COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Surveys and Anatomic Pathology Education Programs

Virtual Peripheral Blood Smear VPBS-A 2022

Participant Summary

Self-Reported Training Available

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2022 VPBS-A
PARTICIPANT SUMMARY

Program Update

Don't Miss Out on this Educational Opportunity!

With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities that may earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

This Survey mailing includes a Self-Reported Training activity*. By reviewing the discussion that begins on page 13, your laboratory staff can participate in this activity which may be used towards fulfilling education and maintenance of certification (MOC) requirements. For your convenience, a form has been included to document your staff's participation in the activity. See page 43.

**CAP Self-Reported Training activities do not offer CE credit but can be used towards fulfilling requirements for MOC by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

Evaluation Criteria

Results for the VPBS Survey are **not** formally evaluated; however statistics will appear in the participant summary for your information.

The quantitative data tables provided in this participant summary include multiple statistical values which may include the median, MIN, and MAX values reported for each peer group. The MIN and MAX values are not the limits of acceptability. The acceptable limits are located on your participant evaluation report.

To provide a timely evaluation of your results, statistics presented in this participant summary reflect participant data received by the due date.

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 41. Laboratories should perform a self-evaluation. For more information, go to cap.org.

1. Hover over Laboratory Improvement and click **Proficiency Testing.**
2. Under Proficiency Testing (PT) Programs, Surveys, click **PT Resources.**
3. Under Existing Customers, click **Performing a Self-Evaluation When PT is Not Graded.**

VPBS-01

Clinical History for VPBS-02 – VPBS-06

This peripheral blood smear is from a 63-year-old man with a history of malignant B-cell lymphoma, now presenting with fatigue. Laboratory data include: WBC = 66.8 x 10E9/L; RBC = 2.11 x 10E12/L; HGB = 6.3 g/dL; HCT = 18.9%; MCV = 90 fL; PLT = 221 x 10E9/L; and RDW = 17%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.
https://www.digitalscope.org/LinkHandler.axd?LinkId=0f4ed923-1162-423b-a4e9-c65ca60e6303

To access the online Hematology Glossary, please click the hyperlink below:
https://documents.cap.org/documents/cap-hematology-and-clinical-microscopy-glossary.pdf

Summary of Participant Survey Results

The following is a statistical summary of all results submitted by participating laboratories. These are provided to allow participants to see their responses in the context of their peers. These results may identify findings or topics for further education or review. Survey results are not intended to represent the correct or desired responses for proficiency testing purposes and the SD and CV% should not be interpreted as acceptable reporting limits. Participants are encouraged to review discrepant results with their medical director.

WBC Differential - %	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1302	4.0	1.9	45.8	4	0	10
Lymphocytes	1271	37.8	32.9	86.9	27	0	100
Lymphocytes, reactive	575	1.2	3.5	*	0	0	21
Monocytes	810	1.0	1.2	*	1	0	5
Eosinophils	533	0.0	0.0	0.0	0	0	0
Basophils	559	0.0	0.0	0.0	0	0	0
Metamyelocytes	564	0.1	0.3	*	0	0	1
Myelocytes	528	0.0	0.0	0.0	0	0	0
Promyelocytes	537	0.0	0.0	0.0	0	0	0
Blasts	892	33.4	36.6	*	14	0	98
nRBC/100 WBC	1220	3.1	1.7	54.5	3	0	8

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1209	2.660	1.189	44.7	2.67	0.00	6.55
Lymphocytes	1188	25.244	21.960	87.0	18.04	0.00	84.64
Lymphocytes, reactive	533	0.848	2.355	*	0.00	0.00	14.03
Monocytes	747	0.665	0.793	*	0.67	0.00	3.35
Eosinophils	496	0.003	0.030	*	0.00	0.00	0.45
Basophils	517	0.000	0.000	0.0	0.00	0.00	0.00
Metamyelocytes	484	0.005	0.052	*	0.00	0.00	0.66
Myelocytes	487	0.000	0.000	0.0	0.00	0.00	0.00
Promyelocytes	495	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	832	22.336	24.555	*	9.35	0.00	82.00

*When low results are reported on an analyte, a high coefficient of variance (CV%) may result. When the mean value is very low, the CV% may be exaggerated.

**Please see discussion on "Calculating Absolute Counts" that appears in this PSR.

Other cells: *All cells not listed on results form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 289 Freq
Malignant lymphoid cell (other than blast)/lymphoma cell	151
Immature/abnormal lymphoid cell	16
Basket cell/smudge cell	13
Atypical mononuclear cell	8
Malignant prolymphocyte	3
Myeloid precursor	1
Would refer for identification	97

Platelet Estimate

Intended Response: Adequate/normal platelets	N = 1318 Freq	 %
Decreased platelets	10	0.8
Adequate/normal platelets	1304	98.9
Increased platelets	3	0.2
Unable to quantitate - platelet clumps present	1	0.1

Note: For proficiency testing purposes only, platelet counts of < 140 x 10E9/L are considered decreased and > 450 x 10E9/L are considered increased**.**

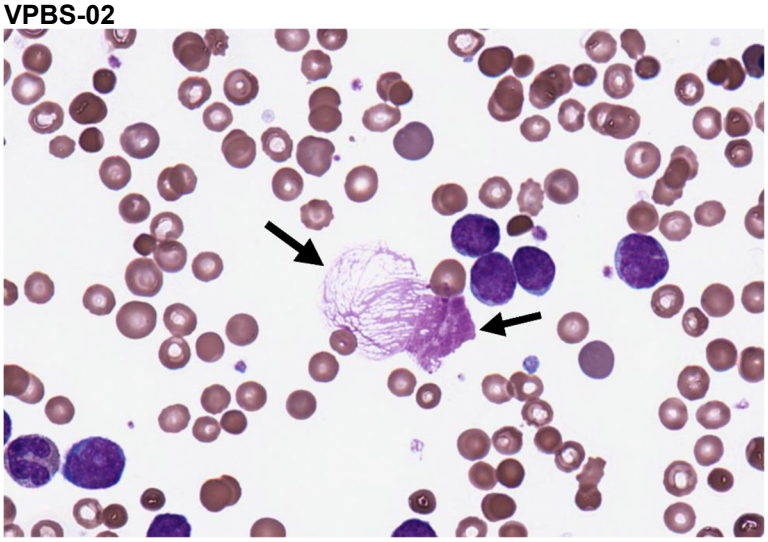
Red Cell Morphology	Total Responses N = 4490 Freq	Total Responses N = 4490 % Total Response	Total Unique Kits N = 1320 % Unique Kits
Polychromatophilic (non-nucleated) red blood cell	1183	26.4	89.6
Spherocyte	1163	25.9	88.1
Echinocyte (burr cell, crenated cell)	498	11.1	37.7
Nucleated red blood cell, normal or abnormal morphology	430	9.6	32.6
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	331	7.4	25.1
Microcyte (with increased central pallor)	295	6.6	22.4
Stomatocyte	163	3.6	12.3
Target cell (codocyte)	113	2.5	8.6
Ovalocyte (elliptocyte)	62	1.4	4.7
Acanthocyte (spur cell)	52	1.2	3.9
Howell-Jolly body	42	0.9	3.2
Erythrocyte, normal	38	0.9	2.9
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	36	0.8	2.7
Teardrop cell (dacrocyte)	28	0.6	2.1
Rouleaux	27	0.6	2.0
Basophilic stippling (coarse)	12	0.3	0.9
Red blood cell agglutinates	6	0.1	0.5
Bite cell (degmacyte)	4	0.1	0.3
Pappenheimer bodies (iron or Wright stain)	3	0.1	0.2
Blister cell/Prekeratocyte	1	0.0	0.1
Erythrocyte with overlying platelet	1	0.0	0.1
Hemoglobin C crystal	1	0.0	0.1
Immature or abnormal cell, would refer for identification	1	0.0	0.1

Committee Comments on the CBC and Peripheral Blood Whole Slide

The provided CBC data indicate normocytic anemia and leukocytosis. Red blood cells show anisocytosis with polychromatophilic red blood cells, spherocytes, and nucleated red blood cells present. Leukocytes are increased, consisting of numerous malignant lymphocytes. Smudge cells are readily identified. Platelets are adequate and demonstrate normal morphology.

Cell Identification

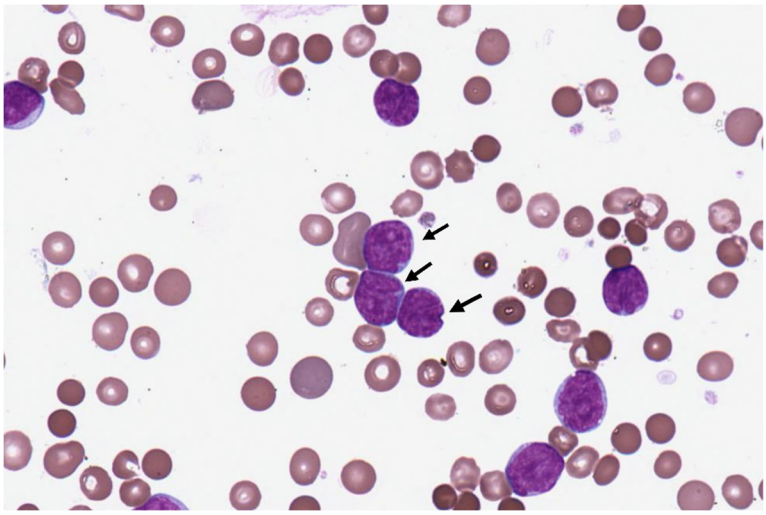
VPBS-02



Identification	Participants Freq	%	Evaluation
Basket cell/smudge cell	1320	99.5	Educational
Stain precipitate	4	0.3	Educational
Blast cell	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational

The arrowed cell is a basket cell or smudge cell, as correctly identified by 99.5% of participants. Basket cells or smudge cells are most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a nondescript chromatin mass or have chromatin strands spreading out from a condensed nuclear remnant, giving the appearance of a basket. The cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

VPBS-03



Identification	Participants Freq	%	Evaluation
Malignant lymphoid cell (other than blast)	682	51.4	Educational
Blast cell	532	40.1	Educational
Lymphocyte	38	2.9	Educational
Immature or abnormal cell, would refer for identification	36	2.7	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	33	2.5	Educational
Lymphocyte, large granular	1	0.1	Educational
Monocyte	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cells are malignant lymphoid cells (other than blast), as correctly identified by 51.4% of participants. 2.7% of participants selected immature/abnormal cell, would refer as the identification which is an acceptable answer. Lymphoma cells can exhibit a variety of appearances depending on the lymphoma subtype, and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 to 30 µm, and the N:C ratio varies from 7:1 to 3:1. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a diagnosis. In blood smears, it may be difficult to distinguish reactive lymphocytes from lymphoma cells. The most important distinction between these cells is the difference in their N:C ratios. The N:C ratio tends to be low in reactive lymphocytes, while it is high in lymphoma cells. In addition, reactive lymphocytes are characterized by their wide range of morphologic appearances within the same blood smear. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances, any individual case tends to show a more monotonous population of the abnormal cells as seen in this image.

40.1% of participants incorrectly identified the arrowed cells as blast cells. A blast is a large, round-to-oval cell, 10 to 20 µm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent red blood cells. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. The

morphologic features of a blast cell do not permit determination of the cell lineage, ie, myeloblast versus lymphoblast. The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage (ie, "myeloblast"). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining (eg, peroxidase or Sudan black) is required to determine the lineage of a given blast cell.

As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Blasts may rarely be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable. While there can be significant morphologic overlap between blasts and malignant lymphoid cells, the more condensed chromatin of the cells pictured, as well as the history of a malignant B-cell lymphoma, make 'malignant lymphoid cell, other than blast' the best answer in this case.

2.9% of participants incorrectly identified the arrowed cells as lymphocytes. While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with round to oval nuclei, absence of nucleoli, and an N:C ratio ranging from 5:1 to 2:1, while the cells pictured here are larger with more irregular nuclear contours and conspicuous nucleoli. Despite the fact that both normal and malignant lymphoid cells can have a high N:C ratio, several morphologic features, including increased cell size, irregular nuclear contours, and conspicuous nucleoli should aid in differentiating the malignant lymphoid cells pictured from normal lymphocytes. In addition, the history of a malignant B-cell lymphoma should serve as a clue to the identity of the cells in these images.

2.5% of participants incorrectly identified the arrowed cells as lymphocytes, reactive. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. These cells have round to oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present.

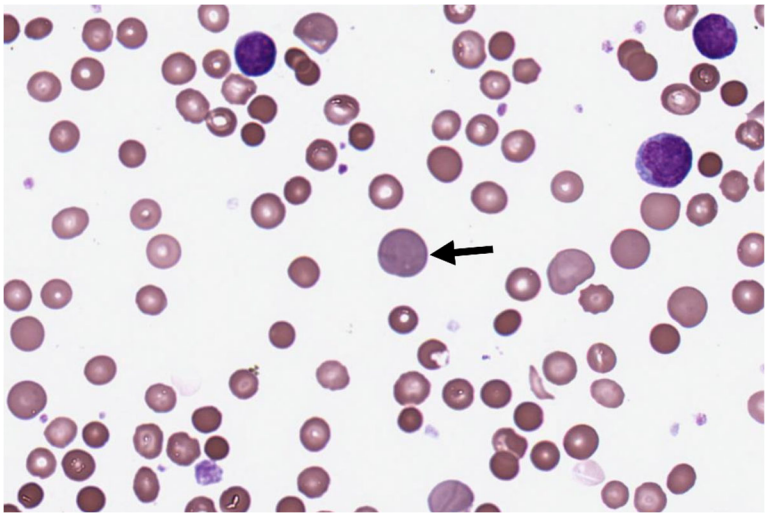
Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The N:C ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells.

Another type of reactive lymphocyte is referred to as a Downey I cell. These cells are rare. These cells possess scant to moderate amounts of basophilic cytoplasm. The nuclei often appear indented, folded, or lobulated. The chromatin is condensed. A few small vacuoles may be present. Granules may also be apparent.

Plasmacytoid lymphocytes resemble plasma cells and are intermediate in size (10 to 20 µm) and round to oblong in shape. They have round nuclei that are centrally placed or slightly eccentric. The chromatin is slightly to moderately coarse and forms small dense masses or a meshwork of strands resembling that of plasma cells. Nucleoli are generally not visible, but some cells may have one or two small irregular nucleoli. The cytoplasm is moderately abundant, homogeneous, and light blue to deep slate-blue, and it may show a perinuclear clear zone, or hof.

The cells present in these images are monotonous and large in size with high N:C ratios and irregular nuclear contours. These features should aid in the distinction between reactive lymphocytes and malignant lymphocytes. In addition, the history of a malignant B-cell lymphoma should serve as a clue to the identity of the cells in these images.

VPBS-04



Identification	Participants Freq	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1232	92.9	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	84	6.3	Educational
Basophilic stippling (coarse)	5	0.4	Educational
Blast cell	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Spherocyte	1	0.1	Educational

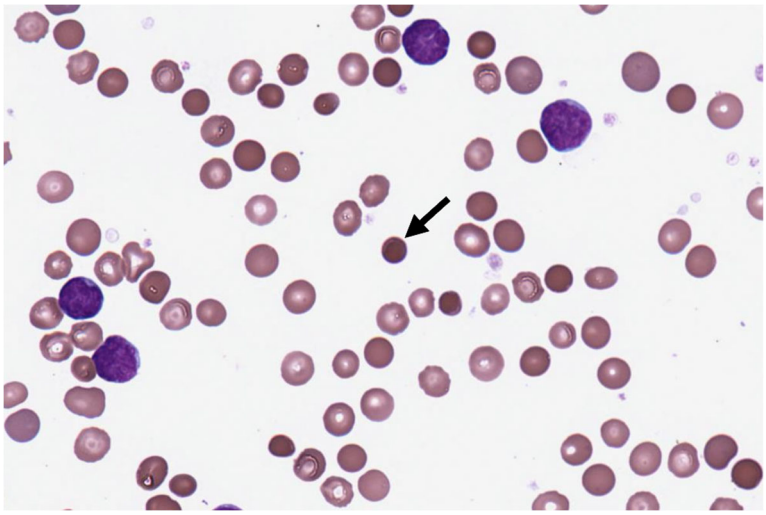
The arrowed cell is a polychromatophilic red blood cell, as correctly identified by 92.9% of participants. The response of polychromatophilic red blood cell is considered the most specific answer for this challenge. A polychromatophilic red blood cell is a non-nucleated, round or ovoid red blood cell that represents the final stage of red blood cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte and usually lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the “substantia reticulofilamentosa.” The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

6.3% of participants incorrectly identified the arrowed cell as a macrocyte, oval or round (excludes polychromatophilic RBC). Macrocytes are abnormally large red blood cells (diameter > 8.5 µm). They are best detected by comparing to other red blood cells in a smear in the context of the MCV. The MCV for this case was 90 fL, which indicates normal-sized red blood cells. Macrocytes may be oval or round. The hemoglobin concentration is normal; macrocytes lack significant polychromasia. If polychromasia is readily identified, as in this case, the term polychromatophilic red blood cell is preferred for proficiency testing purposes. The pink-gray color of the cytoplasm in the arrowed cell supports classification as a polychromatophilic red blood cell.

Round macrocytes are associated with reticulocytosis, liver disease, hypothyroidism, and post-splenectomy states. Oval macrocytes are most commonly associated with vitamin B12 or folic acid deficiency. Abnormal red blood cell maturation (dyserythropoiesis) may also cause oval macrocytosis. Examples include myelodysplastic syndromes and chemotherapy. Oval macrocytes may be mistaken for ovalocytes (elliptocytes).

Ovalocytes are often longer than normal red blood cells and are significantly narrower. The sides of the cells are nearly parallel, unlike the much more rounded edges of oval macrocytes. The hemoglobin of ovalocytes is often concentrated at the ends, unlike the even peripheral distribution of oval macrocytes. Also, oval macrocytes are much larger than ovalocytes.

VPBS-05

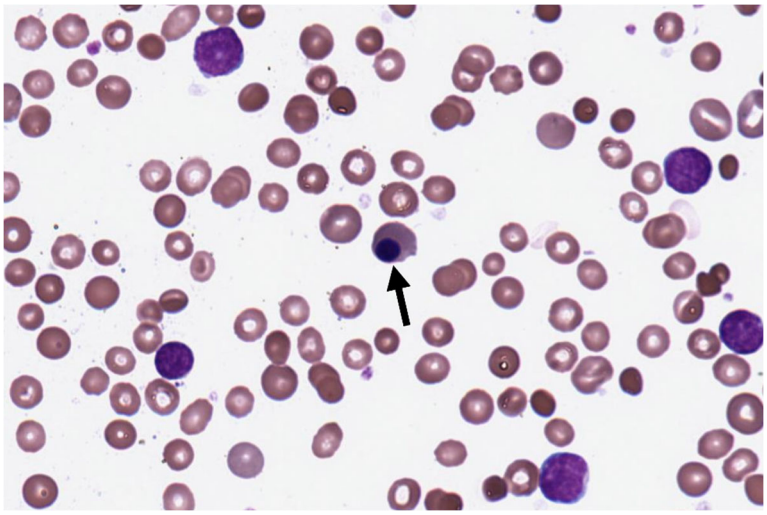


Identification	Participants Freq	%	Evaluation
Spherocyte	1255	94.7	Educational
Microcyte (with increased central pallor)	65	4.9	Educational
Erythrocyte, normal	3	0.2	Educational
Hemoglobin C crystal	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cell is a spherocyte, as correctly identified by 94.7% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and usually without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Microspherocytes (spherocytes measuring 4 µm or less in diameter) are frequently seen in severe burns or microangiopathies and represent rounded-up fragments of red blood cells.

4.9% of participants incorrectly identified the arrowed cell as a microcyte (with increased central pallor). Microcytes are smaller than normal red blood cells, measuring less than 6 µm in diameter and less than 80 fL in volume. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte. When there is little or no variation in RBC size, morphology is less reliable than instrument-generated MCVs in determining if microcytosis is present. The MCV for this case was 90 fL, which indicates normal sized red blood cells. On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. In contrast, the arrowed cell shows complete lack of central pallor. Although other poikilocytes, such as spherocytes and fragmented red blood cells, can be very small in size, these red blood cells lack central pallor and should be specifically identified rather than classified as “microcytes.” Microcytes commonly are seen in iron deficiency anemia, thalassemia, lead poisoning and some cases of anemia of chronic disease.

VPBS-06



Identification	Participants Freq	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1319	99.5	Educational
Polychromatophilic (non-nucleated) red blood cell	5	0.4	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational

The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 99.5% of participants. The term nucleated red blood cell 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. 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).

Case Presentation:

This peripheral blood smear is from a 63-year-old man with a history of malignant B-cell lymphoma, now presenting with fatigue. Laboratory data include: WBC = 66.8 x 10E9/L; RBC = 2.11 x 10E12/L; HGB = 6.3 g/dL; HCT = 18.9%; MCV = 90 fL; PLT = 221 x 10E9/L; and RDW = 17%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: B-cell lymphoma with autoimmune hemolytic anemia

On the peripheral blood smear, there are smudge/basket cells and circulating malignant lymphocytes consistent with peripheralized lymphoma. In addition, there are erythroid changes suggestive of hemolysis (polychromatophilic erythrocytes, spherocytes, and nucleated red blood cells). Collectively, these findings are consistent with hemolysis in the setting of a B-cell lymphoma. Additional laboratory testing, including reticulocyte count, haptoglobin, lactate dehydrogenase, and indirect bilirubin can be used to confirm the presence of hemolysis.

Question 1. What red cell abnormalities are most commonly identified on review of a peripheral blood smear in a patient with warm autoimmune hemolytic anemia?

A. Schistocytes
B. Spherocytes
C. Target cells
D. None of the above

Once hemolysis is confirmed, a direct antiglobulin test (Coombs test) should be used to distinguish immune and non-immune causes. A positive direct antiglobulin test, most commonly for IgG alone, in the absence of recent transfusions, cold agglutinins, or a Doneth-Landsteiner antibody is considered confirmatory of warm autoimmune hemolytic anemia. Less commonly, DAT may be positive for both IgG and C3. Any patient with warm autoimmune hemolytic anemia (AIHA) should undergo additional assessment in an attempt to identify any underlying condition. If there is no apparent cause, a thorough evaluation for a lymphoproliferative disorder is warranted, given the frequency of lymphoma in these patients.

Question 2. What is the most common pattern of positivity for the direct antiglobulin test in warm autoimmune hemolytic anemia?

A. IgG
B. C3d
C. Both
D. Neither

Warm autoimmune hemolytic anemia (AIHA) is hemolysis resulting from autoantibodies directed against red blood cells. This may be caused by a variety of conditions, including viral infections, autoimmune disorders, and lymphoproliferative disorders. Antibodies are typically IgG subtype and react most efficiently at or near physiologic temperature. Patients often present with anemia due to hemolysis, however, hemoglobin may be normal early in the process or following a compensatory increase in red blood cell production. Peripheral blood findings of hemolysis include increased reticulocytes, which may be represented on peripheral blood smears as polychromatophilic erythrocytes, spherocytes, and nucleated red blood cells. This is primarily an extravascular process, and thus red cell fragments or schistocytes, which are seen in intravascular hemolysis, are not typically identified in these cases.

Question 3. What antibody isotype is most frequently associated with warm autoimmune hemolytic anemia?

A. IgA
B. IgG
C. IgM
D. IgE

Unlike warm AIHA, cold agglutinin disease (CAD) occurs when autoantibodies, which react best at approximately four degrees Celsius, cause red cell agglutination and hemolysis. CAD is typically mediated by IgM antibodies, resulting in complement activation and extravascular hemolysis. Similar to warm AIHA, direct antiglobulin test will be positive. However, unlike warm AIHA, it is typically only positive for C3d and not IgG.

Jonathan Galeotti, M.D., M.S.
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. *Am J Hematol.* 2002;69(4):258-271.
2. Brodsky RA. Warm Autoimmune Hemolytic Anemia. *N Engl J Med.* 2019;381(7):647-654.
3. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. *Blood Rev*. 2020;41:100648.
4. Barcellini W. Immune Hemolysis: Diagnosis and Treatment Recommendations. *Semin Hematol.* 2015;52(4):304-312. doi:10.1053/j.seminhematol.2015.05.001.

ANSWERS TO QUESTIONS:

Question 1: B. Spherocytes.
Phagocytosis of a portion of the red cell membrane, due to antibody binding to the cell surface, results in formation of spherocytes in warm autoimmune hemolytic anemia. Schistocytes are seen in intravascular hemolysis, most commonly associated with microangiopathic hemolytic anemia. Target cells are associated with other causes of anemia, including thalassemia, anemia associated with liver disease, and iron deficiency anemia.

Question 2: A. IgG.
In the setting of hemolytic anemia, a positive DAT with IgG reactivity is consistent with a diagnosis of warm autoimmune hemolytic anemia. In some cases, C3d will also be positive, but it is not required for a diagnosis of warm autoimmune hemolytic anemia. Cold agglutinin disease is typically only positive for C3d and not IgG.

Question 3: B. IgG.
IgG autoantibodies are the most common subtype seen in warm autoimmune hemolytic anemia, while IgA and IgM antibodies are only rarely implicated. IgM antibodies are more frequently identified in cold agglutinin disease.

VPBS-07

Clinical History for VPBS-08 – VPBS-12

This peripheral blood smear is from a 73-year-old man with a history of monoclonal gammopathy of undetermined significance (MGUS). Laboratory data include: WBC = 8.1 x 10E9/L; RBC = 3.02 x 10E12/L; HGB = 9.8 g/dL; HCT = 30.1%; MCV = 101 fL; PLT = 194 x 10E9/L; and RDW = 15%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.
https://www.digitalscope.org/LinkHandler.axd?LinkId=4eec0a4a-9cc8-4a34-9528-bc437d900024

To access the online Hematology Glossary, please click the hyperlink below:
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The following is a statistical summary of all results submitted by participating laboratories. These are provided to allow participants to see their responses in the context of their peers. These results may identify findings or topics for further education or review. Survey results are not intended to represent the correct or desired responses for proficiency testing purposes and the SD and CV% should not be interpreted as acceptable reporting limits. Participants are encouraged to review discrepant results with their medical director.

WBC Differential - %	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1311	74.9	3.9	5.3	75	63	86
Lymphocytes	1317	11.6	4.0	34.6	12	0	23
Lymphocytes, reactive	901	3.6	3.2	88.9	3	0	13
Monocytes	1302	6.9	2.4	35.1	7	0	14
Eosinophils	1176	1.5	0.8	52.2	1	0	3
Basophils	1198	1.2	0.6	49.3	1	0	3
Metamyelocytes	664	0.4	0.6	*	0	0	2
Myelocytes	578	0.1	0.4	*	0	0	1
Promyelocytes	535	0.0	0.0	0.0	0	0	0
Blasts	533	0.0	0.0	0.0	0	0	0
nRBC/100 WBC	625	0.1	0.3	*	0	0	1

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1228	6.070	0.335	5.5	6.08	4.86	7.30
Lymphocytes	1230	0.946	0.330	34.9	0.97	0.00	1.94
Lymphocytes, reactive	832	0.293	0.269	91.9	0.24	0.00	1.13
Monocytes	1215	0.554	0.199	35.9	0.57	0.00	1.21
Eosinophils	1106	0.120	0.065	54.0	0.10	0.00	0.32
Basophils	1115	0.101	0.053	52.5	0.08	0.00	0.41
Metamyelocytes	610	0.032	0.048	*	0.00	0.00	0.16
Myelocytes	536	0.012	0.029	*	0.00	0.00	0.11
Promyelocytes	498	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	496	0.000	0.000	0.0	0.00	0.00	0.00

*When min results are reported on an analyte, a max coefficient of variance (CV%) may result. When the mean value is very min, the CV% may be exaggerated.

**Please see discussion on "Calculating Absolute Counts" that appears in this PSR.

Other cells: *All cells not listed on results form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 236 Freq
Plasma cell, morphologically mature/abnormal/containing inclusion (eg. Dutcher body, Russell body)	168
Abnormal/atypical/reactive lymphocyte	4
Immature granulocyte/myeloid precursor	4
Basket/smudge cell	1
Would refer for identification	59

Platelet Estimate

Intended Response: Adequate/normal platelets	N = 1318 Freq	%
Decreased platelets	37	2.8
Adequate/normal platelets	1280	97.1
Increased platelets	1	0.1

Note: For proficiency testing purposes only, platelet counts of < 140 x 10E9/L are considered decreased and > 450 x 10E9/L are considered increased.

Red Cell Morphology	Total Responses N = 4098 Freq	Total Responses N = 4098 % Total Response	Total Unique Kits N = 1309 % Unique Kits
Ovalocyte (elliptocyte)	1183	28.9	90.4
Teardrop cell (dacrocyte)	1132	27.6	86.5
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	355	8.7	27.1
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	332	8.1	25.4
Polychromatophilic (non-nucleated) red blood cell	332	8.1	25.4
Rouleaux	189	4.6	14.4
Echinocyte (burr cell, crenated cell)	152	3.7	11.6
Microcyte (with increased central pallor)	111	2.7	8.5
Erythrocyte, normal	90	2.2	6.9
Erythrocyte with overlying platelet	44	1.1	3.4
Acanthocyte (spur cell)	42	1.0	3.2
Target cell (codocyte)	41	1.0	3.1
Bite cell (degmacyte)	28	0.7	2.1
Spherocyte	22	0.5	1.7
Blister cell/Prekeratocyte	11	0.3	0.8
Stomatocyte	11	0.3	0.8
Nucleated red blood cell, normal or abnormal morphology	10	0.2	0.8
Howell-Jolly body	6	0.1	0.5
Basophilic stippling (coarse)	3	0.1	0.2
Red blood cell agglutinates	2	0.1	0.1
Pappenheimer bodies (iron or Wright stain)	1	0.0	0.1
Sickle cell (drepanocyte)	1	0.0	0.1

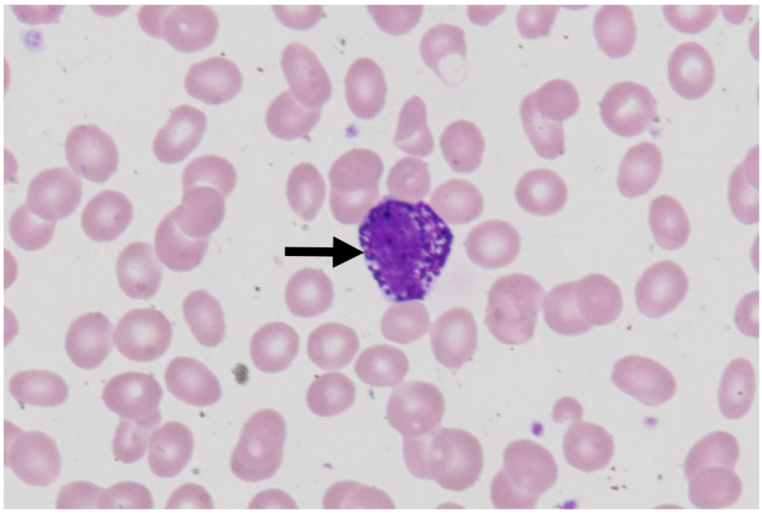
VPBS-07, cont'd.

Committee Comments on CBC and Peripheral Blood Whole Slide Image

The CBC data are indicative of a moderate anemia with marked macrocytosis as well as an unquantifiable platelet count due to platelet clumping. The red blood cells show polychromasia, with increased numbers of polychromatophils. There is no significant size and shape variation among red blood cells (no anisopoikilocytosis). Platelets are frequently clumped, in clusters of 5 - 20 or more aggregated platelets. Some platelets are also seen individually scattered. Platelets show normal granulation and mild size variation. Giant platelets are rare. White blood cell (WBC) manual differential counts of the virtual peripheral blood smear reveal normal numbers of all WBC components (in decreasing order of relative numbers): segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. No significant morphologic abnormalities are observed in any of the WBCs. Lymphocytes show a spectrum of morphology, including occasional large granular lymphocytes and immunoblasts (reactive lymphocytes).

Cell Identification

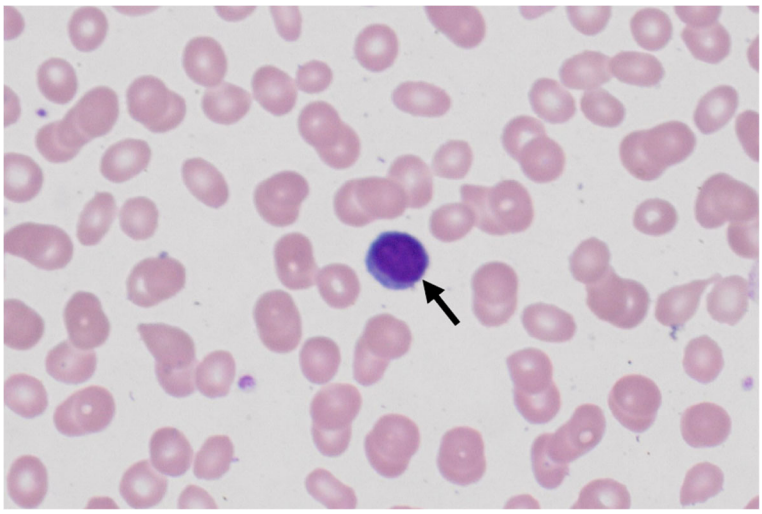
VPBS-08



Identification	Participants Freq	%	Evaluation
Basophil, any stage	1322	99.6	Educational
Basophilic stippling (coarse)	3	0.2	Educational
Lymphocyte, large granular	1	0.1	Educational
Mast cell	1	0.1	Educational

The arrowed cell is a basophil, as correctly identified by 99.6% of participants. Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic myelocyte to the mature segmented basophil, 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, in hypersensitivity reactions, with hypothyroidism, iron deficiency, and renal disease. Basophil granules can be stained with toluidine blue (resulting in a purple color) to differentiate them from the granules of neutrophils.

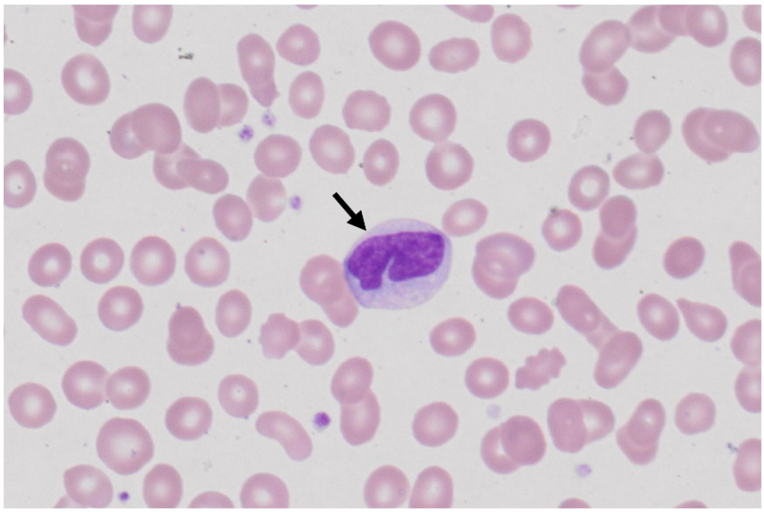
VPBS-09



Identification	Participants Freq	%	Evaluation
Lymphocyte	1309	98.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	12	0.9	Educational
Bacteria (cocci or rod), extracellular	1	0.1	Educational
Bite cell (degmacyte)	1	0.1	Educational
Blast cell	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Monocyte	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

The arrowed cell is a normal lymphocyte, as correctly identified by 98.6% of the participants. While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology. 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 diffusely 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.

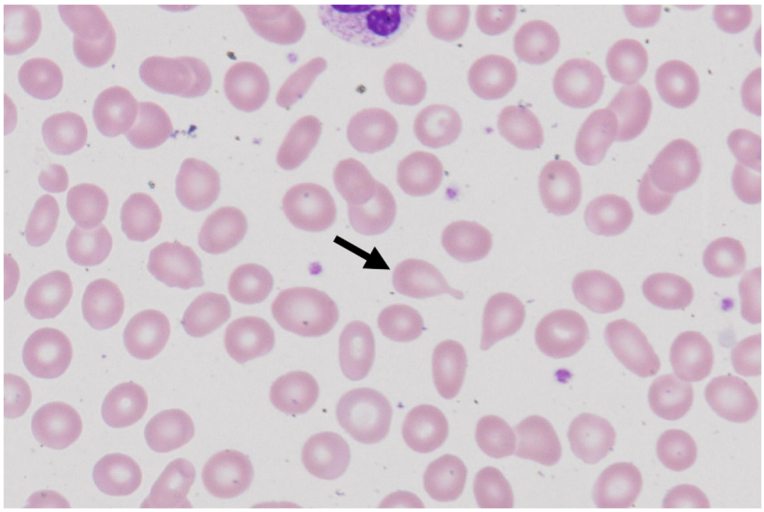
VPBS-10



Identification	Participants Freq	%	Evaluation
Monocyte	1308	98.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	8	0.6	Educational
Monocyte, immature (promonocyte, monoblast)	8	0.6	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cell is a normal monocyte, as correctly identified by 98.6% of the 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 N:C ratio ranges from 4:1 to 2:1. 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.

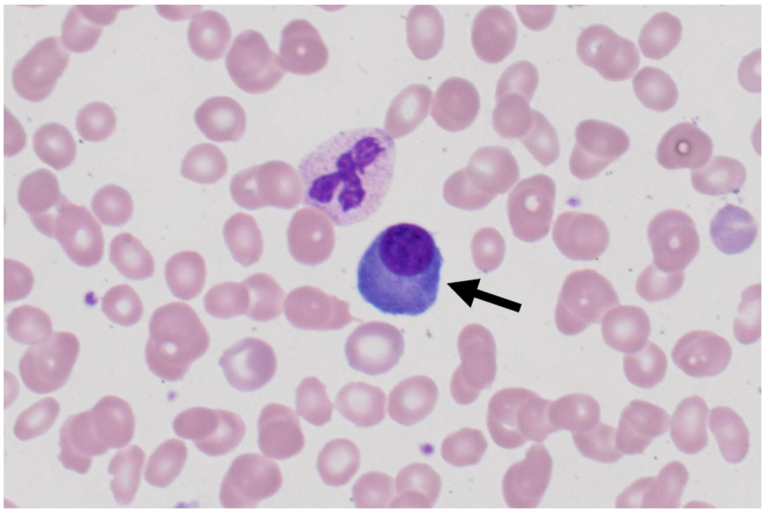
VPBS-11



Identification	Participants Freq	%	Evaluation
Teardrop cell (dacrocyte)	1323	99.7	Educational
Target cell (codocyte)	3	0.2	Educational
Erythrocyte with overlying platelet	1	0.1	Educational

The arrowed cell is a teardrop cell/dacrocyte, as correctly identified by 99.7% of the 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, 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-12



Identification	Participants Freq	%	Evaluation
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1129	85.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	116	8.7	Educational
Nucleated red blood cell, normal or abnormal morphology	42	3.2	Educational
Immature or abnormal cell, would refer for identification	14	1.1	Educational
Lymphocyte	10	0.8	Educational
Malignant lymphoid cell (other than blast)	5	0.4	Educational
Blast cell	3	0.2	Educational
Protozoa (non-malarial)	3	0.2	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a plasma cell, as correctly identified by 85.1% of the participants. 1.1% of participants selected “immature/abnormal cell, would refer” which is also an acceptable answer. Plasma cells represent terminally differentiated B-lymphocytes and are a normal constituent of the bone marrow where they usually comprise less than 5% of the cellularity. They are rarely seen in normal peripheral blood. They range in size from 10 to 20 µm, and they are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. Occasional benign plasma cells are binucleated. Nucleoli are absent. The cytoplasm stains gray blue to deeply basophilic. A prominent hof or perinuclear zone of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus. This area corresponds to the Golgi zone, which is prominent in cells that produce large amounts of protein, such as immunoglobulin in the case of plasma cells. Cytoplasmic granules are absent, and scattered vacuoles of varying size may be seen.

Immature or atypical plasma cells in the bone marrow or, less commonly, in the blood, are associated with a variety of plasma cell dyscrasias, including multiple myeloma (plasma cell myeloma), lymphoplasmacytic lymphoma (Waldenstrȍm macroglobulinemia), and amyloidosis. Malignant plasma cells show a wide spectrum of morphologic features and may include some or all forms of plasmablasts, immature plasma

cells, and mature plasma cells. The cells range from those that are easily recognized as plasma cells to those that are difficult to classify without ancillary studies or clinical data. Binucleated and multinucleated forms may be frequent and, when present, often display immature nuclear characteristics. Atypical mitotic figures may also be found. Malignant plasma cells in the peripheral blood may be numerous in cases of plasma cell leukemia.

Plasma cells normally produce and secrete immunoglobulins. This protein product may appear in different forms within the cytoplasm. When production within a particular plasma cell is increased or when there is a blockage in secretion, accumulation of immunoglobulin occurs. This finding can occur in mature, immature, or malignant plasma cells. These plasma cells range from 10 to 25 µm, and the N:C ratio varies from 1:2 to 1:3. Accumulations of immunoglobulin sometimes appear as intranuclear inclusions called Dutcher bodies. While Dutcher bodies appear to be within the nucleus, they are actually pseudoinclusions that occur when a cytoplasmic globule invaginates through the nucleus or is surrounded by the nucleus. The immunoglobulin globules may also appear as large cytoplasmic eosinophilic globules called Russell bodies. When multiple Russell bodies are present, the cell is called a Mott cell. Occasionally, immunoglobulin inclusions in plasma cells may form crystalline structures in the cytoplasm.

8.7% of participants incorrectly identified this cell as a lymphocyte, reactive. There are some morphologic similarities between this cell and a plasmacytoid lymphocyte (a morphologic subset of reactive lymphocytes). However, this cell is best classified as a plasma cell due to its markedly eccentric nucleus and prominent hof (perinuclear clear zone); these features are less pronounced in plasmacytoid lymphocytes.

3.2% of participants incorrectly identified this cell as a nucleated red blood cell, normal or abnormal morphology. While nucleated red blood cells can sometimes have eccentric nuclei as in this case, the chromatin pattern in this case is that of a plasma cell rather than a nucleated red blood cell. In addition, the prominent hof (perinuclear clear zone) in this cell is a key finding in plasma cells that is not seen in nucleated red blood cells.

Case Presentation:

This peripheral blood smear is from a 73-year-old man with a history of monoclonal gammopathy of undetermined significance (MGUS). Laboratory data include: WBC = 8.1 x 10E9/L; RBC = 3.02 x 10E12/L; HGB = 9.8 g/dL; HCT = 30.1%; MCV = 101 fL; PLT = 194 x 10E9/L; and RDW = 15%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Monoclonal gammopathy of undetermined significance

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic, premalignant condition of clonal plasma cells or clonal lymphocytes with plasmacytic differentiation.

MGUS is divided into two subtypes based on the type of monoclonal immunoglobulin produced by the plasmacytic disorder. IgM MGUS is characterized by production of a monoclonal IgM protein, as its name suggests, and is associated with a lymphoplasmacytic lymphoproliferative disorder. Non-IgM MGUS is more common and is associated with a plasma cell neoplasm, typically without lymphocytic differentiation. Non-IgM MGUS is characterized by the production of a monoclonal IgG (60%), IgA (15%), IgD (1%), or IgE (1%) protein; and approximately 20% non-IgM MGUS are characterized by production of an immunoglobulin light chain (kappa or lambda) only.

Question 1. The most common monoclonal protein produced in non-IgM MGUS is:

A. IgA
B. IgE
C. IgG
D. IgM

MGUS is typically identified incidentally in the evaluation of a different disorder since patients are asymptomatic by definition. The diagnosis of MGUS is made when laboratory testing identifies a monoclonal protein on SPEP that is < 30 g/L (or an abnormal free light chain ratio and an increase of the involved light chain with complete loss of heavy chain expression in the serum in the case of a non-IgM light chain MGUS). A bone marrow biopsy must show <10% clonal plasma cells (non-IgM MGUS) or lymphoplasmacytic infiltrate (IgM MGUS) and additional laboratory evaluation is also required to exclude symptoms of end organ damage (hypercalcemia, renal insufficiency, anemia, bone lesions). Importantly, the end organ damage must be attributable to the plasmacytic neoplasm. For example, patients with anemia due to iron deficiency who otherwise meet criteria for MGUS would still be considered to have MGUS despite their anemia.

Question 2. Which of the following are necessary for the diagnosis of MGUS:

A. Anemia attributable to a clonal plasmacytic process
B. Less than 10% clonal plasma cell in a bone marrow biopsy
C. Lytic bone lesions
D. Monoclonal protein quantitated at more than 30 g/L on SPEP

MGUS is considered a premalignant neoplasm. Approximately 1% of cases of MGUS progress to multiple myeloma or related malignancies every year. MGUS is relatively common in older populations; 3 - 4% of people

over the age of 50 have MGUS. The vast majority of cases do not progress to myeloma or related malignancies. Peripheral blood smear findings in MGUS are generally normal, or demonstrate changes due to unrelated conditions. Rarely, rouleaux formation can be seen.

The differential diagnosis of MGUS includes both neoplastic and reactive conditions. Multiple myeloma also shows a monoclonal immunoglobulin (or excess of monoclonal light chains), but is differentiated from MGUS by >10% clonal plasma cells in the bone marrow and/or the presence of end organ damage. Mature B cell lymphomas such as lymphocytic lymphoma can also show plasmacytic differentiation and produce a monoclonal immunoglobulin; they are differentiated from MGUS by > 10% clonal lymphoplasmacytic infiltrate in the bone marrow, or a significant monoclonal lymphoplasmacytic population circulating in the peripheral blood. Reactive plasmacytosis is also included in the differential; this entity does not produce a monoclonal immunoglobulin on SPEP, but rather increased but polyclonal immunoglobnulins. Reactive plasmacytosis is characterized by a polyclonal plasma cell proliferation.

The presence of circulating plasma cells in this case is not typical of MGUS and is concerning for progression to a plasma cell neoplasm such as multiple myeloma; correlation with bone marrow findings and evaluation for symptoms of end organ damage are required for final classification.

Question 3: True or false: MGUS causes symptoms that prompt patients to seek medical attention in most cases.

A. False
B. True

Philipp W. Raess, MD, PhD, FCAP
Hematology and Clinical Microscopy Committee

REFERENCES:

1. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Rajkumar SV et al. *Lancet Oncol.* 2014 Nov;15(12):e538-48.
2. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, rev. 4th ed. IARC; 2016.

ANSWERS TO QUESTIONS:

Question 1: C. IgG
Non-IgM MGUS with a monoclonal IgG accounts for 60% of cases of non-IgM MGUS. Non-IgM MGUS is more common than IgM MGUS.

Question 2: B. Less than 10% clonal plasma cells on bone marrow biopsy
MGUS must have less than 10% clonal plasma cells on the bone marrow biopsy; if more than 10% clonal plasma cells are present, a diagnosis of plasma cell myeloma should be considered.

Question 3: A. False
Most cases of MGUS are discovered in the evaluation for other entities. By definition, MGUS should not have evidence of end organ damage attributable to the underlying plasmacytic disorder that could cause symptoms.

VPBS-13

Clinical History for VPBS-14 – VPBS-18

This peripheral blood smear is from a 56-year-old woman who presents with fever and chills after recently traveling throughout Ethiopia for the past 6 months. Laboratory data include: WBC = 9.6 x 10E9/L; RBC = 5.05 x 10E12/L; HGB = 14.6 g/dL; HCT = 41.1%; MCV = 88 fL; PLT = 83 x 10E9/L; and RDW = 14%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.
https://www.digitalscope.org/LinkHandler.axd?LinkId=4828da75-3e20-47b4-95e8-e070566f011d

To access the online Hematology Glossary, please click the hyperlink below:
https://documents.cap.org/documents/cap-hematology-and-clinical-microscopy-glossary.pdf

Summary of Participant Survey Results

The following is a statistical summary of all results submitted by participating laboratories. These are provided to allow participants to see their responses in the context of their peers. These results may identify findings or topics for further education or review. Survey results are not intended to represent the correct or desired responses for proficiency testing purposes and the SD and CV% should not be interpreted as acceptable reporting limits. Participants are encouraged to review discrepant results with their medical director.

WBC Differential - %	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1311	68.4	4.3	6.3	68	56	81
Lymphocytes	1308	12.0	4.9	40.7	11	0	27
Lymphocytes, reactive	1121	7.2	4.5	62.5	7	0	20
Monocytes	1306	12.9	4.0	30.8	13	1	24
Eosinophils	579	0.1	0.3	*	0	0	1
Basophils	547	0.0	0.0	0.0	0	0	0
Metamyelocytes	548	0.1	0.3	*	0	0	1
Myelocytes	532	0.0	0.0	0.0	0	0	0
Promyelocytes	536	0.0	0.0	0.0	0	0	0
Blasts	530	0.0	0.0	0.0	0	0	0
nRBC/100 WBC	573	0.0	0.0	0.0	0	0	0

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1226	6.561	0.427	6.5	6.53	5.00	8.10
Lymphocytes	1224	1.163	0.484	41.6	1.10	0.00	2.70
Lymphocytes, reactive	1036	0.693	0.435	62.8	0.67	0.00	2.02
Monocytes	1220	1.245	0.380	30.5	1.25	0.10	2.40
Eosinophils	535	0.012	0.033	*	0.00	0.00	0.10
Basophils	506	0.000	0.000	0.0	0.00	0.00	0.00
Metamyelocytes	511	0.008	0.026	*	0.00	0.00	0.10
Myelocytes	497	0.000	0.000	0.0	0.00	0.00	0.00
Promyelocytes	500	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	495	0.000	0.000	0.0	0.00	0.00	0.00

*When low results are reported on an analyte, a high coefficient of variance (CV%) may result. When the mean value is very low, the CV% may be exaggerated.

**Please see discussion on "Calculating Absolute Counts" that appears in this PSR.

Other cells: *All cells not listed on results form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 121 Freq
Plasmodium sp. (malaria)	60
Basket cell/smudge cell	6
Would refer for identification	55

Platelet Estimate

Intended Response: Decreased platelets	N = 1309 Freq	%
Decreased platelets	1283	97.3
Adequate/normal platelets	33	2.5
Increased platelets	2	0.1
Unable to quantitate - platelet clumps present	1	0.1

Note: For proficiency testing purposes only, platelet counts of < 140 x 10E9/L are considered decreased and > 450 x 10E9/L are considered increased**.**

Red Cell Morphology	Total Responses N = 2352 Freq	Total Responses N = 2352 % Total Response	Total Unique Kits N = 1101 % Unique Kits
Erythrocyte, normal	611	26.0	55.5
Polychromatophilic (non-nucleated) red blood cell	339	14.4	30.8
Erythrocyte with overlying platelet	284	12.1	25.8
Macrocyte, oval or round (excluding polychromatophilic red bood cell)	203	8.6	18.4
Spherocyte	203	8.6	18.4
Microcyte (with increased central pallor)	118	5.0	10.7
Rouleaux	124	5.3	11.3
Teardrop cell (dacrocyte)	106	4.5	9.6
Ovalocyte (elliptocyte)	101	4.3	9.2
Echinocyte (burr cell, crenated cell)	72	3.1	6.5
Stomatocyte	70	3.0	6.4
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	47	2.0	4.3
Bite cell (degmacyte)	15	0.6	1.4
Howell-Jolly body	13	0.6	1.2
Acanthocyte (spur cell)	12	0.5	1.1
Immature or abnormal cell, would refer for identification	9	0.4	0.8
Red blood cell agglutinates	8	0.3	0.7
Basophilic stippling (coarse)	5	0.2	0.5
Target cell (codocyte)	5	0.2	0.5
Pappenheimer bodies (iron or Wright stain)	3	0.1	0.3
Blister cell/Prekeratocyte	2	0.1	0.2
Nucleated red blood cell, normal or abnormal morphology	2	0.1	0.2

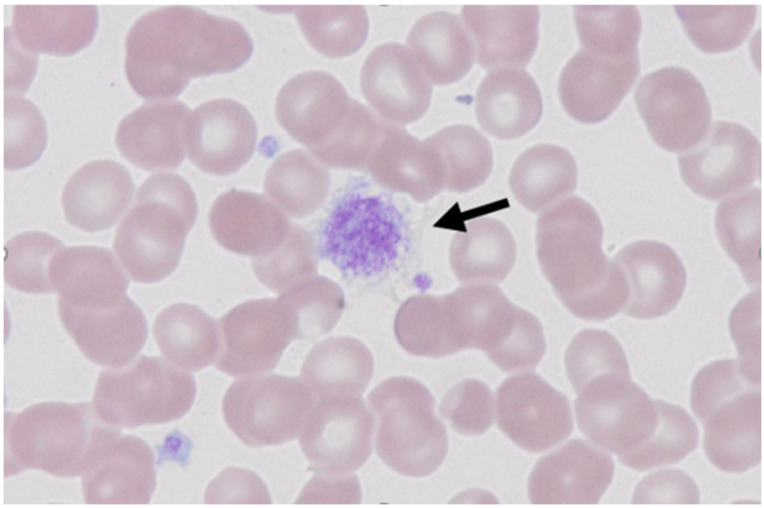
VPBS-13, cont'd.

Committee Comments on the CBC and Peripheral Blood Whole Slide Image

Review of this peripheral blood smear reveals a moderate thrombocytopenia. Several reactive lymphocytes are noted with heterogeneous morphology. In addition, micro-organisms, consistent with *Plasmodium vivax*, are noted. These include ring form trophozoites, trophozoites, and gametocytes. Infected red blood cells are frequently larger than other RBC.

Cell Identification

VPBS-14



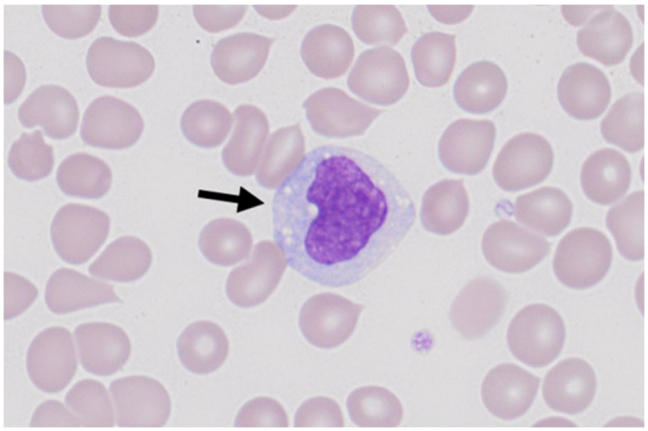
Identification	Participants Freq	%	Evaluation
Platelet, giant (macrothrombocyte)	1283	96.7	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	36	2.7	Educational
Basket cell/smudge cell	4	0.3	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Monocyte	1	0.1	Educational
Plasmodium sp. (malaria)	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational

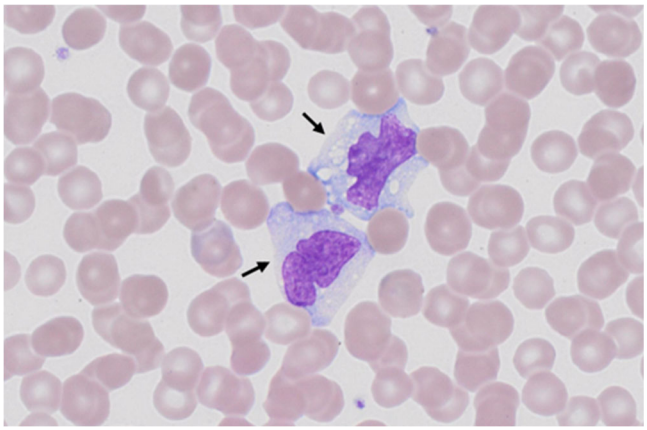
The arrowed object is a platelet, giant (macrothrombocyte), as correctly identified by 96.7% of participants. Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term giant platelet is used when the platelet is larger than the size of the average red blood cell in the field, assuming a normal MCV. The periphery of the giant platelet may be round, scalloped, or stellate. The cytoplasm may contain a normal complement of fine azurophilic granules, or the granules may fuse into giant forms. Giant platelets are a rare finding in normal peripheral blood, but may be seen in many different reactive, neoplastic, and inherited conditions. Reactive causes include conditions in which platelet turnover is markedly increased, such as immune thrombocytopenia or severe leukemoid reactions. Giant platelets are most often seen in myeloproliferative neoplasms and myelodysplastic syndromes. The inherited conditions associated with giant platelets are rare and have associated thrombocytopenia. This group of disorders is termed congenital macrothrombocytopenias and includes May Hegglin anomaly and Bernard-Soulier syndrome among multiple other disorders.

2.7% of participants incorrectly identified this arrowed object as a megakaryocyte. Megakaryocyte “nuclei” or “nuclear fragments” are uncommonly observed in the peripheral blood. After subtotally discharging their cytoplasm to form platelets, mature megakaryocytes may enter the bloodstream, and sometimes be observed in peripheral smears. Circulating megakaryocytes are not necessarily indicative of neoplasia, as they can be seen in neonates; on the other hand, they are more commonly detected in leukoerythroblastic reactions and myeloproliferative neoplasms (such as in chronic myeloid leukemia and primary myelofibrosis). The cell nucleus in the peripheral blood smear is single-lobed or, less commonly, multilobated. The chromatin is smudged or “puddled” and is surrounded by a scant amount of cytoplasm. At times, the cytoplasm is barely perceptible on light microscopy, giving the impression of a “bare” or “naked” megakaryocyte nucleus. However, upon closer inspection, a small amount of wispy, frilly, or fragmented

cytoplasm can still be seen. There may be a few localized areas of cytoplasmic blebs or adherent platelets. If the nuclear characteristics are not appreciated, megakaryocyte "nuclei" may be mistakenly identified as lymphocytes. Finding megakaryocyte cytoplasmic fragments and giant platelets in the blood film are helpful clues to the origin of the "bare" nucleus. It is important to remember that these cells are not degenerating cells, and, therefore, the chromatin pattern does not have the characteristics of basket cells. Rarely, circulating mature or precursor megakaryocytes appear in peripheral blood smears with ample amounts of pink-purple to wine-red, flocculent cytoplasm, as would be observed in bone marrow aspirate smears of normal individuals. For CAP proficiency testing purposes in peripheral blood smears, it is sufficient to identify any mature megakaryocyte – whether it is normal, abnormal, a nuclear "fragment," or a circulating micromegakaryocyte – as a megakaryocyte. Although large, the arrowed object is smaller than typical for a megakaryocyte. Moreover, it lacks a nucleus. Therefore, the arrowed object is not consistent with a megakaryocyte.

VPBS-15



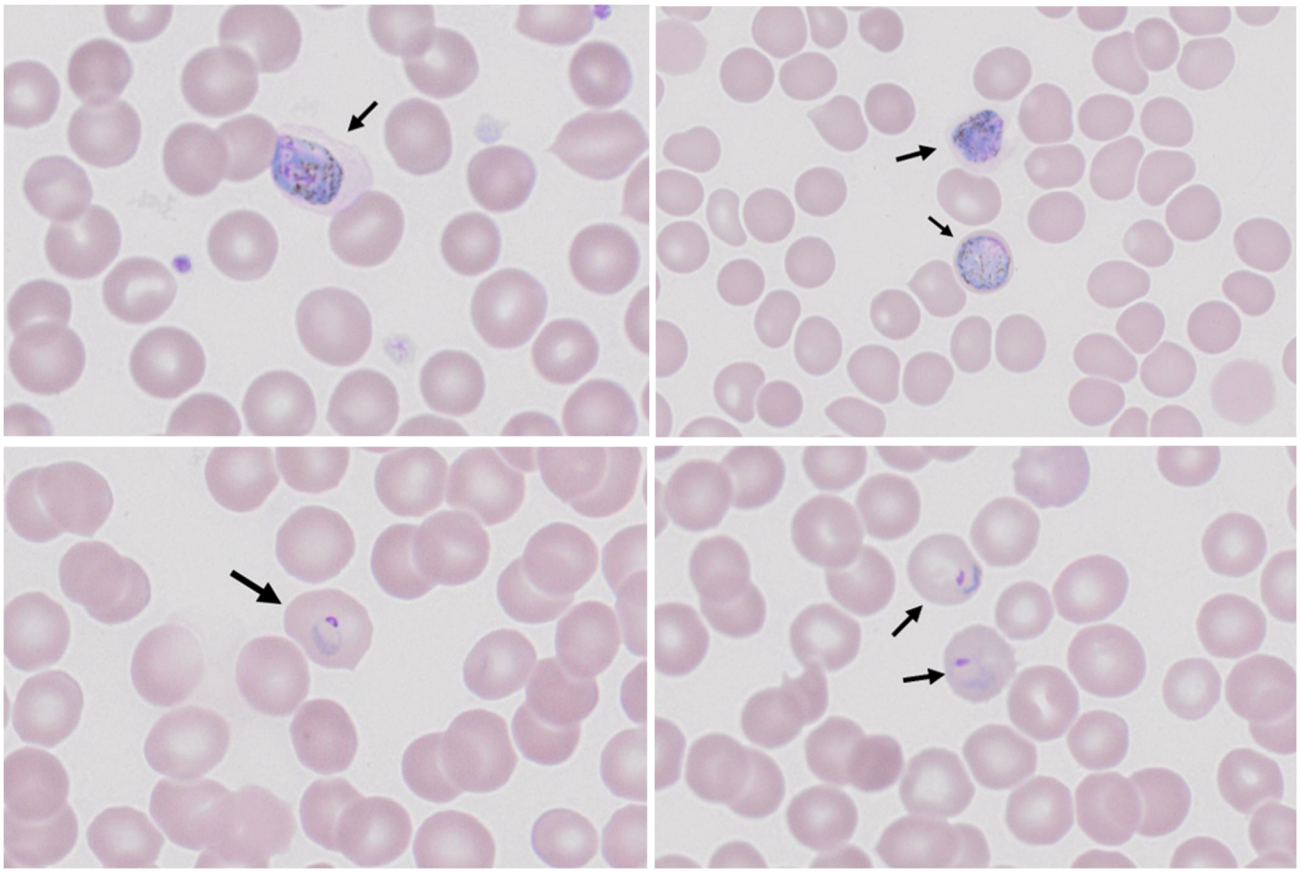


Identification	Participants Freq	%	Evaluation
Monocyte	1306	98.4	Educational
Monocyte, immature (promonocyte, monoblast)	13	1.0	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	6	0.5	Educational
Microcyte (with increased central pallor)	1	0.1	Educational
Plasmodium sp. (malaria)	1	0.1	Educational

The arrowed cells are monocytes, as correctly identified by 98.4% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. Most 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 nuclear-to-cytoplasmic (N:C) ratio ranges from 4:1 to 2:1. 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.

1.0% of participants incorrectly identified the arrowed cells as monocyte, immature. For the purposes of proficiency testing, selection of the response “monocyte, immature (promonocyte, monoblast)” should be reserved for malignant cells in the context of acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndromes. While immature monocytes may be normally identified in marrow aspirates, they are generally inconspicuous and do not resemble the cells described in this section. The malignant monoblast is a large cell, usually 15 to 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests (eg, cytochemistry and/or flow cytometry) are required to accurately assign blast lineage. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts. The chromatin pattern of the arrowed cells is condensed (ie, mature) and not finely dispersed. Moreover, the other morphologic features in this blood smear are not compatible with a myeloid neoplasm such as acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndrome. Therefore, the identification of monocyte, immature is incorrect.

VPBS-16



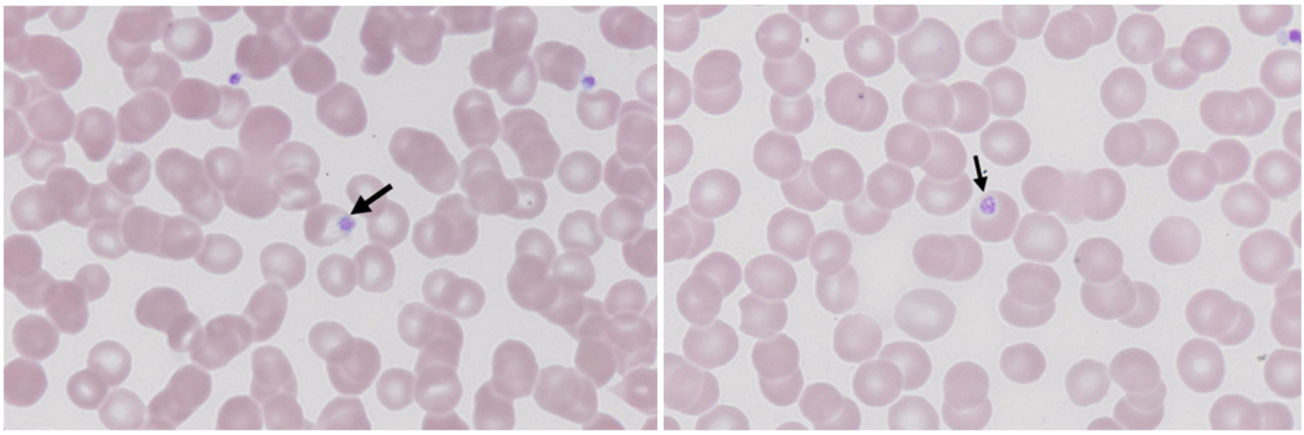
Identification	Participants Freq	%	Evaluation
Plasmodium sp. (Malaria)	1163	87.7	Educational
Parasite(s) seen, referred for definitive identification	150	11.3	Educational
Babesia sp.	7	0.5	Educational
Immature or abnormal cell, would refer for identification	3	0.2	Educational
Erythrocyte with overlying platelet	1	0.1	Educational
Howell-Jolly body	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational

The arrowed objects are *Plasmodium* sp. (Malaria), as correctly identified by 87.7% of participants. There are five species of *Plasmodium* that cause the clinical disease known as malaria: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. Species may be distinguished by different shapes and appearance of the various stages of development. The ring forms of all five types of malaria are usually less than 2 µm in diameter. Trophozoites range from 3 to 8 µm, depending on the species. Schizonts and gametocytes range from approximately 5 to 11 µm. Erythrocytes infected by *P. ovale* and *P. vivax* are enlarged. Schüffner stippling (a golden brown to black pigment in the cytoplasm of the infected erythrocyte) is most conspicuous in infections with *P. ovale* and *P. vivax*. Multiple stages of organism development are seen in the peripheral blood with all species except *P. falciparum*, where the peripheral blood usually contains only ring forms and gametocytes (unless infection is very severe). Multiple ring forms within one erythrocyte are also most common with *P. falciparum* and are not seen with *P. malariae*. Mixed infections may occur. Potential look-alikes include platelets overlying red blood cells, clumps of bacteria or platelets that may be confused with

schizonts, masses of fused platelets that may be confused with a gametocyte, precipitated stain, *Babesia* infection, and contaminating microorganisms (bacteria, fungi, etc.). Often infected cells are present in low numbers and difficult to identify in thin blood films. Use of a thick smear or concentration method increases the ability to identify malarial parasites in the blood.

11.3% of participants identified the arrowed objects as parasite seen, referred for definitive identification. This is an acceptable response only if you would routinely send identification in question to an outside laboratory with another CLIA number.

VPBS-17

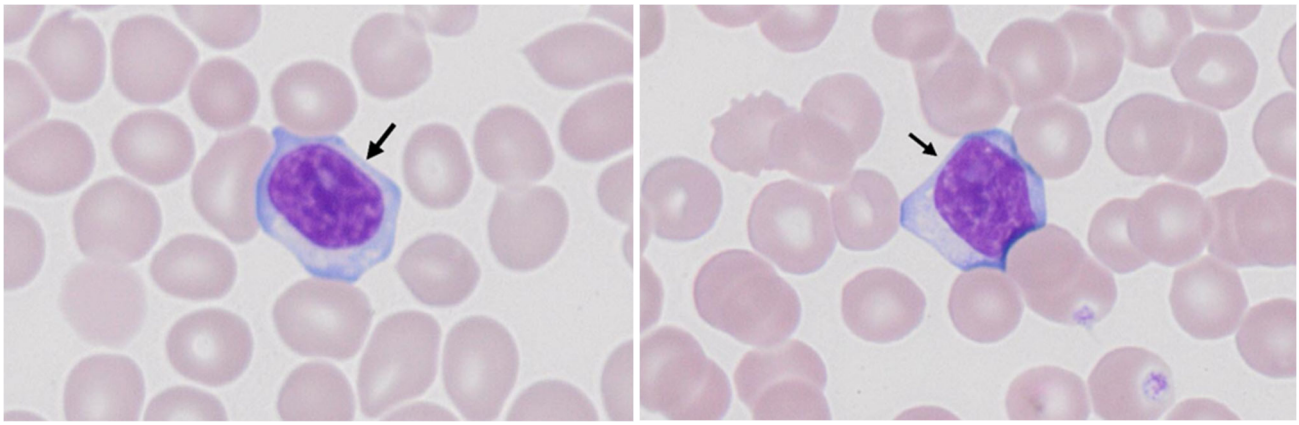


Identification	Participants Freq	%	Evaluation
Erythrocyte with overlying platelet	1242	93.6	Educational
Platelet, normal	65	4.9	Educational
Plasmodium sp. (malaria)	9	0.7	Educational
Parasite(s) seen, referred for definitive identification	4	0.3	Educational
Howell-Jolly body	3	0.2	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Mitotic figure	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed cells are erythrocytes with overlying platelet, as correctly identified by 93.6% of participants. In preparing a peripheral blood smear, platelets may adhere to or overlap red blood cells, suggesting a red blood cell inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the red blood cell. Many times, the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine red blood cell inclusions.

4.9% of participants incorrectly identified these arrowed objects as platelet, normal. Although the arrowed objects do contain platelets, they also include a red blood cell underlying the platelets. Therefore, they are more accurately identified as erythrocytes with overlying platelet.

VPBS-18



Identification	Participants Freq	%	Evaluation
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1223	92.2	Educational
Lymphocyte	85	6.4	Educational
Lymphocyte, large granular	11	0.8	Educational
Blast cell	2	0.1	Educational
Monocyte	2	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Parasite(s) seen, referred for definitive identification	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cells are lymphocytes, reactive, as correctly identified by 92.2% of participants. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described, and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. These cells have round to oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present.

Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The N:C ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells. Another type of reactive lymphocyte is referred to as a Downey I cell. These cells are rare. These cells possess scant to moderate amounts of basophilic cytoplasm. The nuclei often appear

indented, folded, or lobulated. The chromatin is condensed. A few small vacuoles may be present. Granules may also be apparent. Plasmacytoid lymphocytes resemble plasma cells and are intermediate in size (10 to 20 µm) and round to oblong in shape. They have round nuclei that are centrally placed or slightly eccentric. The chromatin is slightly to moderately coarse and forms small dense masses or a meshwork of strands resembling that of plasma cells. Nucleoli are generally not visible, but some cells may have one or two small irregular nucleoli. The cytoplasm is moderately abundant, homogeneous, and light blue to deep slate-blue, and it may show a perinuclear clear zone, or hof.

6.4% of participants incorrectly identified the arrowed cells as lymphocytes. 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 diffusely 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. Although the arrowed cells are lymphocytes, they show reactive features such as abundant amoeboid cytoplasm that partially surrounds adjacent red cells with a darker-staining, furled margin. Therefore, lymphocyte, reactive is a more accurate identification.

Case Presentation:

This peripheral blood smear is from a 56-year-old woman who presents with fever and chills after recently traveling throughout Ethiopia for the past 6 months. Laboratory data include: WBC = 9.6 x 10E9/L; RBC = 5.05 x 10E12/L; HGB = 14.6 g/dL; HCT = 41.1%; MCV = 88 fL; PLT = 83 x 10E9/L; and RDW = 14%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: Malaria (*Plasmodium vivax*)

This case illustrates a thin peripheral blood smear from a patient with *Plasmodium vivax* infection. Malaria is a mosquito-borne disease caused by a *Plasmodium* parasite. Infected patients often experience fever, chills, and flu-like illness. If left untreated, patients may develop severe complications resulting in death. In 2019, an estimated 229 million cases of malaria occurred worldwide, and 409,000 people died (mostly children in Africa). About 2,000 cases of malaria are diagnosed in the US each year. Most cases in the US are in travelers and immigrants returning from countries where malaria transmission occurs, such as sub-Saharan Africa and South Asia.

Question 1. Which of the following statements relating to malaria is CORRECT?

A. Malaria can present with fever, chills, and other flu-like symptoms
B. Malaria infection is typically self-limited without need for therapy
C. Malaria is caused by *Babesia microti*
D. Most cases of malaria in the US are acquired from mosquito bites in patients residing in the southeast

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There are five clinically important species of *Plasmodium: P. falciparum* and *P. vivax* (the two most common), *P. ovale, P. malariae,* and *P. knowlesi*. The latter can infect both animals and humans. Again, a prompt diagnosis is critical because the disease can rapidly progress to a severe form with multi-organ failure and death.

Question 2. Which of the following statements relating to malaria is CORRECT?

A. *P. falciparum and vivax* account for most cases of malaria
B. *P. malariae* account for most cases of malaria
C. *P. malariae* can infect animals
D. *P. ovale* can infect animals

Laboratory findings accompanying uncomplicated malaria may include anemia and thrombocytopenia. Along with parasites, reactive lymphocytes may be seen in the blood smear, as seen in this case. Microscopy is the gold standard for malaria diagnosis, although rapid diagnostic tests (RDTs) and nucleic-acid based tests (NATs) also play important roles. Two types of smears are recommended, thin and thick.

The thin smear is a conventional Giemsa-stained blood smear and is particularly useful for evaluating morphology and species identification, in addition to parasitemia estimation. For optimal results, morphology should be examined on thin peripheral blood smears prepared within one hour of blood collection as delays in smear preparation can lead to changes in parasite morphology. According to the Centers for Disease Control and Prevention, at least 500 red blood cells (RBCs) should be counted and examined for the presence of intracellular parasites. When examining malaria thin smears, it is important to distinguish parasites from artifacts such as

platelets overlying RBCs (as seen in this case), clumps of bacteria/platelets/precipitated stain that may be confused with schizonts, masses of fused platelets that may be confused with a gametocyte, or other parasites such as babesia, which can appear morphologically similar and present with a similar clinical picture.

The thick smear is prepared by lysing RBCs prior to staining, which allows the specimen to be concentrated on the slide. Although this technique increases sensitivity for detection, it alters parasite morphology and eliminates RBC features that can be used for species identification. Thorough review of both thin and thick smears is critical.

Question 3. Which of the following statements relating to malaria is CORRECT?

A. The gold standard evaluation for malaria is a rapid diagnostic test
B. Thick smears for malaria allow for parasitemia enumeration
C. Thin smears for malaria are more sensitive than thick smears
D. Thin smears for malaria should be made within one hour of blood draw

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Roughly 95% of initial smears are positive in symptomatic patients. If initial smears are negative in a patient with suspected malaria, repeat smears should be performed every 6 to 12 hours for 48 hours to definitively rule out malaria. In addition to detection of malaria parasites, accurate species identification is also important. *P. vivax* and *P. ovale* have dormant liver stages that require prolonged treatment with primaquine to prevent reactivation.

Distinction of *Plasmodium sp*. by microscopy is based on recognition of certain features of both parasites and infected RBCs that are suggestive of, but not entirely specific for, a given species. In the present case, there are morphologic clues to the specific species. Morphologic features favor *P. vivax* due to the ameboid shape of trophozoites, the large size of infected RBCs, and gametocytes that are round to oval and fill the host cell.

Natasha M. Savage, MD
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Centers for Disease Control and Prevention. Malaria. CDC Web site. http://cdc.gov/malaria. Accessed October 3, 2021.
2. Kurzer J. Blood changes in infectious disease. In: Pereira I, George TI, Arber DA, eds. Atlas of Peripheral Blood: The Primary Diagnostic Tool. Lippincott Williams & Wilkins; 2012:173-184.

ANSWERS TO QUESTIONS:

Question 1: A. Malaria can present with fever, chills, and other flu-like symptoms
Malaria is a mosquito-borne disease caused by a *Plasmodium* parasite, not *Babesia*. Infected patients often experience fever, chills, and flu-like illness, and if left untreated, patients may develop severe complications and death. Most cases in the US are in travelers and immigrants returning from countries where malaria transmission occurs.

Question 2: A. *P. falciparum and vivax* account for most cases of malaria
P. falciparum and *P. vivax* are the most common causes of malaria. *P. knowlesi* can infect both animals and humans.

Question 3: D. Thin smears for malaria should be made within one hour of blood draw
Thick and thin smears with morphologic review are the gold standard for malaria detection. Thick smears are more sensitive for detection than thin smears but do not allow for parasitemia enumeration. Both should be made within one hour from blood draw for optimal morphology.

Calculating Absolute Counts:

The absolute count (ABS) for any leukocyte subset is calculated utilizing the following formula:

$$\text{ABS } (\times 10E9/L) = \frac{\% \times \text{WBC } (\times 10E9/L)}{100}$$

where % is the percentage of the leukocyte (neutrophils, lymphocytes, etc. obtained from the manual differential), WBC represents the total white blood cell count (×10E9/L) for the particular case, and ABS represents the absolute count for the subset in question (neutrophils, lymphocytes, etc.). For example, if the total white count (WBC) is 8.0 × 10E9/L and lymphocytes are 25% of the differential, then the absolute lymphocyte count (ABS) is obtained as follows:

$$\text{ABS } (\times 10E9/L) = \frac{25\% \times 8.0 \ (\times 10E9/L)}{100}$$

$$= 2.00$$

REFERENCE:

1. CAP Hematology checklist item HEM.36820

NOTE: For WBC differential counts, the CAP recommends that laboratories report absolute cell counts, along with their corresponding reference intervals. The CAP discourages the reporting of percent cell counts without absolute counts on WBC differentials. Laboratories reporting only percent cell counts must provide laboratory established reference intervals.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review, and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include, but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	Response was not formally graded due to insufficient peer group data. Please see the participant summary for additional information.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the participant summary and compared its results to a similar method, all method, all participant statistics, or data tables for groups of 3-9 laboratories, if provided. Perform and document the corrective action of any unacceptable results. If self-evaluation is not possible, it is up to the laboratory director/designee to determine an alternative performance assessment.
21	Specimen problem	Document that the laboratory has reviewed the proper statistics supplied in the participant summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range	Document the comparison of results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
24	Incorrect response due to failure to provide a valid response code	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the participant summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial	Document the investigation of the results as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge	Review participant summary for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation.
27,31	Lack of participant or referee consensus	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the participant summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific committee decision	Applies to a response that is not penalized based on scientific committee decision. Document that the laboratory has reviewed the proper statistics supplied in the participant summary.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
33	Specimen determined to be unsatisfactory after contacting the CAP	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the participant summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response	The participant summary indicates which tests are graded (see evaluation criteria) and which tests are not evaluated/educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. If a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the kit instructions and/or the result form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant medical staff and committees (eg, infectious diseases, pharmacy and therapeutics, infection control).
77	Improper use of the exception code for this mailing	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 46, 88, 92	Various codes	No action required.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the VPBS-A 2022 CAP Program
Product Mailing, Year

Participant Summary/Final Critique report and can self-report this activity towards fulfilling education and maintenance of certification (MOC) requirements. Time spent on activity* _____.

Participant	Date	Participant	Date
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

_____ _____

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

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NOTES

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This concludes the report.

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