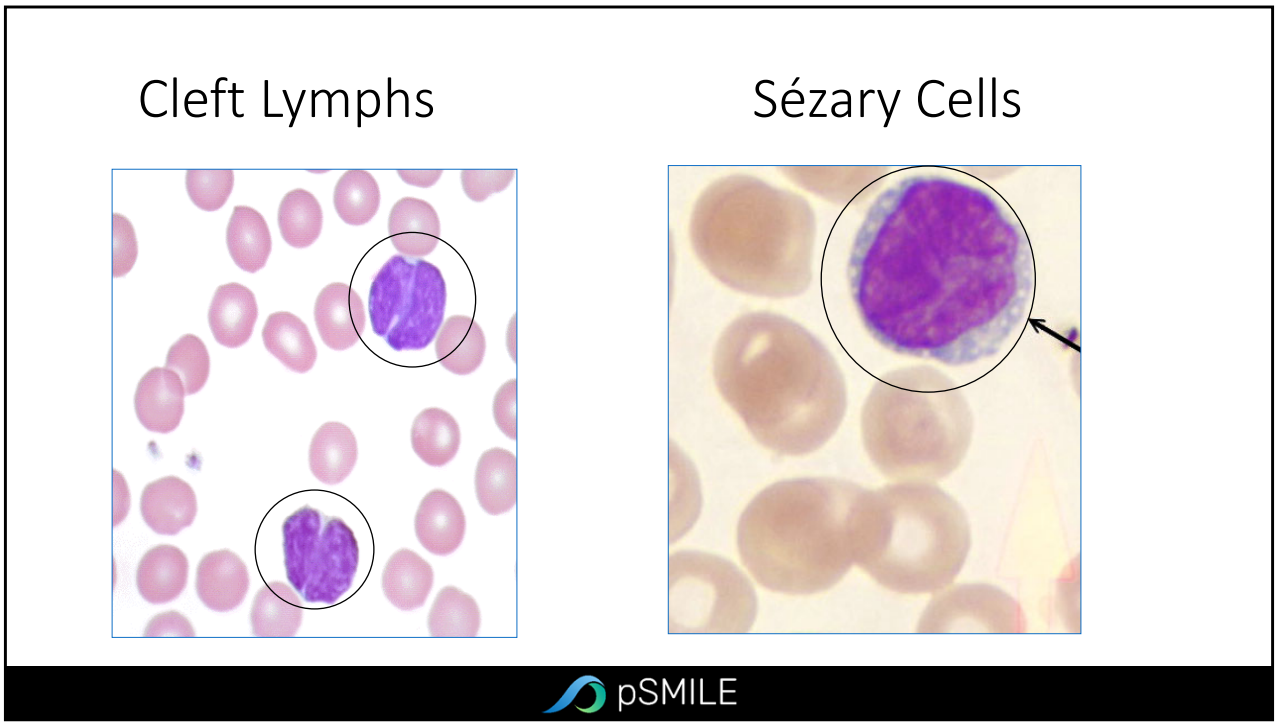
I use this same technique when looking at the cells on a body fluid slide too.



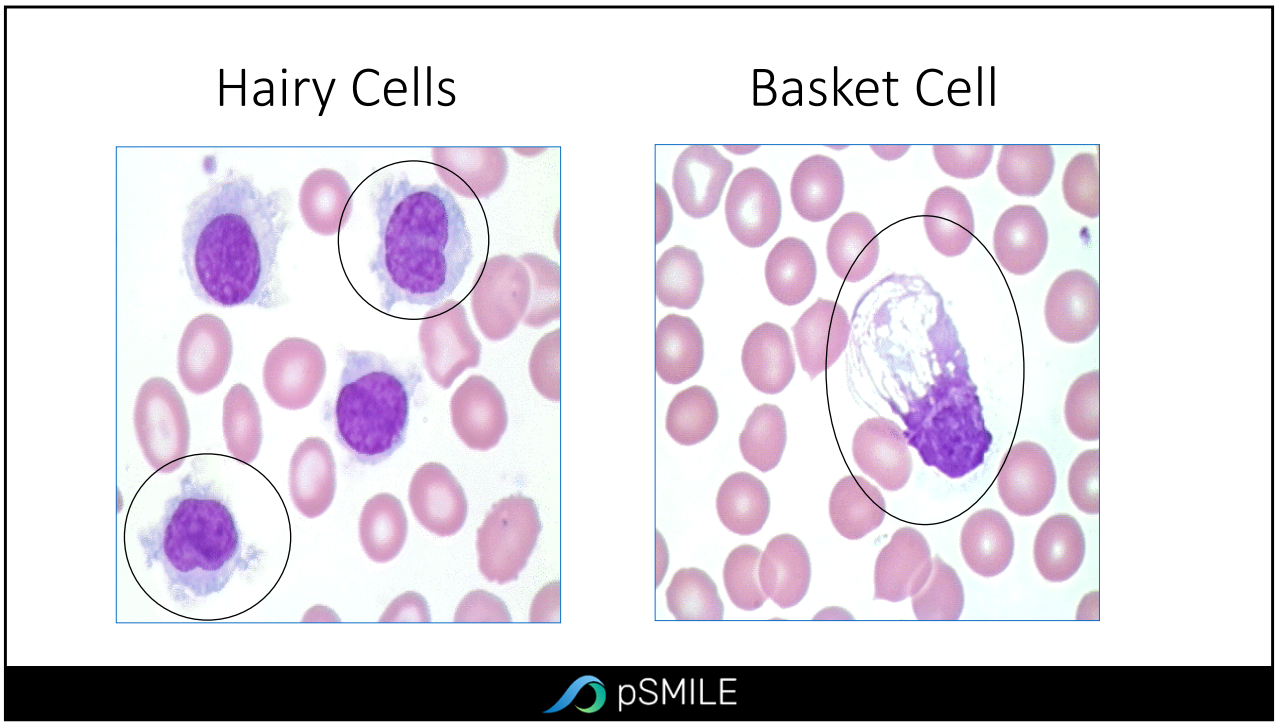
On these slides we have two cell lines that also confused technologist

On the right we have plasma cells. Plasma cells will have this lighter hof area. Plasma cells are rarely seen in the normal blood stream. They are medium size, round to oval cells with abundant cytoplasm and an eccentric nucleus. The ones that have a pink-red cytoplasm are called flame cells. They can be seen in multiple myeloma.

On the other side are plasmacytoid lymph. They are intermediate size and have a round to oblong shape. They have a round nucleus that is usually slightly eccentric. The chromatin of the nucleus is usually coarse. They can contain a hof area but not as distinct as seen with true plasma cells. The cytoplasm is usually very dark blue. This is just a reactive lymphocyte.

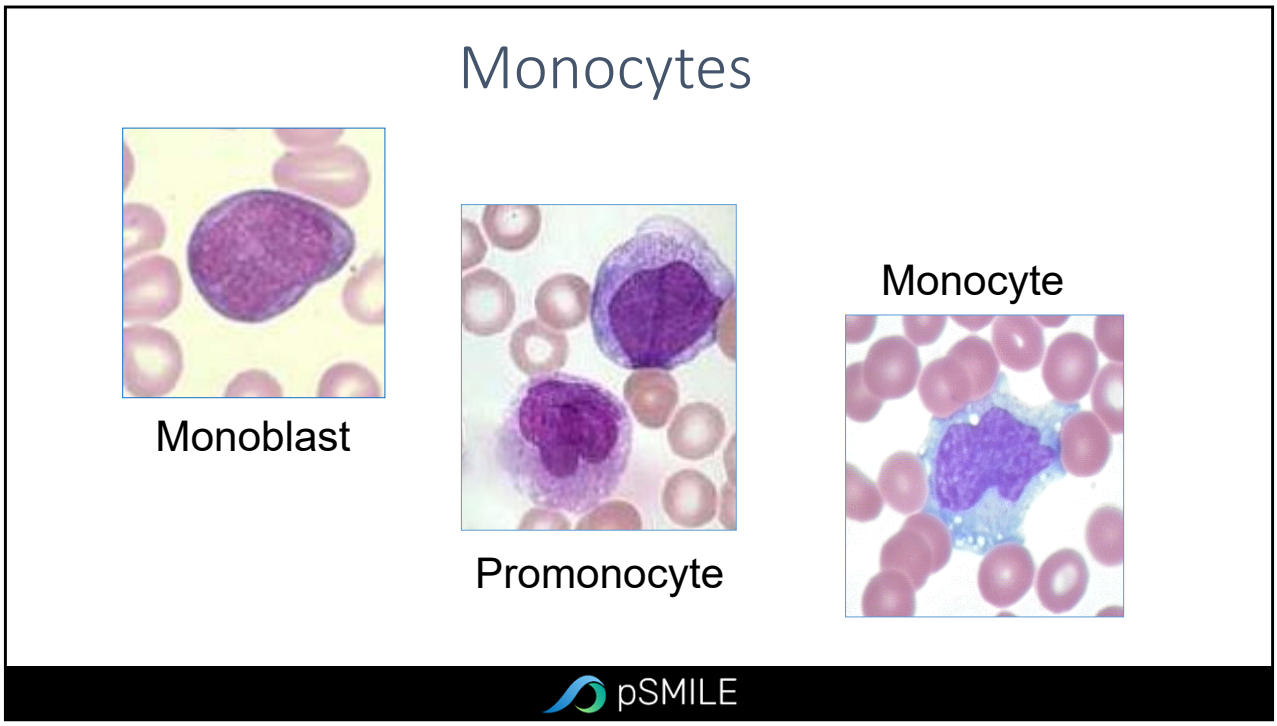


On the left we have cleft cells or as my husband calls them the “butt-cracked cells”. Depending on the cause these can be seen in certain infections such as whopping cough others can be seen certain types of lymphomas.
On the right we have Sezary cells. These are found in patients with mycosis fungoides which is a cutaneous T cell lymphoma. They are usually round to oval with folded, grooved, or convoluted nuclear membranes that give them a cerebral-form appearance. The cytoplasm is usually pale, blue to gray and can have many small vacuoles adjacent to the nucleus giving it a “pearl necklace appearance.”



On the left we have Hairy-Cell Leukemia lymphs. These are seen in a chronic lymphoproliferative disease of the B-Cell origin called Hairy Cell Leukemia. It is normally seen in middle-aged elderly males. The classic hairy cell is round to ovoid lymphoid cell that are slightly larger than normal mature lymphs. The usually contain more cytoplasm that is pale blue to grayish blue and the border have the characteristic elongated, fine-hairy cytoplasmic projections. To me it looks like they are trying to push the other cells away. The nucleus is usually finer and can have folds in it. What is the special stain you use to differentiate these cells – TRAP - Tartrate Resistant Acid Phosphatase Stain

On the left frame we have a Basket cell or smudge cell. This name is used for cell death most commonly associated with cells that are fragile and easily damaged in the process of making a blood smear. The cell is usually a lymphocyte. You end up with the nucleus that is either smudged or the chromatin strands spread out from a condensed nuclear remnant giving it the basket-like appearance. Usually there is no cytoplasm or very indistinct. They are associated in some disease states characterized by lymphocyte fragility such as infectious mononucleosis and CLL. One way to avoid these on smears is adding a drop of 22% bovine serum albumin to four or five drops of blood before making the slide. The albumin will cushions the cells and prevent the fragments. Sometimes you can also get fragmented cells on pediatric slides due to trying to spread the thicker blood they may have due to a high Hemoglobin. You end up putting too much pressure on the cells and cause them to fragment. So try using the albumin technique to get a better slide
So let's go onto the Monocyte line

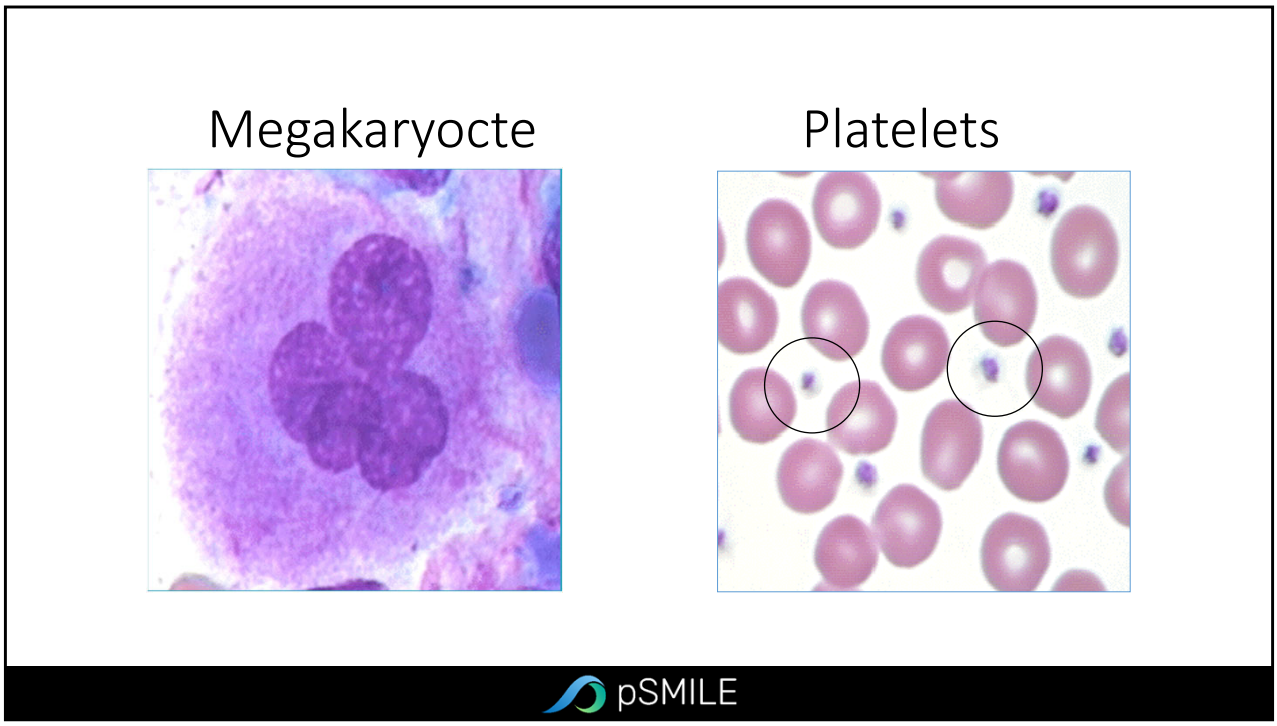


There are three maturation stages for the Monocyte.
First is the Monoblast. It usually is only seen in the bone marrow. It looks similar to other blast except usually it has a little more cytoplasm and the nucleus can have folds. The only way to differentiate between all the blast is with special stains and flow which check the cell markers. For Monoblast nonspecific esterase stain will differentiate these from other blast.

Next is the Promonocyte. It is a little smaller than the blast. The nucleus can be indented and it may or may not have nucleoli. They have moderate amount of gray blue cytoplasm and can have granules.

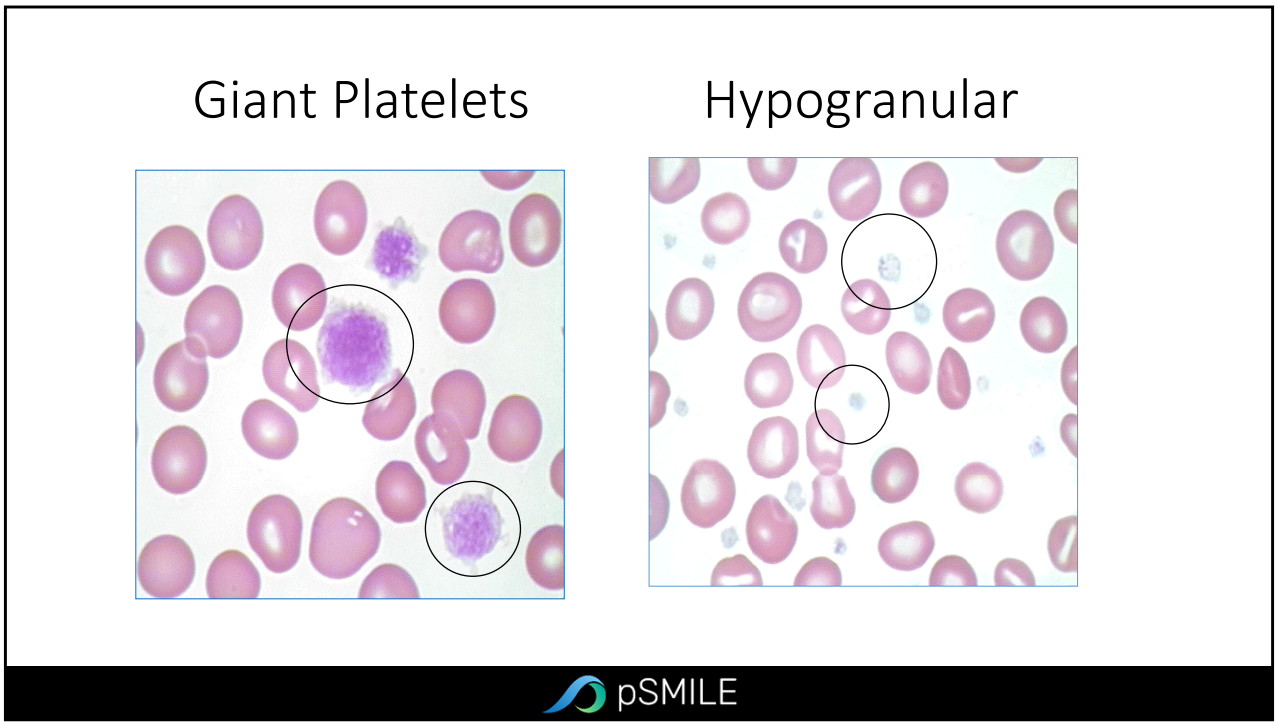
Next is the mature monocyte. It is usually larger than the neutrophil. It usually has abundant grey/gray blue cytoplasm that may contain granules and vacuoles. The cytoplasm can have pseudopods showing its movement through the blood stream. The cytoplasm can have a variety of shapes. It can be kidney shaped, folded or indented. The chromatin is usually described as brain-like convolutions.
What is a monocyte called when it goes into the tissues? Histocyte or Macrocyte.

Let’s go to the last cell line to review - Platelets



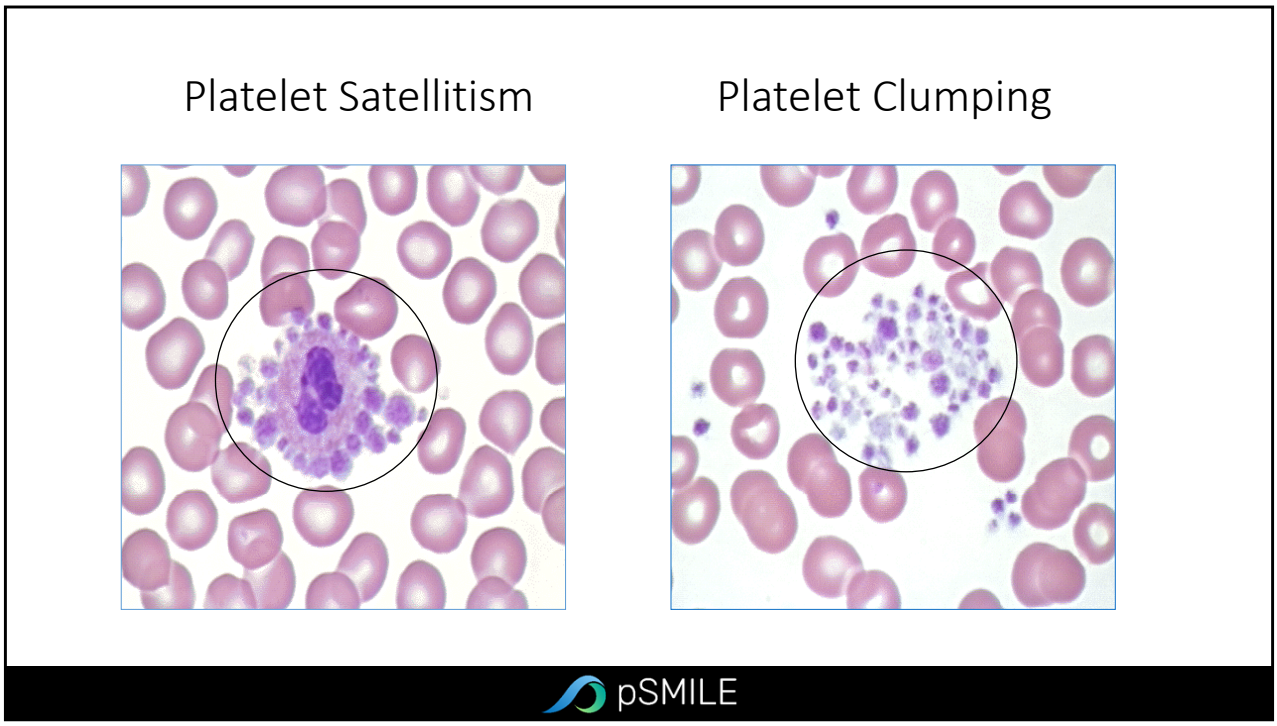
On the left we have a the momma platelet maker a megakarocyte in its the final stage of maturation. It usually contains a multilobed nucleus and its cytoplasm is actually the matured platelets . They exit the bone marrow and get lodge in the lungs where the rapid blood flow causes the megakaryocte cytoplasm to fragment into what we know as the platelets.

So platelets are actually cytoplasmic fragments that contain no nucleus. They are small round or oval blue-gray in color. They are usually single but may form aggregates. Normal platelets contain at least some azurophilic granules.
Usually a normal range is equal to 8-15 per 100x oil immersion.
Next we will look at some variations of platelets that can be seen.



On the left we have large or giant platelets. For Cell ID proficiency that have a normal MCV you should call it a large/giant platelet when the platelet is larger than the average RBC. They are seen in myeloproliferative disorders and myelodysplastic syndromes.

On the other side are hypogranular or agranular platelets. As its name implies it has reduced or absent granularity. They may be normal in size or enlarged and/or misshapen. The cytoplasm stains pale blue or blue gray. They can be associated with myeloproliferative disorders and acute leukemia's or CML

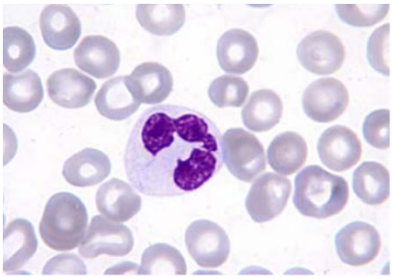


And here we see two examples of clumped platelets.
On the left we have Platelet Satellitism. It always reminded me of an old western where the wagon train is surrounded. This clumping is usually around banded and segmented neutrophils. It can be associated with an interaction with EDTA.
On the other slide shows platelet clumping too.

In both cases because of the clumping of the platelets your instruments printout can show a decrease platelet count and an increase in either RBC or WBC depending the size of the clump.
If the clumping is caused by EDTA drawing the blood in a blue top can help alleviate the clumping. You just have to make sure to mix the blue top and of course you can't use one that had been already spun and had plasma take off for coagulation testing. You need to multiple the results by 1.1 and add it to the total to accommodate the dilution factor of the anti-coagulant in the blue tops. Later in the presentation you will hear other ways to deal with platelet clumping.
In cases of severe clumping only an estimate should be reported not a numerical answer. Again this should be a laboratory policy.

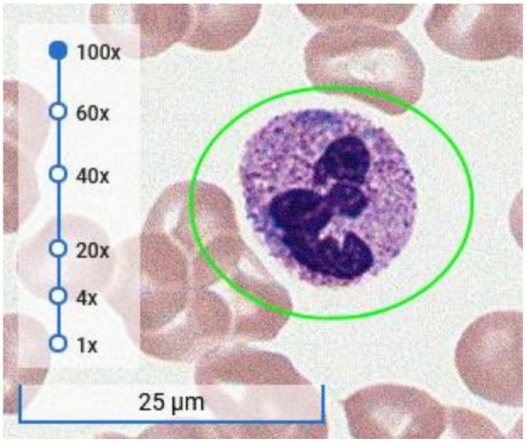
QUIZ TIME

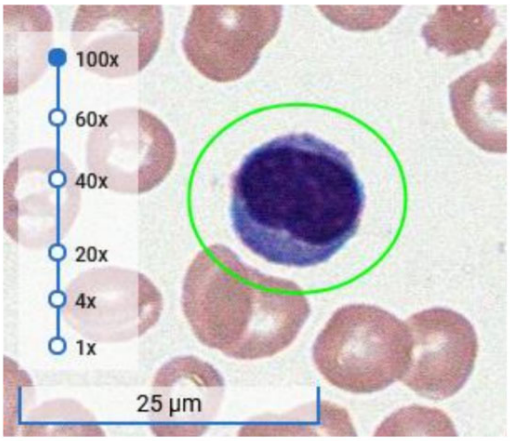
Can you Identify the Cell?



pSMILE

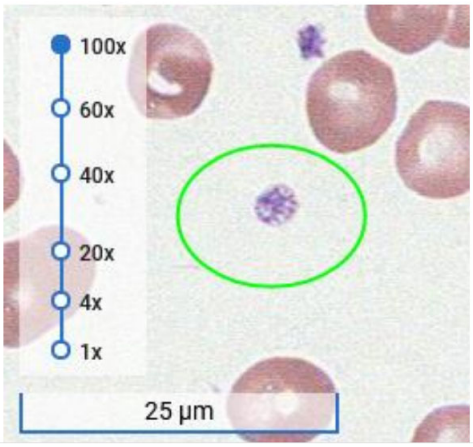
OWA SLIDES

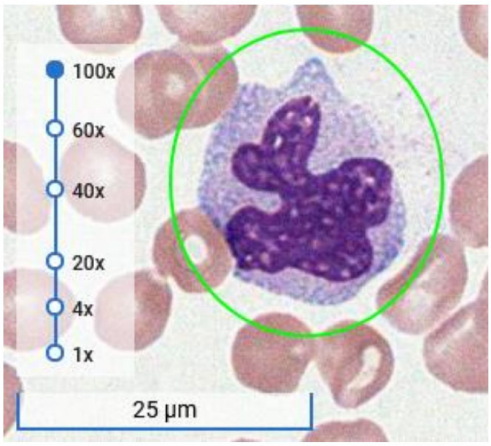




Cell on left- Neutrophil seg/band with Dohle and toxic granulation
Cell on right- lymphocyte

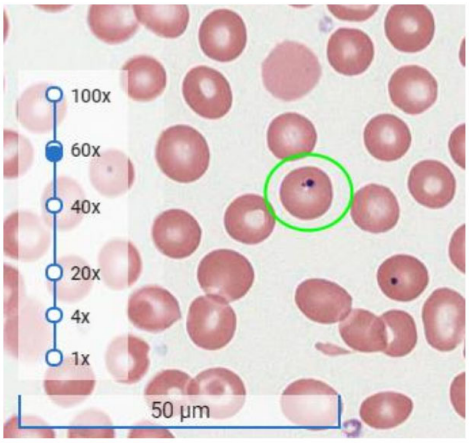
OWA Slides

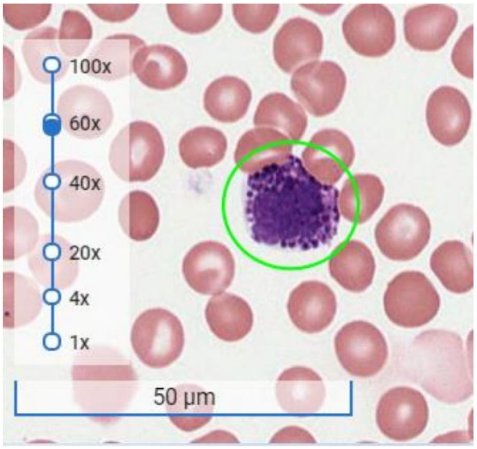




Cell on left-platelet
Cell on right- monocyte with pseudopod

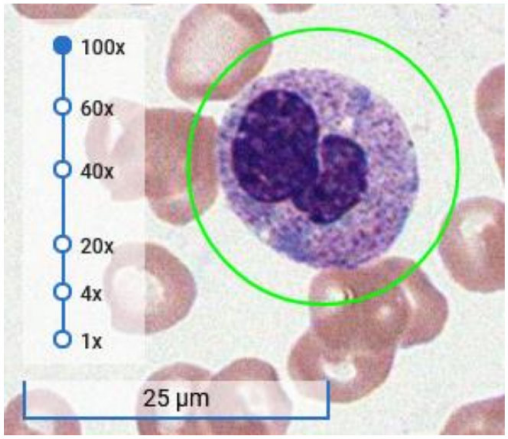
OWA Slides

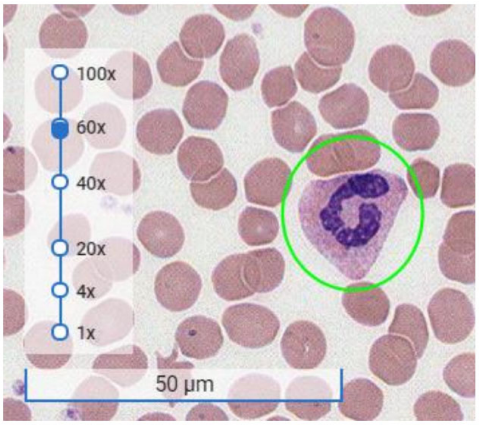




Cell on left- Howell Jolly Body
Cell on right- Basophil

OWA Slides

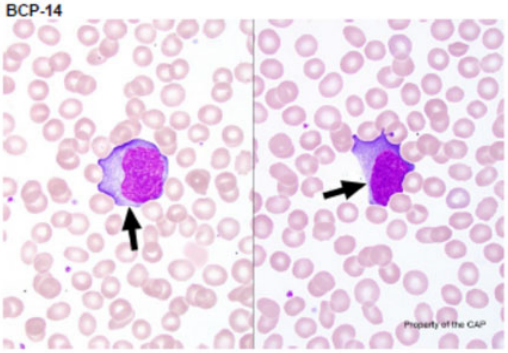




Cell on left- neutrophil seg/band with Dohle and toxic granulations
Cell on right- Band

Blood Cell Identification – Graded

BCP-14



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Laboratory Quality Solutions

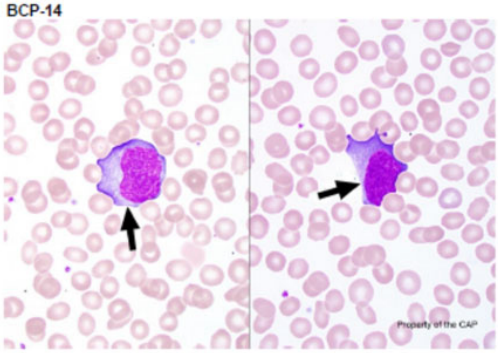
Name this cell: size 15um

1. **Monocyte**
2. **Abnormal/Reactive Lymph**
3. **Plasma Cell**
4. **Blast**

pSMILE

Blood Cell Identification – Graded

BCP-14



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Why is this a Reactive Lymphocyte:

1. Round to ovoid 10-25 um

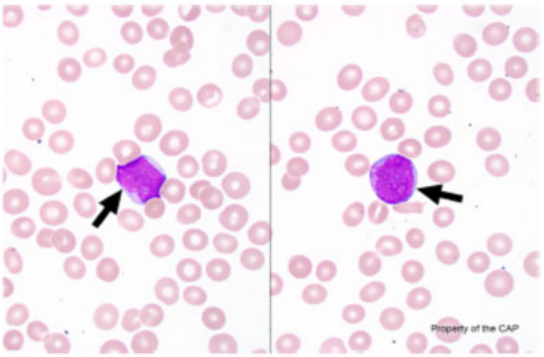
2. N:C Ratio varies- 3:1 - 1:2

3. Large nuclei that has moderately condensed chromatin and absent nucleoli

4. Amoeboid cytoplasm surrounding adjacent rbcs

Blood Cell Identification – Ungraded

BCP-09



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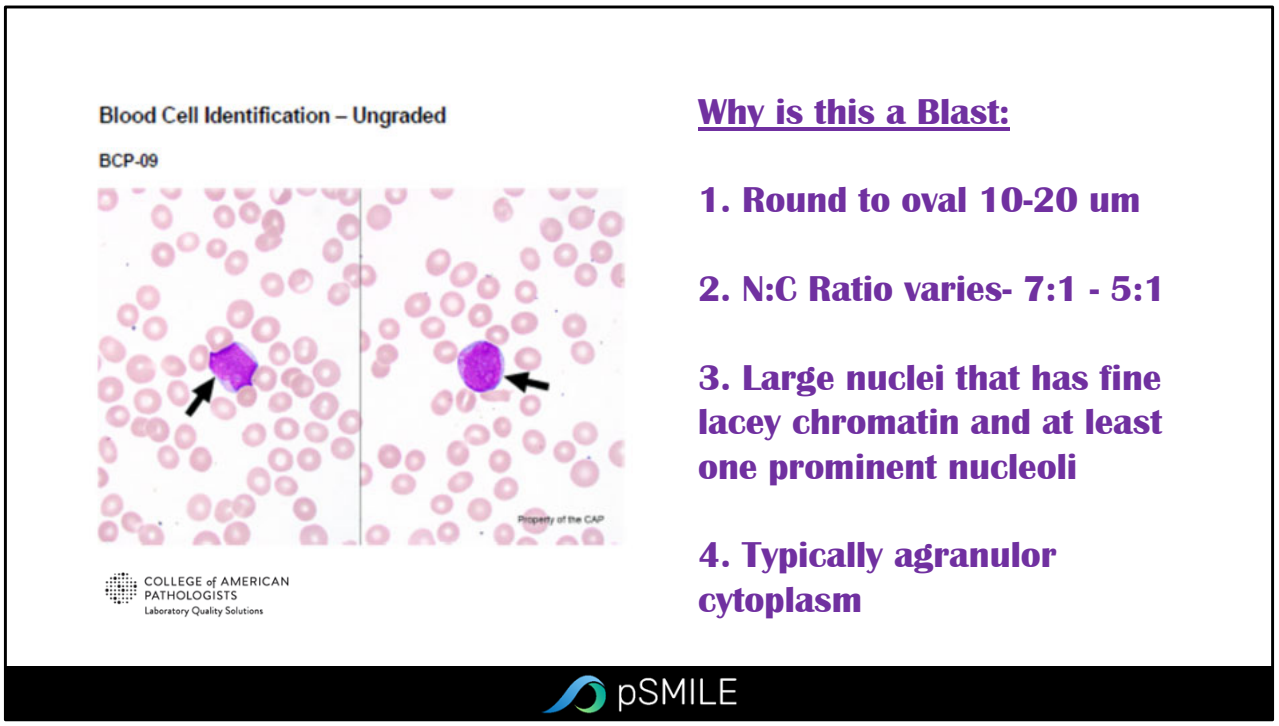
Laboratory Quality Solutions

Name this cell: size 20um

1. **Monocyte**
2. **NRBC**
3. **Blast**
4. **Abnormal/Reactive Lymph**

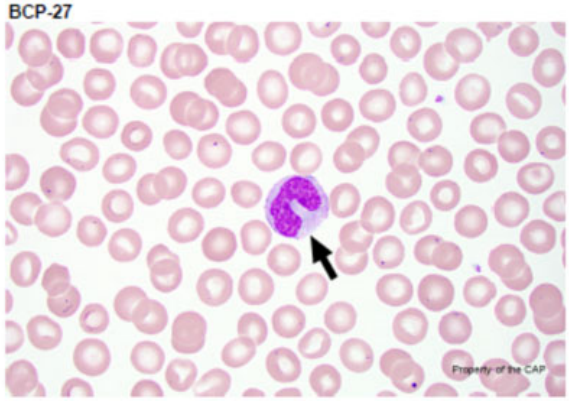
pSMILE



Huge nucleus
At least one prominent nucleoli
Very difficult to classify a blast- generally if auer rods are present it is in the myeloid lineage (neutrophil)

Blood Cell Identification – Ungraded

BCP-27



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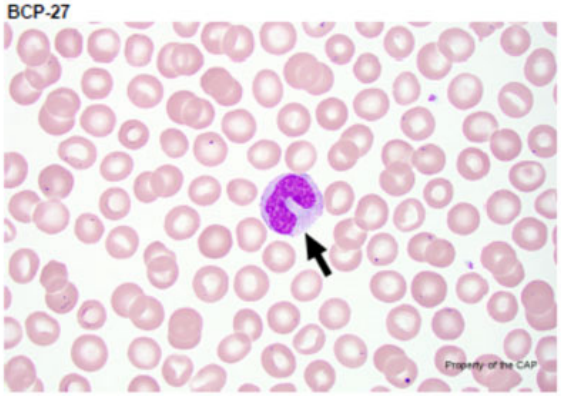
Name this cell: 20um

1. **Band**
2. **Lymphocyte**
3. **Monocyte**
4. **Segmented Neutrophil**

pSMILE

Blood Cell Identification – Ungraded

BCP-27



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Why is this a Monocyte:

1. Round 12-25 um (sometimes have pseudopod extenstions)

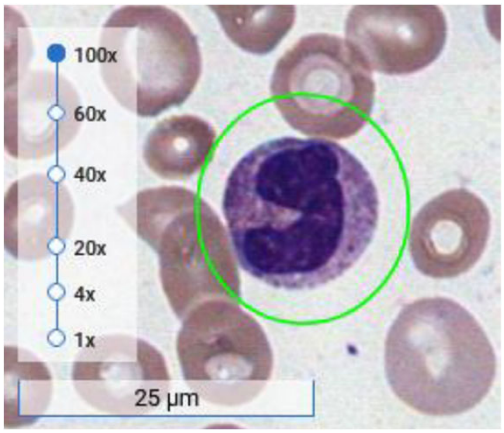
2. N:C Ratio varies- 4:1 - 2:1

3. Band-like nuclei that has condensed chromatin and absent nucleoli.

4. Blueish gray cytoplasm ground glass appearance

pSMILE

12-20 Micrometers

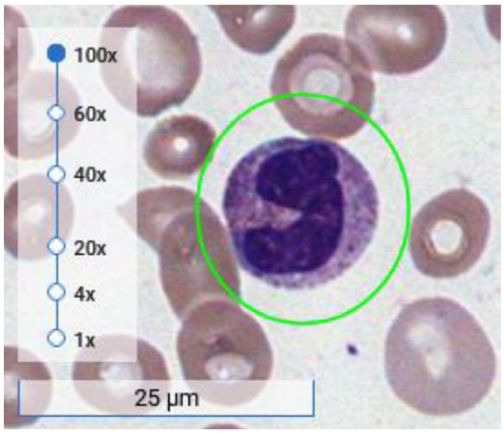


oneworld ACCURACY™

Name this cell:

1. **Metamyelocyte**
2. **Band/Segmented neutrophil**
3. **Lymphocyte**
4. **Monocyte**

pSMILE



oneworld ACCURACY™

Why is this a Band/Segmented Neutrophil :

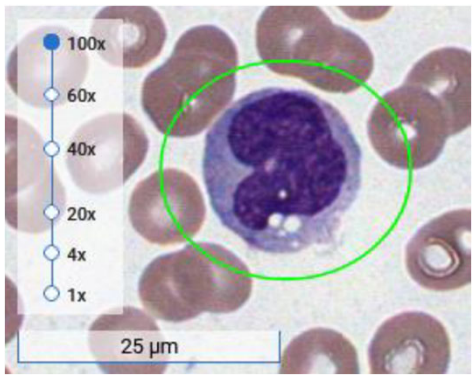
1. Round 25 um

2. N:C Ratio 1:2

3. Band-like nuclei that has condensed chromatin and absent nucleoli. Could be folded on top of itself making it a possible seg.

4. Purple blue cytoplasm

pSMILE

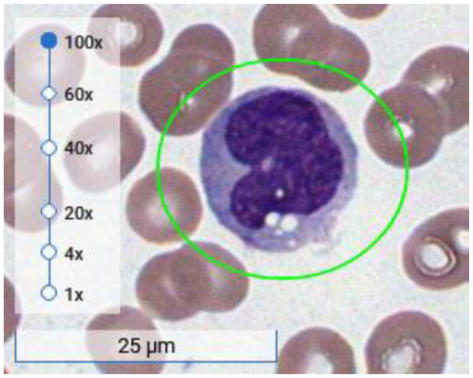


oneworld ACCURACY™

Name this cell:

1. Lymphocyte
2. Band/Segmented Neutrophil
3. Monocyte
4. Blast

pSMILE



oneworld ACCURACY™

Why is this a Monocyte :

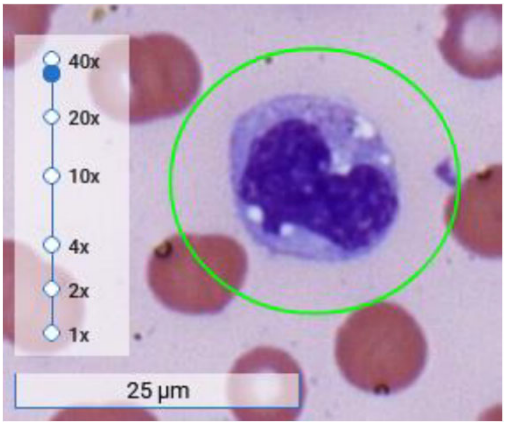
1. Round 15-25 um

2. N:C Ratio varies- 4:1 to 2:1

3. Nuclei that has condensed chromatin and absent nucleoli.

4. Blueish gray cytoplasm ground glass appearance with vacuoles

pSMILE

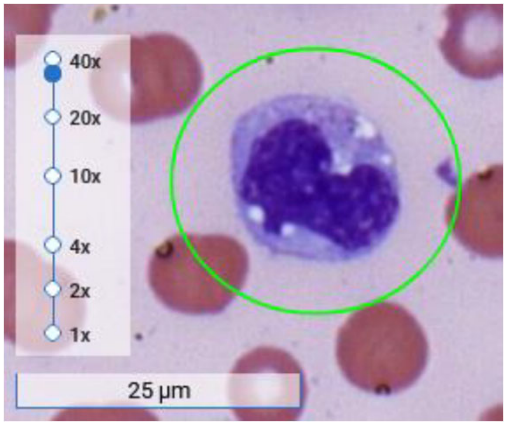


oneworld ACCURACY

Name this cell:

1. **Band**
2. **Lymphocyte**
3. **Plasma Cell**
4. **Monocyte**

pSMILE



oneworld ACCURACY™

Why is this a Monocyte :

1. Round 15-25 um

2. N:C Ratio varies- 4:1 to 2:1

3. Nuclei that has condensed chromatin and absent nucleoli.

4. Blueish gray cytoplasm ground glass appearance with vacuoles

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Monocytes	Lymphocytes
Large and spherical-shaped cells.	The size varies while encountering an infectious agent.
Makeup 2-8% of circulating WBCs.	Makeup 20-30% of circulating WBCs.
The cytoplasm is cloudy, opaque and blue-grey in colour with fine lilac granules.	The cytoplasm is clear, transparent and sky blue in colour without granules.
Presence of Infrequent vacuoles.	Presence of frequent vacuoles.
The lifespan of circulating monocytes is about 24 hours.	Lymphocytes are long-lived cells which may live for months or years.
Destroy pathogens through phagocytosis.	Destroy pathogens by producing antibodies.
The nucleus of a monocyte is soft, spongy, and oval-shaped with a pale bluish violet colour stain.	The nucleus of a lymphocyte is dense, oval-shaped and stretched with a deep purplish-blue colour stain.
Dendritic cells and Macrophages are two types of Monocytes.	B lymphocytes (B cells) and T lymphocytes (T cells) are two types of lymphocytes.
Monocytes are the tools for Innate Immunity.	Lymphocytes are tools for Adaptive Immunity

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- Dr. Lori Sokoll- Principal Investigator
- NIH/DAIDS- Daniella Livnat
- Mark Swartz- Project Manager

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Contract Number: 75N93020C00001

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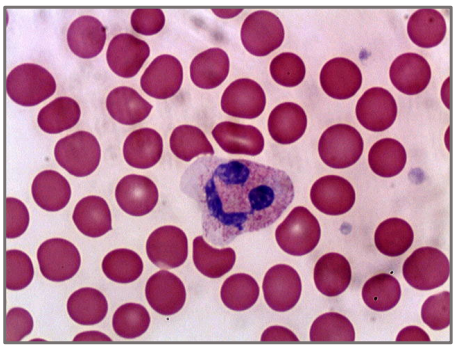
Questions



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