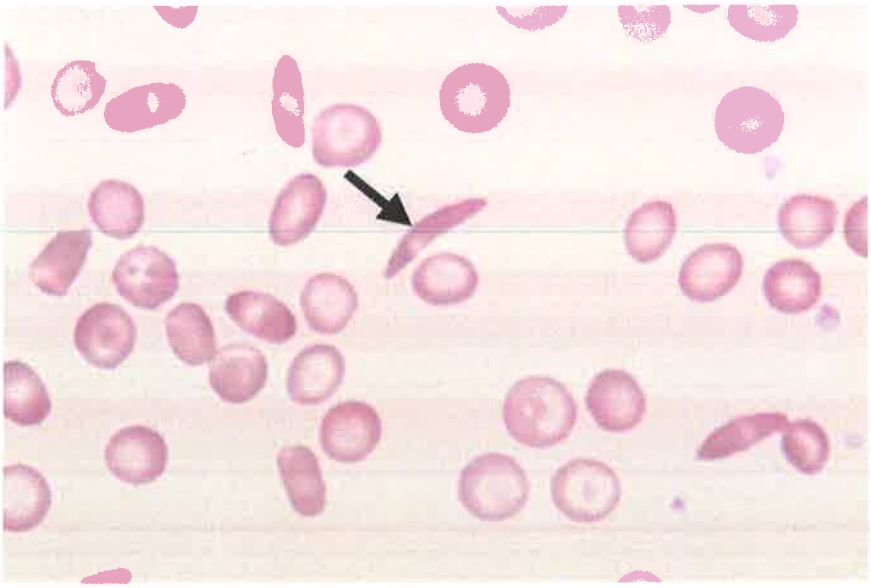
Cell Identification

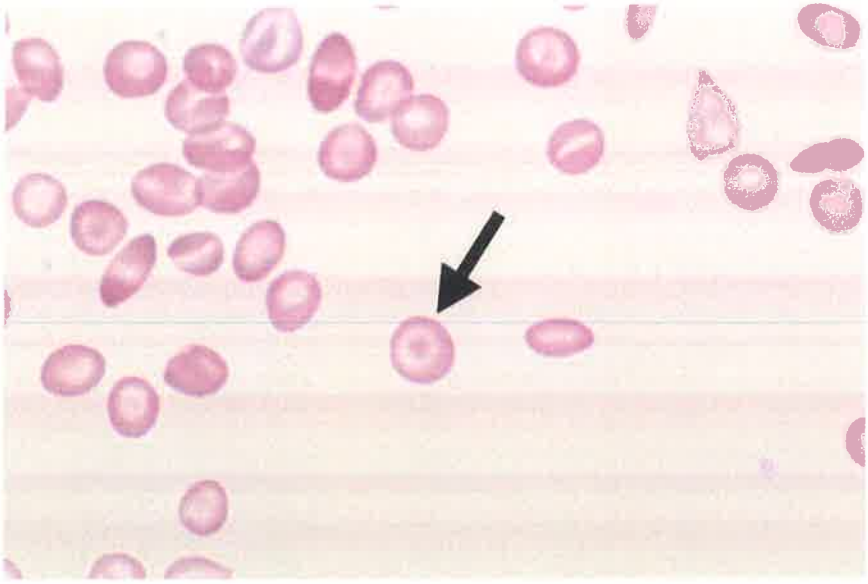
VPBS-20



Identification	Participants No.	Participants %	Evaluation
Sickle cell (drepanocyte)	1253	99.8	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational
Spherocyte	1	0.1	Educational

The arrowed cell is a sickle cell (drepanocyte), as correctly identified by 99.8% of participants. Sickle cells are long, thin red cells that appear crescentic with two pointed ends and usually lack central pallor. They are formed due to the crystallization of Hemoglobin S upon deoxygenation. Thus, sickle cells may be seen in hemoglobin SS disease (sickle cell anemia) as well as in other hemoglobinopathies where the sickle cell mutation is present in conjunction with other beta-hemoglobin mutations (eg, SC disease, SD disease and S-beta-thalassemia). Since sickle cells may be rapidly removed if a functional spleen is present, they are most commonly seen in appreciable numbers with splenic hypofunction or asplenia.

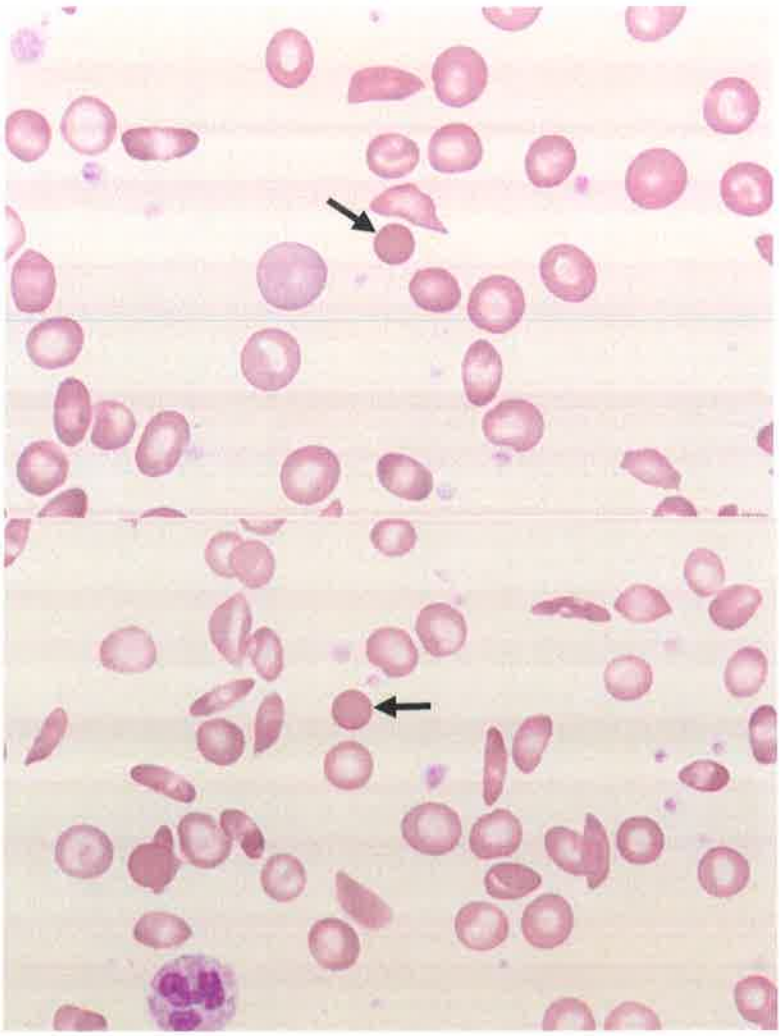
VPBS-21



Identification	Participants No.	Participants %	Evaluation
Target cell (codocyte)	1250	99.5	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Erythrocyte, normal	1	0.1	Educational
Plasmodium *sp.*	1	0.1	Educational
Stomatocyte	1	0.1	Educational
Teardrop cell (dacrocyte)	1	0.1	Educational

The arrowed cell is a target cell (codocyte), as correctly identified by 99.5% of participants. Target cells are thin red blood cells with increased surface to membrane volume ratio and decreased hemoglobin content. This causes the cells to flatten out in a blood film, creating the appearance of a target with a central hemoglobinized area, surrounding zone of pallor, and a peripheral hemoglobinized zone. Target cells have also been described as having the appearance of a Mexican hat or bull's-eye. Target cells are usually associated with hemoglobinopathies, such as sickle cell anemia or thalassemia, but may also be seen in iron deficiency anemia, post-splenectomy, or associated with chronic liver disease. Artifactual target cells may also be seen due to slow drying of smears in a humid environment or when excessive EDTA is present.

VPBS-22



Identification	Participants No.	Participants %	Evaluation
Spherocyte	1235	98.3	Educational
Microcyte (with increased central pallor)	14	1.1	Educational
Acanthocyte (spur cell)	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cells are spherocytes, as correctly identified by 98.3 % of participants. Spherocytes are densely staining spherical red blood cells that are smaller in diameter than a normal red cell (usually less than 6.5 µm in diameter). The cells almost always lack evidence of central pallor. Spherocytes are seen in hereditary spherocytosis and autoimmune hemolytic anemias, where they are formed by removal of red cell membrane by the spleen with retention of cellular hemoglobin. Spherocytes may also be seen in

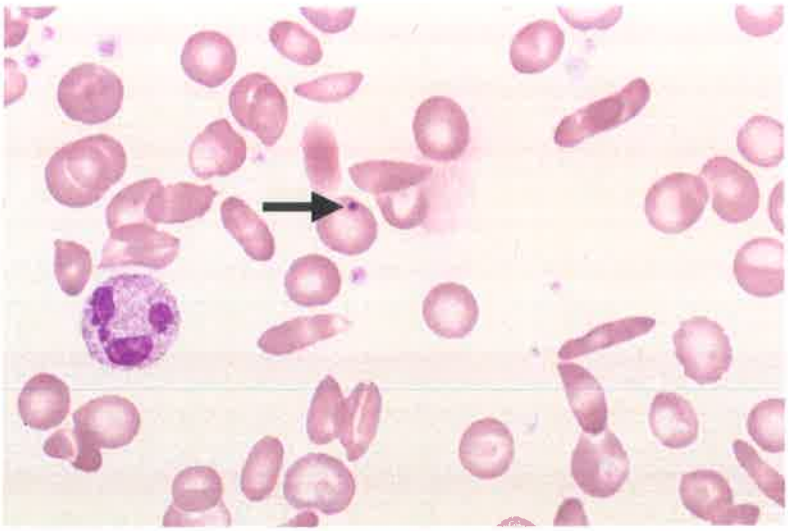
VPBS-22, (cont.)

VPBS-22 Discussion, Cont'd:

burns, microangiopathies, and in association with other red cell abnormalities where they represent rounded up red blood cell fragments.

1.1 % of participants identified these cells as microcytes with increased central pallor; however, central pallor is lacking.

VPBS-23

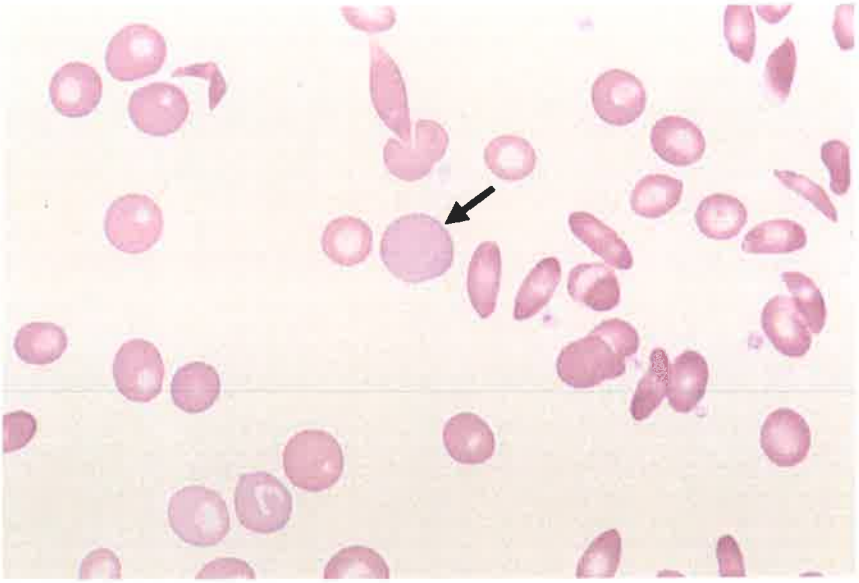


Identification	Participants No.	Participants %	Evaluation
Howell-Jolly body	1236	98.4	Educational
Erythrocyte with overlying platelet	14	1.1	Educational
Hemoglobin C crystal	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational
Platelet, normal	1	0.1	Educational
Plasmodium *sp.*	1	0.1	Educational
Spherocyte	1	0.1	Educational

The arrowed red cell inclusion is a Howell-Jolly body, as correctly identified by 98.4 % of participants. Howell-Jolly bodies are small, round, dark purple homogenous masses that measure about 1.0 µm in diameter seen in the cytoplasm of red blood cells. They represent a fragment of nuclear DNA that is left behind when the nucleus is extruded from the red blood cell. Usually these red cell DNA fragments are removed by the spleen, but in cases where the spleen is hypofunctional or absent, such as in sickle cell anemia, they may be readily found in the blood film. Usually only a single Howell-Jolly body is seen in a red blood cell; multiple Howell-Jolly bodies may be occasionally seen in some red cells in cases of megaloblastic anemia.

1.1 % of participants identified the arrowed inclusion/object as an erythrocyte with overlying platelet. When erythrocytes contain overlying platelets, the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine red blood cell inclusions. The halo is nonexistent in this image.

VPBS-24



Identification	Participants No.	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	984	78.4	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	249	19.7	Educational
Erythrocyte, normal	10	0.8	Educational
Basophilic stippling (coarse)	6	0.5	Educational
Spherocyte	2	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational
Blister cell/Prekeratocyte	1	0.1	Educational
Howell-Jolly body	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 78.4% of participants. The polychromatophilic red blood cell is a round to oval non-nucleated red blood cell that lacks appreciable central pallor. The polychromatophilic red blood cell is usually larger than a normal red blood cell and often homogenously stains pale purple to pink gray in a Wright stain blood film due to an increased amount of RNA in the cell cytoplasm. The intensity of the polychromasia will vary with the amount of RNA and the age of the cell, with younger cells appearing more purple or blue and relatively more mature cells appearing more pink-gray (as in the arrowed cell). Polychromatophilic red blood cells represent the final stage of red blood cell maturation after extrusion of the cell nucleus and exiting the bone marrow. Increased numbers of polychromatophilic red blood cells may be seen in severe anemia due to a wide variety of causes as the bone marrow tries to compensate for decreased red cell numbers.

VPBS-24, (cont.)

VPBS-24 Discussion, Cont'd:

19.7% of participants incorrectly identified the arrowed cell as a macrocyte, oval or round (excluding polychromatophilic red cell). The identified cell shows pink-grey cytoplasm of a later polychromatophilic red cell rather than the fully hemoglobinized red to orange colored cytoplasm that would be expected in a non-polychromatophilic cells and would be similar in tint to surrounding, normocytic red cells in the field. Additionally, non-polychromatophilic macrocytes tend to have at least some slight indication of central pallor, which is lacking in this cell. In contrast, polychromatophilic red cells often lack central pallor. Another hint that this may not represent a true macrocyte is the patient's MCV of 81 fL, which is normocytic, and the clinical history of sickle cell anemia, which is associated with rapid red cell turn-over due to hemolysis which often leads to increased numbers of polychromatophilic red cells.

Clinical Presentation:

This peripheral blood smear is from a 13-year-old boy presenting with bone pain. The patient has a history of sickle cell disease. Laboratory data includes: WBC = 12.0 x 10E9/L; RBC = 2.40 x 10E12/L; HGB = 6.9 g/dL; HCT = 19.2%; MCV = 81 fL; PLT = 405 x 10E9/L; and RDW = 29%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Sickle Cell Anemia

Sickle cell anemia is one of the most common hemoglobinopathies. It is most commonly seen in persons of African descent, where the mutation is thought to provide some protection against malarial infection. In the United States, the incidence in African Americans is approximately 1:500 although up to 8% of the population may carry the sickle cell mutation. The mutation is inherited as an autosomal recessive trait.

Hemoglobin S (HbS) is an abnormal hemoglobin caused by a point mutation leading to a substitution of glutamic acid for a valine amino acid (β6 Glu → Val) in the beta-globin chain that forms hemoglobin. This mutation causes a decrease in solubility of hemoglobin when deoxygenated or in a low pH environment (pH < 7.2), leading to protein polymerization and the formation of long, crystalline arrays that deform the cell into a sickle shape. Increased hemoglobin polymerization and red cell sickling may also occur when HbS is in the presence of other abnormal hemoglobins such as HbC, HbD, or HbO-Arab. When a single beta-globin chain is mutated (heterozygous state), the patient will have sickle cell trait, which is normally clinically asymptomatic. Significant clinical disease occurs when there are homozygous mutations in both beta-globin genes (sickle cell anemia or HbSS) or when other abnormal hemoglobins with mutations in the beta globin gene are present (eg, HbSC disease or sickle-β-thalassemia).

Question 1: Sickle cell anemia is due to:

A. A heterozygous deletion leading to an amino acid loss in the beta-globin gene
B. A homozygous point mutation leading to an amino acid change in the beta-globin gene
C. A large heterozygous deletion involving several amino acids in the alpha-globin gene
D. Homozygous point mutations leading to amino acid deletions in the alpha-globin gene

---

Patients with sickle cell anemia will present in childhood with moderate hemolytic anemia, arising due to the premature removal of the abnormal red cells by both intravascular and extravascular hemolysis. This leads to development of moderate anemia before one year of age. The blood smear will show numerous abnormally shaped cells, with crescentic, boat-shaped, or oval sickle cells being the most commonly seen. Target cells are prominent and other abnormal shapes including holly-leaf shapes, envelope cells and occasional spherocytes may be seen. Evidence of a marrow response to hemolysis is usually present including increased polychromasia, basophilic stippling, and nucleated red cells. Because the abnormally shaped cells are rigid, they tend to lodge in the spleen and other organs leading to infarction or ischemic damage. By early childhood, most patients have infarcted the spleen to the degree that it is no longer functional (autosplenectomy). This will lead to signs of hyposplenism in the smear including the presence of Howell-Jolly bodies, Pappenheimer bodies, and increased abnormally shaped cells.

Question 2: Blood findings (in addition to identifying sickle cells) which are suggestive of sickle cell anemia include:

A. Macrocytic anemia with many hypersegmented neutrophils
B. Microcytic anemia with numerous spherocytes
C. Normocytic anemia with many Howell-Jolly bodies
D. Normocytic anemia with normal red cell morphology

Diagnosis of sickle cell anemia is based on clinical findings in combination with demonstration of HbS by hemoglobin analysis by gel electrophoresis, capillary electrophoresis, or high-performance liquid chromatography (HPLC). Screening tests that indicate the presence of HbS (but do not distinguish between heterozygous sickle trait and homozygous sickle cell disease or the presence of other abnormal hemoglobins in association with HbS) include the sickle test (mixing blood with sodium metabisulfite and observing sickle cells in a blood film) or the solubility test (mixing blood with dithionite reagent and looking for precipitation of hemoglobin). Genetic testing may be used for prenatal screening to directly identify the mutation.

Question 3: A diagnosis of sickle cell anemia may be made based on a positive sickle test.

A. True
B. False

Treatment of patients with sickle cell disease is dependent on the severity of the symptoms, which may vary widely from patient to patient and is often aimed at supportive care and minimizing exposure to physiologic triggers of sickling, such as high altitude. Other therapies are aimed at decreasing the severity of painful, ischemic crises due to lodging of the abnormal sickle cells in the microvasculature of joints and organs (such as the lung, kidney, or brain) by decreasing the relative amounts of HbS in the red cell. Hydroxyurea or hydroxycarabamide treatments will increase the relative amount of fetal hemoglobin (HbF) in the red cell, leading to decreased sickling. Several other regulators of hemoglobin synthesis are also under investigation. Other approaches aimed at curing the disease, such as bone marrow stem cell transplantation and gene therapy, are also emerging.

Sherrie L. Perkins, MD, PhD
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Ware RE, de Montalambert M, Tshilolo L, Abboud MR. Sickle cell disease. *Lancet.* 390:3110323, 2017.
2. Azar S, Wong TE. Sickle cell disease: a brief update. *Med Clin North Amer.* 101:375-393, 2017.
3. Meier ER. Treatment options for sickle cell disease. *Pediatr Clin North Amer.* 65:427-443, 2018.
4. Williams TN, Thein SL. Sickle cell anemia and its phenotypes. *N Engl J Med.* 376:1561-1573, 2017.
5. Glassy EF. Color Atlas of Hematology. *An Illustrated Field Guide Based on Proficiency Testing*, 2nd ed. College of American Pathologists. 2018:104-109.

ANSWERS TO QUESTIONS:

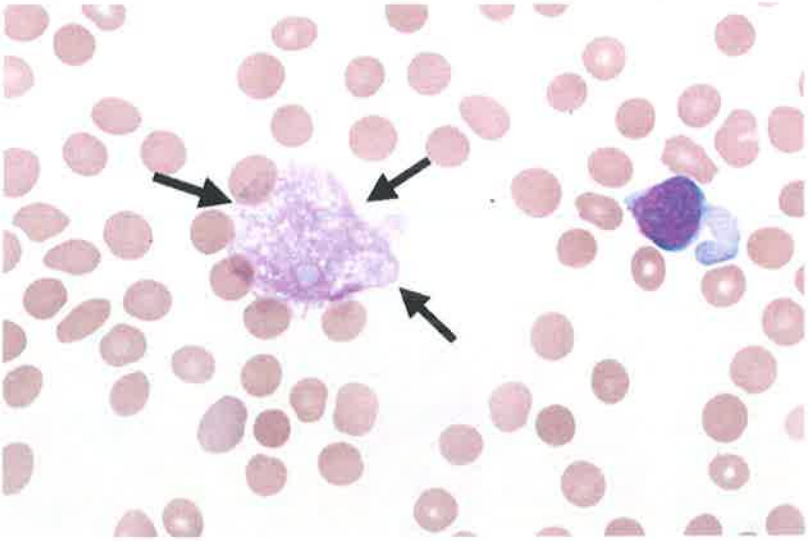
Question 1: **B. A homozygous point mutation leading to an amino acid change in the beta-globin gene.**
HbS is formed due to a point mutation (β6 Glu → 6Val) that leads to substitution of a glutamic acid to a valine amino acid in the beta globin gene. A heterozygous mutation will lead to sickle trait, which is often clinically silent, whereas homozygous mutations will lead to sickle cell anemia.

Question 2: C: Normocytic anemia with many Howell-Jolly bodies.
Sickle cell anemia gives rise to a normocytic anemia due to intravascular and extravascular hemolysis. Due to infarction of the spleen in early life due to the sickle cell's rigidity and abnormal structure, findings of asplenia/splenic hypofunction such as increased Howell-Jolly bodies, Pappenhiemer bodies, and abnormally shaped red cells are commonly seen in the blood film in addition to sickle cells, target cells and polychromasia.

Question 3: **B False**.
The sickle test and solubility test are screening assays that detect the presence of HbS in blood cells. However, they will be positive in cases of sickle trait as well as mixed hemoglobinopathies with a HbS component (ie, HbSC, sickle-β-thalassemia, etc). To make a diagnosis of sickle cell anemia, specific testing that demonstrates HbS as the predominant hemoglobin species is required. Thus, a definitive diagnosis of sickle cell anemia will require hemoglobin gel electrophoresis, capillary gel electrophoresis, or high-performance liquid chromatography (HPLC) to identify all the hemoglobin subtypes present in a patient sample.

Cell Identification

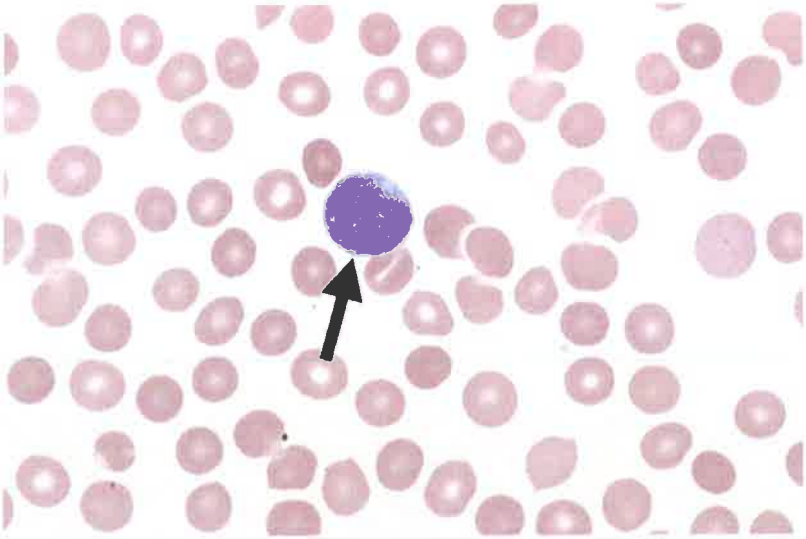
VPBS-26



Identification	Participants No.	%	Evaluation
Basket cell/smudge cell	1237	98.4	Educational
Stain precipitate	10	0.8	Educational
Malignant lymphoid cell (other than blast)	2	0.2	Educational
Blast cell	1	0.1	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Mast cell	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational

The arrowed cell is a basket/smudge cell, as correctly identified by 98.4% of participants. Basket/smudge cells are artifacts produced when fragile cells, typically lymphocytes, are subjected to the shearing forces of the peripheral smear production process. The "basket" appearance results when chromatin strands are spread-out from a condensed nuclear remnant. Basket/smudge cells are most commonly encountered in (though not limited to) disorders of increased lymphocyte fragility, such as infectious mononucleosis or chronic lymphocytic leukemia.

VPBS-27

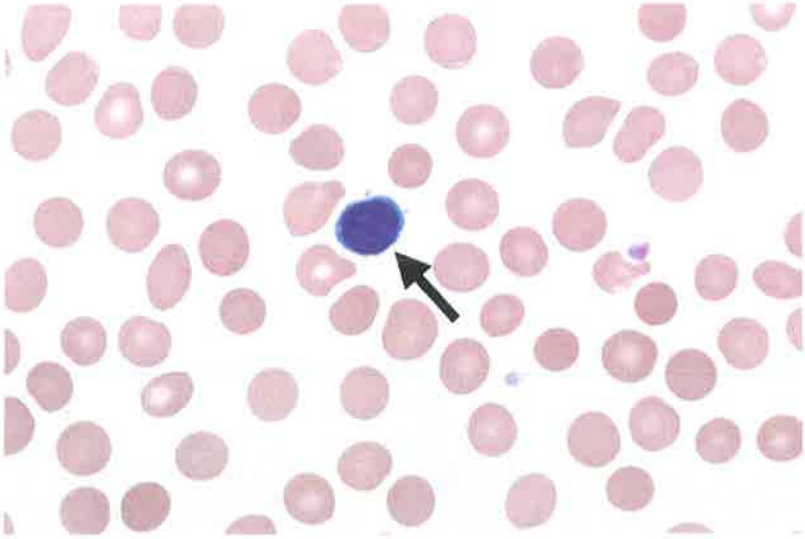


Identification	Participants No.	%	Evaluation
Blast cell	984	78.4	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	112	8.9	Educational
Malignant lymphoid cell (other than blast)	87	6.9	Educational
Lymphocyte	40	3.2	Educational
Immature or abnormal cell, would refer for identification	18	1.4	Educational
Lymphocyte, large granular	5	0.4	Educational
Monocyte, immature (promonocyte, monoblast)	3	0.2	Educational
Neutrophil, myelocyte	2	0.2	Educational
Bacteria (spirochete), extracellular	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Sickle cell (drepanocyte)	1	0.1	Educational

The arrowed cell is a blast, as correctly identified by 78.4% of participants. Blasts are large, round-to-oval cells with high nuclear-to- cytoplasmic ratio. They often have a round to oval nucleus, sometimes with indentations or folds. Blasts demonstrate characteristically immature chromatin, described as fine, lacy, or reticular. One or more nucleoli may be seen. The cytoplasm may be variably basophilic and typically agranular, although some myeloid blasts may demonstrate cytoplasmic granules or Auer rods (the latter being myeloid lineage-specific). The morphologic features of blasts often do not permit determination of the cell lineage (ie, differentiation between myeloblast versus lymphoblast), and the use of other techniques (such as immunophenotypic analysis) is required for proper classification. To this end, classification of the arrowed cells as "immature/abnormal cell," as selected by 1.4% of participants, while less specific, is not strictly incorrect if these participants routinely send slides containing cells such as these to an outside laboratory for confirmatory assessment.

The arrowed cell was incorrectly identified as some form of lymphocyte by 19.0% of participants (lymphocyte by 3.2%; lymphocyte, reactive, by 8.9%; and malignant lymphoid cell by 6.9% of participants). The delicate chromatin pattern, presence of a prominent nucleolus, and high N:C ratio are features that would be inconsistent with mature cells such as lymphocytes (including reactive forms) and (mature) malignant lymphoid cells.

VPBS-28



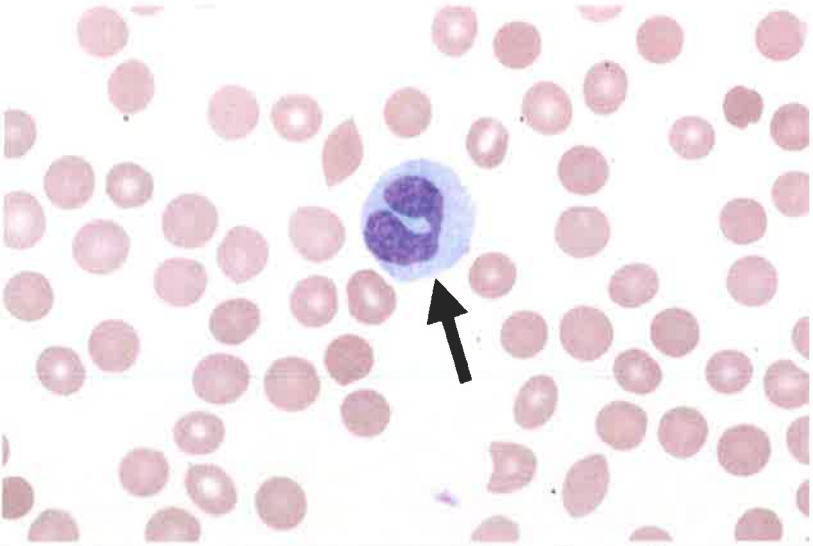
Identification	Participants No.	%	Evaluation
Lymphocyte	1172	93.4	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	57	4.5	Educational
Nucleated red blood cell, normal or abnormal morphology	13	1.0	Educational
Malignant lymphoid cell (other than blast)	10	0.8	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	0.2	Educational
Monocyte	1	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 93.4% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

The arrowed cell was incorrectly identified as a lymphocyte, reactive, by 4.5% of participants. In contrast to reactive lymphocytes, resting lymphocytes such as this typically demonstrate less abundant cytoplasm (with a relatively higher N:C ratio) and typically demonstrate a paler (less basophilic) cytoplasm.

The arrowed cell was incorrectly identified as a nucleated red blood cell, normal or abnormal morphology, by 1.0% of participants. Nucleated red blood cells typically demonstrate a more eosinophilic cytoplasm relative to lymphocytes. Resting lymphocytes also typically have a higher N:C ratio relative to circulating nucleated red cells. Also, the chromatin pattern of nucleated red cells is typically distinct, often reminiscent of an ink drop or "crumbled cookie" pattern

VPBS-29

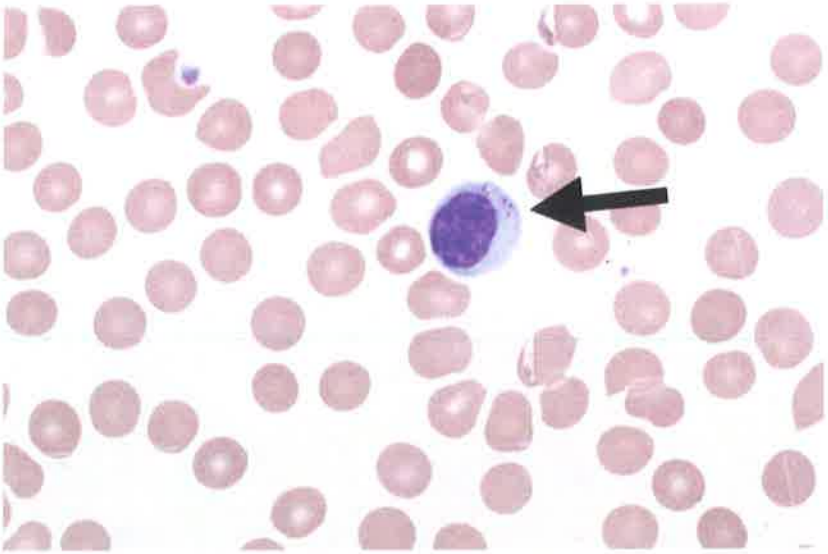


Identification	Participants No.	%	Evaluation
Monocyte	1033	82.2	Educational
Neutrophil, segmented or band	154	12.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	26	2.0	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	15	1.2	Educational
Monocyte, immature (promonocyte, monoblast)	11	0.9	Educational
Neutrophil, giant band or giant metamyelocyte	7	0.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	0.2	Educational
Neutrophil, metamyelocyte	2	0.2	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Microfilaria	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 82.2% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

The arrowed cell was incorrectly identified as a neutrophil by 15.4% of participants (neutrophil with dysplastic nucleus and/or hypogranular cytoplasm by 2.0%; neutrophil with Pelger-Huët nucleus (acquired or congenital) by 1.2%; and neutrophil, segmented or band, by 12.2%). While the cytoplasmic pallor of the arrowed cell might suggest a dysplastic granulocyte, the cellular size, nuclear lobulation, and chromatin pattern would be atypical. Likewise, the lack of a uniformly thin filamentous process connecting two nuclear lobes makes classification as a Pelger-Huët cell inappropriate. Finally, the lack of cytoplasmic granularity would make classification of this cell as a segmented or band neutrophil inappropriate.

VPBS-30



Identification	Participants No.	Participants %	Evaluation
Lymphocyte, large granular	1103	87.8	Educational
Lymphocyte	96	7.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	22	1.8	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	11	0.9	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	5	0.4	Educational
Leukocyte with intracellular bacteria	4	0.3	Educational
Immature or abnormal cell, would refer for identification	3	0.2	Educational
Neutrophil, myelocyte	3	0.2	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	2	0.2	Educational
Basophilic stippling (coarse)	1	0.1	Educational
Blast cell	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational
Stain precipitate	1	0.1	Educational

The arrowed cell is a large granular lymphocyte (LGL), as correctly identified by 87.8% of participants. LGLs are medium to large cells with round nuclei, dense chromatin, and no visible nucleoli. Their cytoplasm is moderate to abundant, clear-to-lightly basophilic, and contains several variable coarse, unevenly distributed, small azurophilic granules.

LGLs may be confused with blasts (identified by 0.1% of participants), lymphocytes (identified as such by 7.6% of participants), reactive lymphocytes (identified as such by 1.8% of participants), or monocytes (identified by 0.1% of participants). In most cases, the nuclear chromatin pattern and nucleus-to-cytoplasm ratio can be used to distinguish LGLs from blasts. Reactive lymphocytes may be as large as LGLs but tend to demonstrate a more basophilic cytoplasm and less prominent cytoplasmic granules; both reactive lymphocytes and LGLs tend to be larger than resting lymphocytes.

Clinical Presentation:

This peripheral blood smear is from a 59-year-old woman with persistent cough, fatigue, and fever. Laboratory data includes: WBC = 25.0 x 10E9/L; RBC = 3.84 x 10E12/L; HGB = 11.6 g/dL; HCT = 35.2%; and PLT = 36 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Acute Myeloid Leukemia (AML) with thrombocytopenia

Epidemiology

AML represents only 1.2% of incident cancers in the US, but poses a significant mortality risk, with 5-year survival rates of only 28% based on recent SEER data [1]. In contrast, breast cancer is the most common cancer, with an incidence over ten times greater than AML, but with a 5-year survival of nearly 90%. AML is generally a disease of adults, with the median age at diagnosis of 68, and fewer than 5% of cases occurring at ages under 20 [1].

Question 1: **Which statement relating to AML epidemiology is CORRECT?**

A. AML is a common cancer, but with a relatively poor 5-year survival rate.
B. AML is a common cancer with a generally good prognosis.
C. AML is a rare cancer with a relatively poor 5-year survival rate.
D. AML is a rare yet highly survivable cancer.

Clinical Presentation

Symptomatic features in AML are often nonspecific, typically representing manifestations of anemia, thrombocytopenia or leukopenia; these may include fatigue and bleeding tendency. Most cases of AML present with at least one cytopenia, though they often involve several lineages. Rare cases may present with tumoral masses (so-called myeloid sarcomas). Some AML subtypes may also demonstrate abnormalities of specific leukocytes including eosinophilia or morphological changes such as granulocytic dysplasia.

Diagnosis

The classification of AML is complex, requiring a combination of clinical, morphological, phenotypic and molecular genetic data [2]. In most instances, a blast count in excess of 20% in the peripheral blood or bone marrow is necessary for a diagnosis of acute leukemia, after which the myeloid lineage must then be confirmed [2]. Some cytogenetic changes (eg. AML with t(8;21), inv(16) or t(16;16), or t(15;17) involving *PML-RARA* fusion) are also considered sufficient for AML, even in the absence of at least 20% blasts [2]. Lineage is typically established by immunophenotypic methods. Occasional cases may also demonstrate Auer rods, which are morphologically diagnostic of myeloid lineage [2].

Question 2. Which statement relating to AML clinical and/or diagnostic features is CORRECT?

A. AML classification is solely based on cytogenetic data.
B. AML commonly presents with tumoral masses.
C. AML diagnosis always requires enumeration of at least 20% blasts.
D. Fatigue and/or bleeding are relatively common symptoms in patients with AML.

Work-up

Most acute leukemias require immunophenotyping for determination of lineage. In many centers, flow cytometric evaluation is employed and can provide rapid assessment and lineage assignment. Additional studies are generally required after lineage is established, including cytogenetic and molecular genetic studies. Full karyotyping is still recommended as the primary means of subclassification and prognostication [2]. Single-gene testing for important classifier variants, such as *NPM1*, *CEBPA, FLT3,* and *RUNX1* mutations are also considered the standard of care [2]. Many centers also employ next-generation sequencing-based assays to allow for assessment for numerous genetic alterations simultaneously.

Treatment

A wide variety of treatment options are available, with choices depending on age, comorbid status, disease subtype, and prognostic category. Otherwise, healthy young patients with low risk disease might be expected to respond well to chemotherapy alone, whereas individuals with high risk disease might require allogeneic transplant.

Question 3. Which statement relating to the work-up of AML is CORRECT?

A. Cytogenetic studies are required for classification, but do not afford prognostic information.
B. Flow cytometric analysis can facilitate the confirmation of myeloid lineage.
C. Next-generation sequencing is required for the work-up of AML.
D. Owing to advanced molecular methods, cytogenetic studies are generally not required as part of the work-up of AML.

Etienne Mahé, MD, MSc, FRCPC, FCAP
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Howlander N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2016. April 2019. https://seer.cancer.gov/csr/1975_2016.
2. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th edition. (Swerdlow SH, Campo E, Harris NL, et al., eds.). International Agency for Research on Cancer; 2017.

ANSWERS TO QUESTIONS:

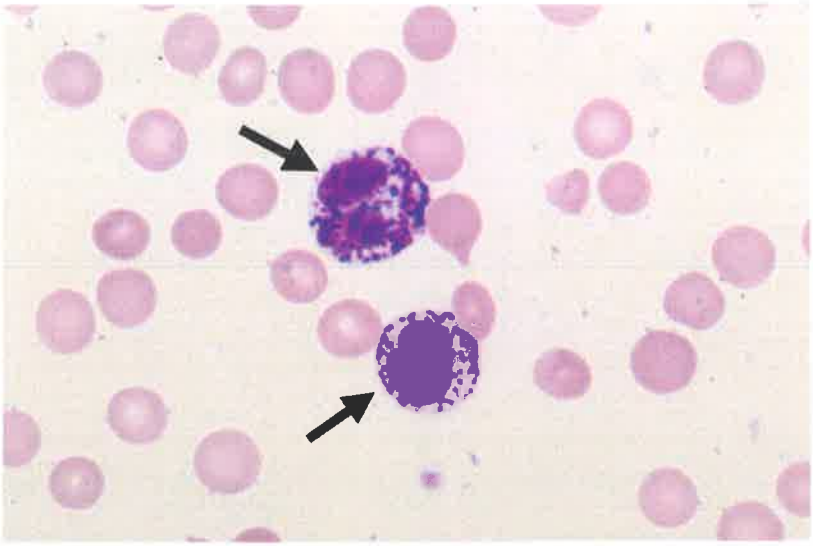
Question 1: C. AML is a rare cancer with a relatively poor 5-year survival rate.
Based on SEER data, AML is a relatively uncommon cancer, and one with a relatively poor 5-year survival rate.

Question 2: D. Fatigue and/or bleeding are relatively common symptoms in patients with AML.
Owing to frequent anemia and/or thrombocytopenia, fatigue and/or bleeding are relatively common symptoms in AML.

Question 3: B. Flow cytometric analysis can facilitate the confirmation of myeloid lineage.
The high-throughput multiparameter nature of flow cytometry makes it a useful immunophenotypic tool for lineage assignment in the setting of acute leukemia.

Cell Identification

VPBS-32



Identification	Participants No.	%	Evaluation
Basophil, any stage	1137	90.6	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	56	4.4	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	25	2.0	Educational
Leukocyte with intracellular bacteria	12	1.0	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	8	0.6	Educational
Basophilic stippling (coarse)	3	0.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.2	Educational
Immature or abnormal cell, would refer for identification	2	0.2	Educational
Leukocyte with intracellular fungi	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Mast cell	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	1	0.1	Educational
Stain precipitate	1	0.1	Educational
Teardrop cell (dacrocyte)	1	0.1	Educational

The arrowed cells are basophils, as correctly identified by 90.6 % of participants. Basophils are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, and the nuclear-to-cytoplasm (N:C) ratio ranges from 1:2 to 1:3. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, as in this case of primary myelofibrosis. Basophilia can also be associated with hypersensitivity reactions, hypothyroidism, iron deficiency, and renal disease.

VPBS-32, (cont.)

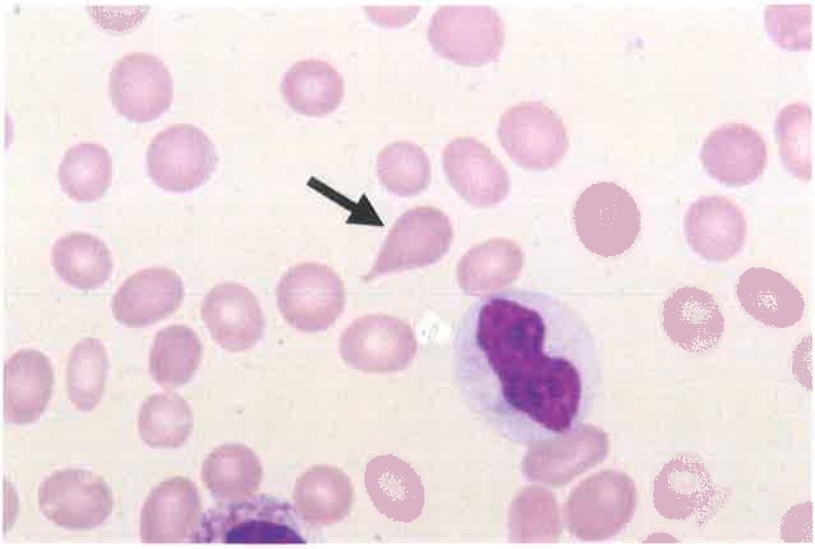
VPBS-32 Discussion, Cont'd:

4.4 % of participants identified the arrowed cells as neutrophils with toxic granulation. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Unlike the granules in basophils, toxic granules are smaller, evenly distributed within the cytoplasm and do not overlay and obscure the nucleus.

2.0 % of participants identified the arrowed cells as leukocyte containing Chediak-Higashi inclusions. Giant, often round, red, blue, or greenish gray granules of variable size are seen in the cytoplasm of otherwise typical leukocytes (granulocytes, lymphocytes, and monocytes) and sometimes erythrocyte precursors normoblasts or megakaryocytes in patients with Chediak-Higashi syndrome. In the blood the disease is manifested by the presence of medium to large peroxidase positive inclusions in the leukocytes. These may be single or in aggregates and do not overlay and/or obscure the nucleus.

1.0 % of participants identified the arrowed cells as leukocyte with intracellular bacteria. It is very unusual to see bacteria on a routine blood film. This finding usually represents an overwhelming infection. When present, the bacteria may be ingested by neutrophils or monocytes and can be seen within the cytoplasm of these cells. When present within neutrophils, bacteria can be difficult to distinguish from toxic granulation. However, toxic granulation tends to involve nearly all of the cytoplasm of the neutrophil, whereas engulfed bacteria are usually few in number. In addition, bacteria are typically larger than toxic granules, measuring around 1 µm in size, and are more defined in shape, ranging from cocci to bacilli and arranged singly, as diplococci, in clusters or in chains. They can be accentuated and confirmed with a Gram stain.

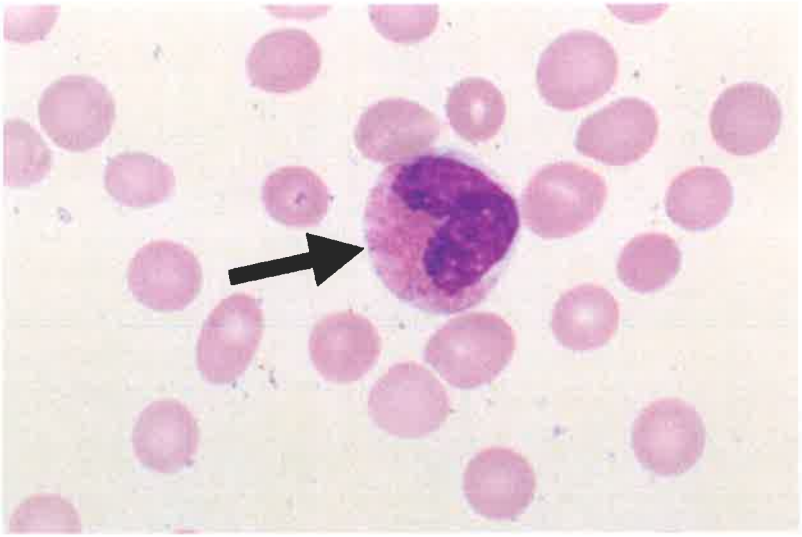
VPBS-33



Identification	Participants No.	%	Evaluation
Teardrop cell (dacrocyte)	1246	99.3	Educational
Neutrophil, segmented or band	3	0.2	Educational
Pappenheimer bodies (iron or Wright stain)	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Target cell (codocyte)	1	0.1	Educational

The arrowed cell is a teardrop cell (dacrocyte), as correctly identified by 99.3% of participants. Red blood cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. These are commonly seen in patients with bone marrow fibrosis (as in this case), but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized due to the fact that their "tails" all point in the same direction.

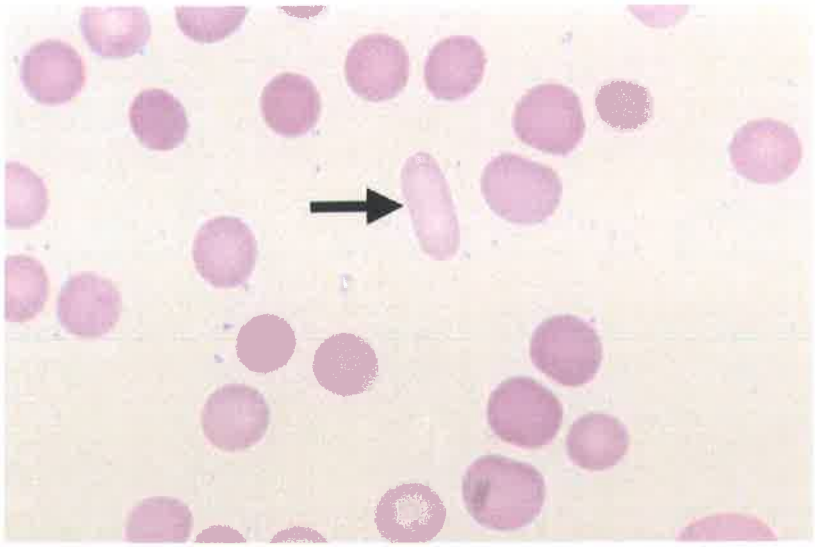
VPBS-34



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	1230	98.0	Educational
Neutrophil, segmented or band	15	1.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	7	0.6	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational

The arrowed cell is a mature eosinophil, as correctly identified by 98.0% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in immature forms. The eosinophil N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is evenly filled with numerous coarse, orange-red granules of uniform size. Mild uneven cytoplasmic granulation seen in rare eosinophils is likely associated with treatment with hydroxyurea. The eosinophilic granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. In the most mature eosinophil form, the nucleus segments into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato-shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes (as seen on the smear) and an occasional cell will exhibit four to five lobes.

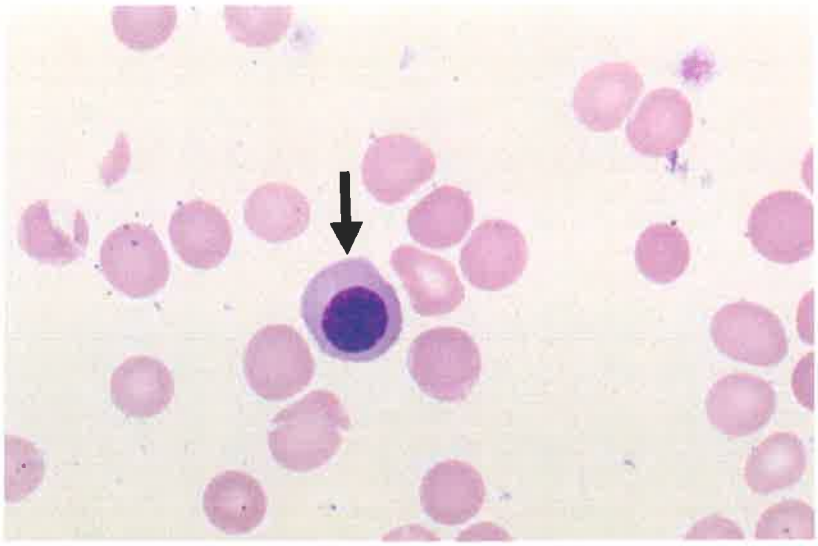
VPBS-35



Identification	Participants No.	%	Evaluation
Ovalocyte (elliptocyte)	1252	99.8	Educational
Nucleated red blood cell, normal or abnormal morphology	2	0.2	Educational

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 99.8% of participants. The terms elliptocytes and ovalocytes are used to describe red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent, as in this case of primary myelofibrosis. Some ovalocytes may superficially resemble oval macrocytes but are not as large as macrocytes and tend to be less oval with sides that are nearly parallel. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

VPBS-36



Identification	Participants No.	Participants %	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1237	98.6	Educational
Lymphocyte	6	0.5	Educational
Erythrocyte, normal	3	0.2	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	3	0.2	Educational
Basophil, any stage	1	0.1	Educational
Blast cell	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a normal nucleated red blood cell (nRBC), as correctly identified by 98.6% of participants. The term nucleated red blood cell (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation (as seen on the smear). Both megaloblastic and dysplastic changes can be seen in these circulating red blood cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red blood cell when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

COLLEGE of AMERICAN PATHOLOGISTS

Don't Miss Out On This Opportunity to Earn Continuing Education Credit

***** Enter the information below and distribute to your laboratory staff. *****

Program Mailing and Year:	VPBS-B 2020
Activity Start Date:	August 31, 2020
Activity Expiration Date:	August 30, 2021

How to Access Your Online Education Activities

1. **Access Your Account**
   a. Go to cap.org and click **Login**.
   b. **If you are associated with one laboratory** that purchased kits for this activity, the system will register you into the activity. A kit will be associated to your registration.
   c. **If you are associated with more than one laboratory** that purchased kits for this activity, you will need to select the laboratory by clicking the **Select or Change Laboratory** button. The system will register you into the activity. A kit will be associated to your registration.
   d. **If you are not associated with a laboratory**, you need to add a laboratory affiliation by following these steps:
      - Under the Login menu, click **Update My Profile**.
      - Click the **Business/Professional** tab.
      - Click **+ Add Affiliation.**

2. **Access Your Online Education Activities**
   a. Go to cap.org and click **Login**.
   b. Click on the **Learning** tab.
   c. Enter the Program code in the Search box (eg, BMD, CGL), then click the binoculars icon.
   d. Click **Register**.
   e. After reviewing the Activity Details page, click **Register.**
   f. Click **Resume** to access the Activity.
   g. Click the confirmation checkbox at the bottom of the Activity Overview page, then click **Continue**.
   h. If you choose to return to the activity later, it can be found on the In-Progress Learning tab. Click the activity title to return to the activity.

Important: Before viewing review the System Requirements page on cap.org. Pop-up blockers must be turned off to complete the activity.

For assistance, call the Customer Contact Center at 800-323-4040 or 847-832-7000, option 1.