We missed one image on the most recent virtual differential survey. I misread the case history and steered the tech in the wrong direction. Let's see if you all can get this one, and then we will discuss.

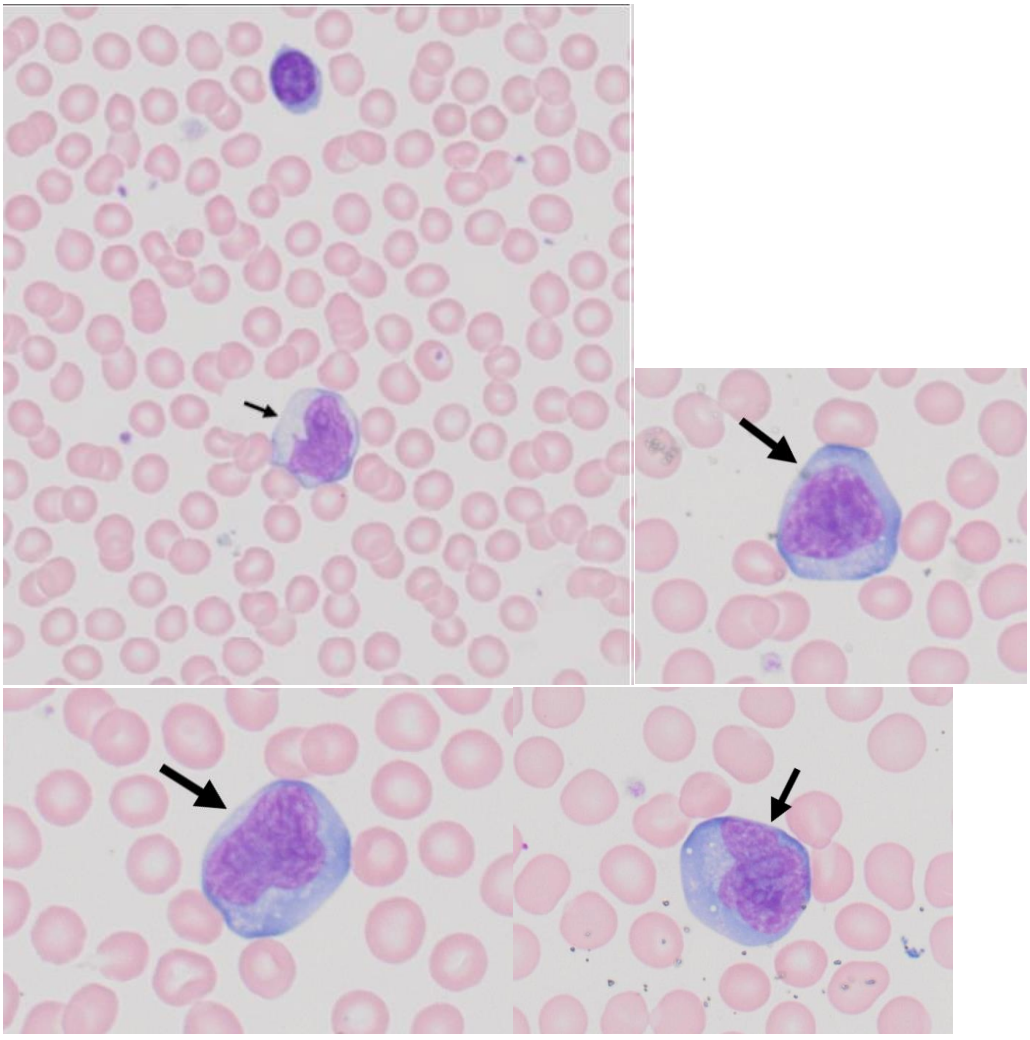
Case History

This peripheral blood smear is from a 20-year-old woman presenting with cervical lymphadenopathy, sore throat, and fatigue.

Laboratory data include:

WBC = 7.3 x 10E9/L;
RBC = 4.92 x 10E12/L;
HGB = 14.3 g/dL;
HCT = 43.4%;
MCV = 89 fL;
PLT = 151 x 10E9/L; and
RDW = 13%.

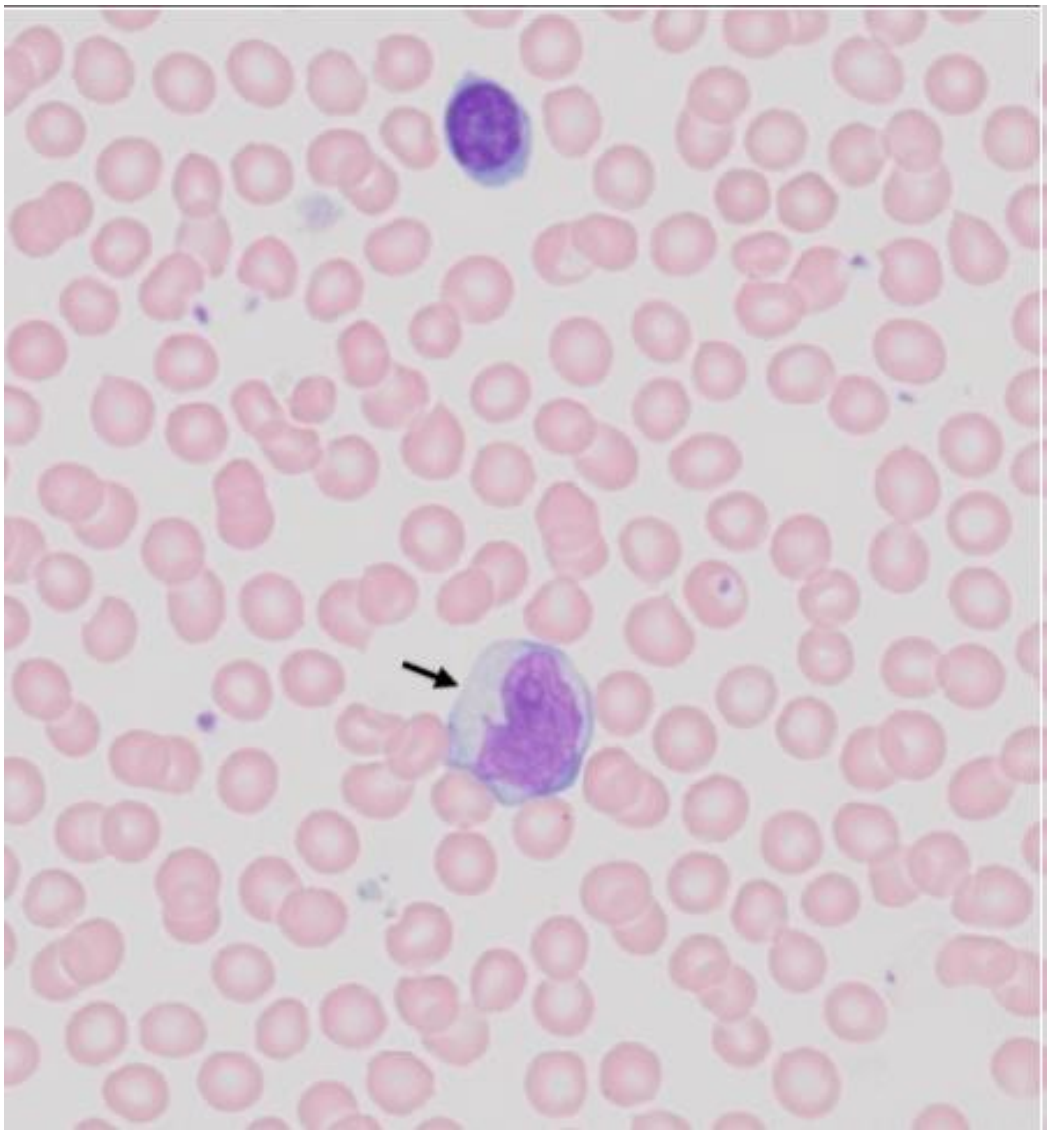
Identify the cell in the following images.



No cheating and just scrolling to the next page for an answer 😊! Try.
What cell type is in the images above?

What diagnosis do you think they could possibly have?

Did you answer this time? Or you still trying to skip ahead? 😊😊

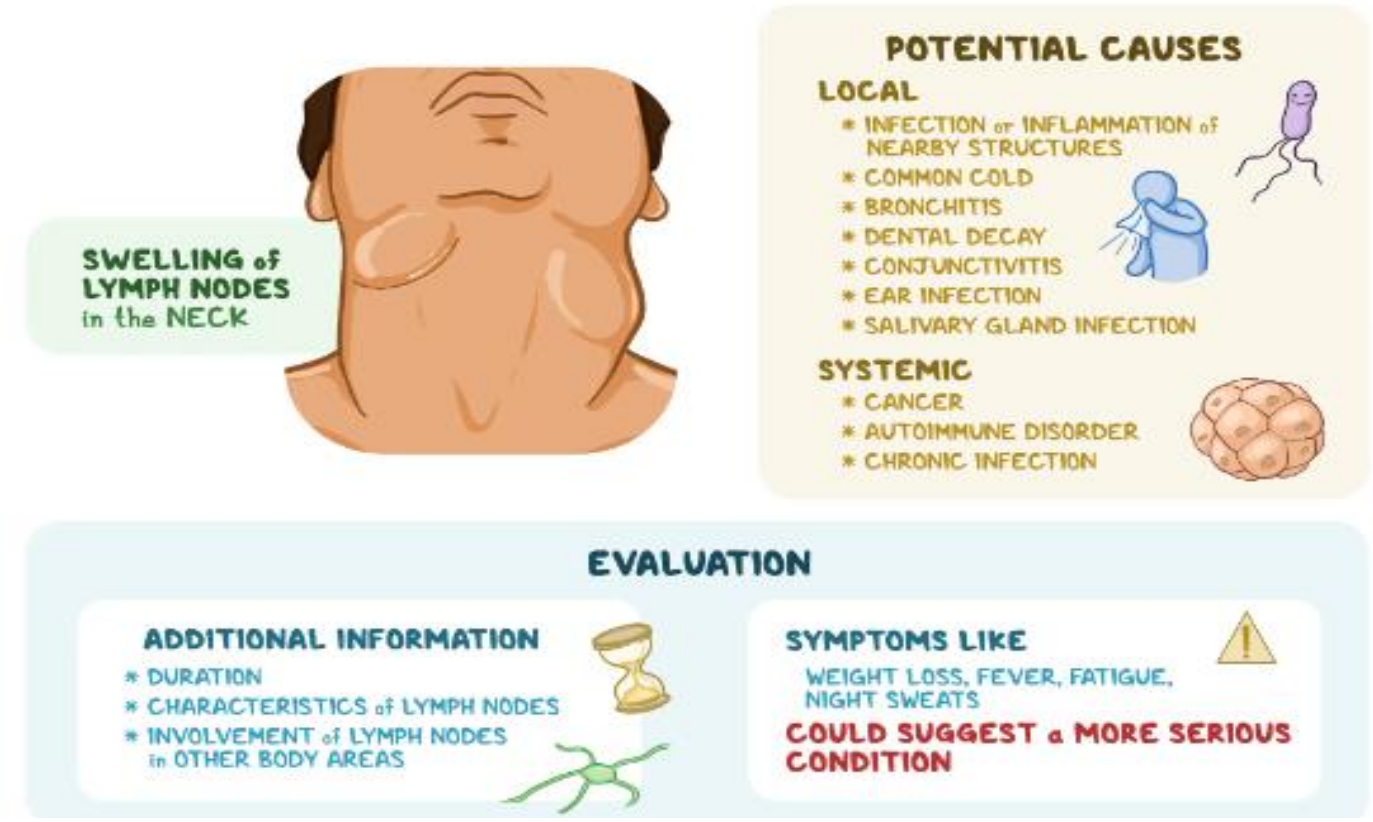
What is it? 

Let’s look at the clues given to us in the case history.

First, note that the WBC count is normal at 7.3. This doesn't automatically exclude leukemia (blast cells), but it doesn't support it either.

Second, the patient is a 20-year-old female. Again, this doesn't completely exclude lymphoma or leukemia, but it does make it much less likely. The young (< 8yo) and old (not going to put an age to save my skin) are more likely to develop lymphomas and leukemias.

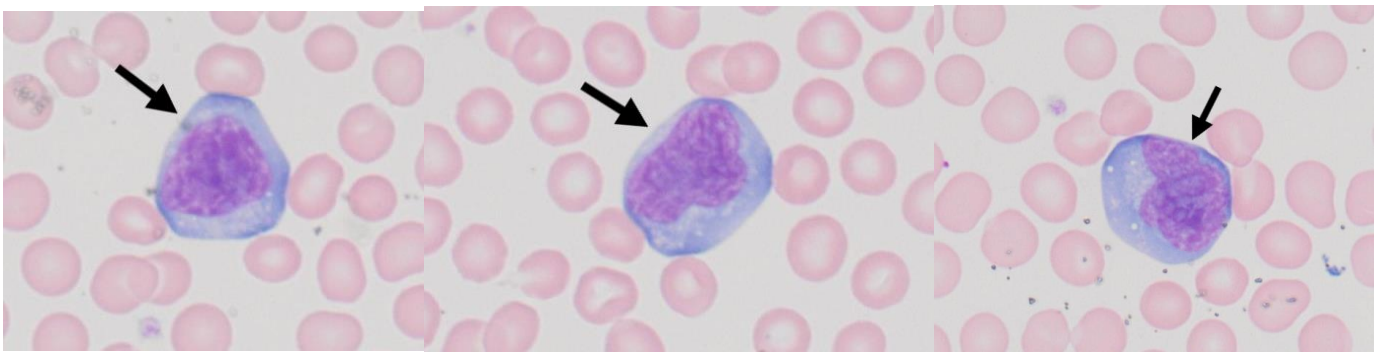
Third, when given a series of medical terms like cervical lymphadenopathy, we must search the whole term to get an idea of the symptom or diagnosis. This was my main error. I only looked up lymphadenopathy, and I focused in on lymphoma being a possible cause. I put blinders on once I read that malignancies can cause the condition. See the image below for a quick synopsis of cervical lymphadnopathy:



Finally, put it all together with what we see as a whole. If you examined the whole slide, you would see reduced PMNs and an increase in lymphocytes, including the funky cells you see in the images.

Swollen and sore throat, fatigue, increase in lymphocyte-like cells, but with a normal WBC count. Final chance, what do you think? Lymphoma or strep throat or mono? And following, are these lymphoma cells or reactive lymphs or myelocytes or blasts or monocytes?

The correct answer according to CAP is that these are reactive lymphs from a patient with infectious mono.

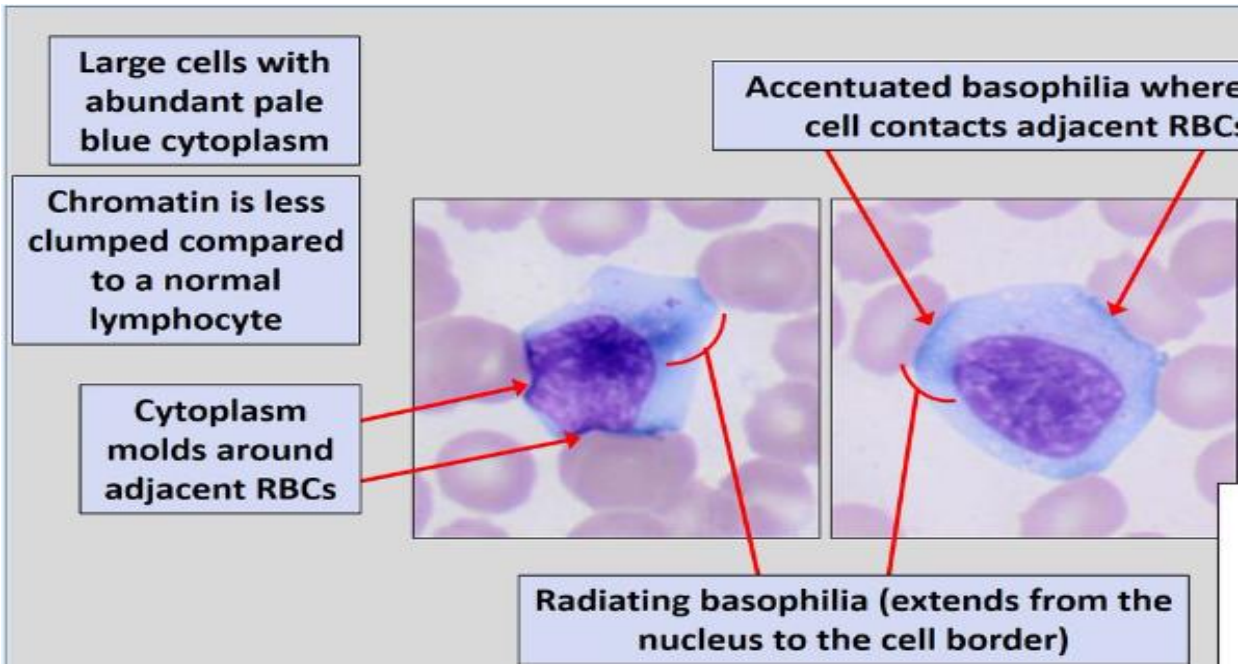


Are these what we are used to seeing when thinking of reactive lymphs? Why or why not?

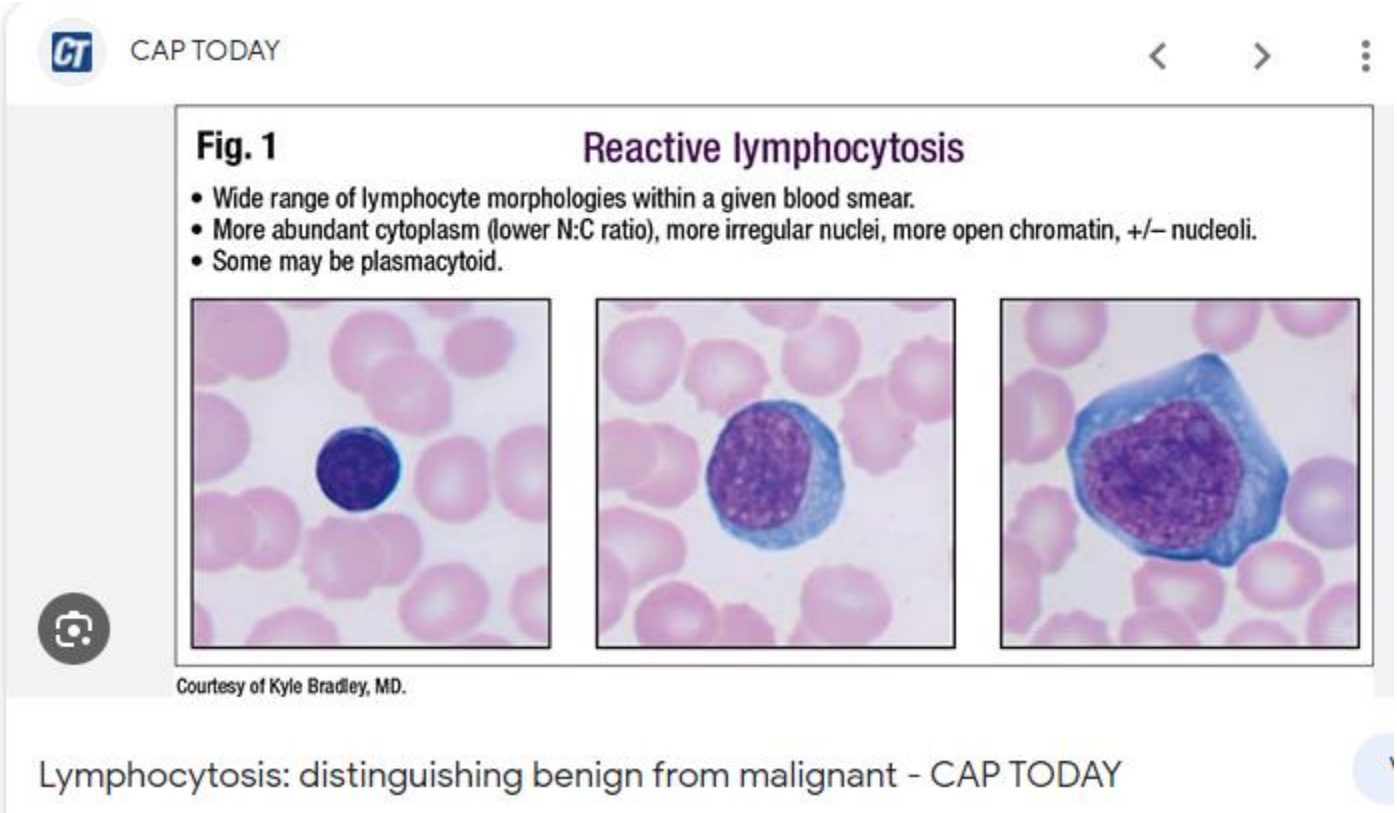
My answer is that they don't appear typical, as I'm not used to seeing plainly visible nucleoli and slightly grainy and sometimes vacuolated cytoplasm. Also, the nucleus looks less clumped, lighter in color, and more "fluffy" than I think of when picturing lymphocytes. I think of reactive lymphs as having a more clear blue cytoplasm, and the edges being a much deeper blue color. I also think of the nucleus as being much more clumped and mature-looking and deeper blue/purple.

Here's why: we are used to seeing Downey Type II reactive lymphs, and the ones seen in the CAP sample are Downey Type III reactive lymphs.

See the image on the following page.

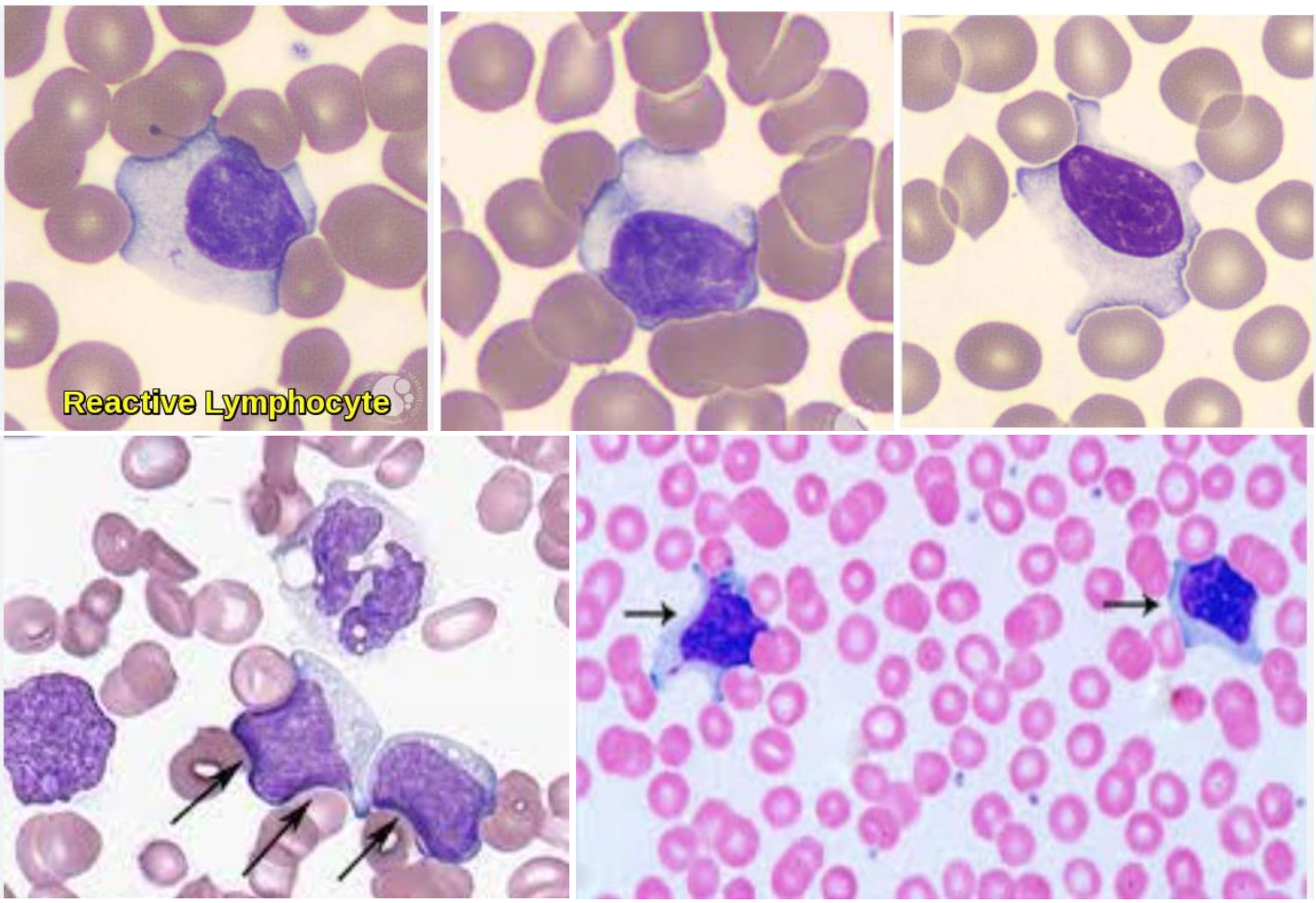
Type II: 

Here is another definition of reactive lymphocytes that include descriptions of what we see in the images from VPBS-34 and the type on the right is much more like a Downey Type III cell.

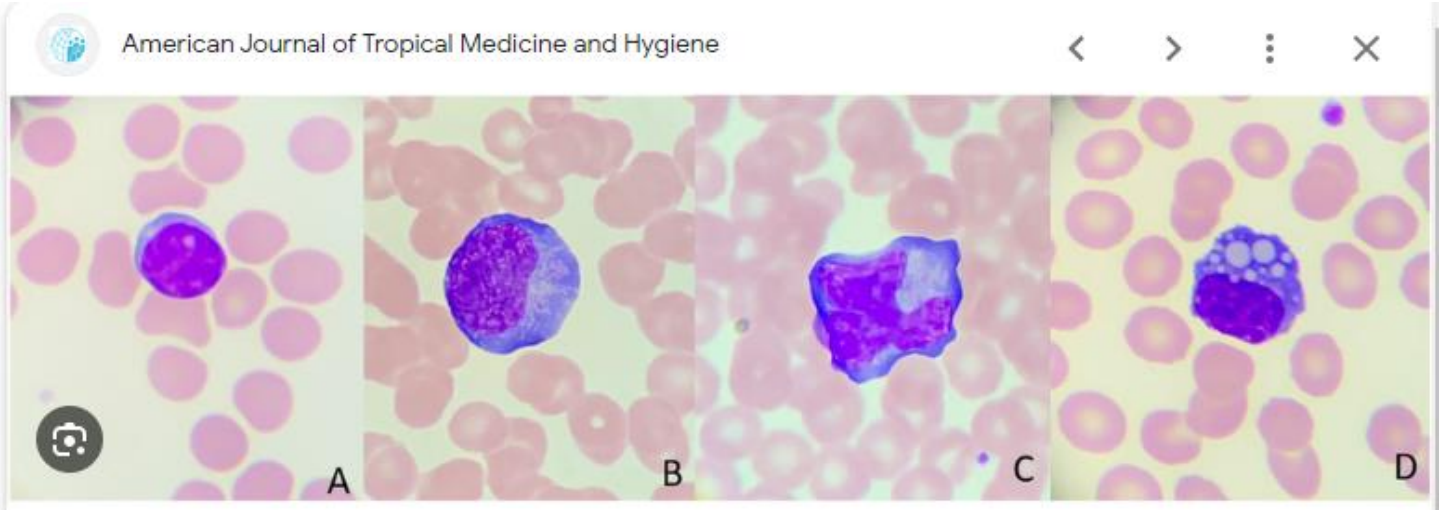


Notice how grainy the cytoplasm can look, and how clearly you can see some nucleoli. They are really just irregular lymphocytes, but ones we don’t see as often.

Here are a few more reactive lymphocyte examples.

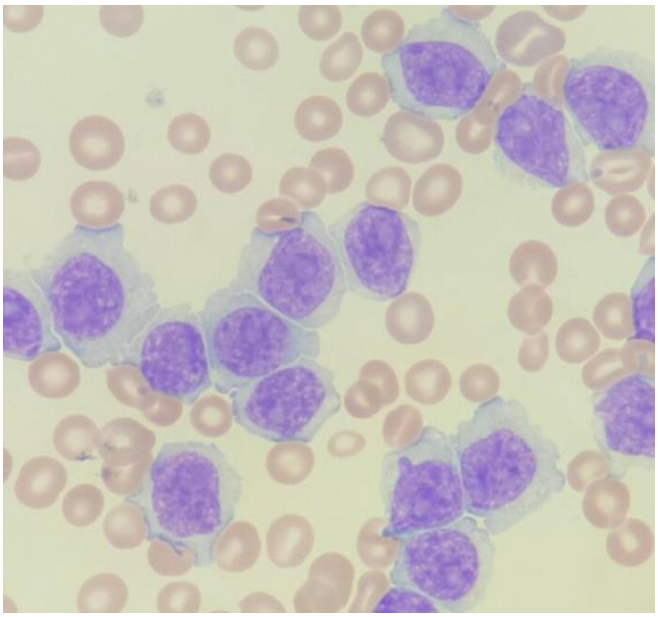


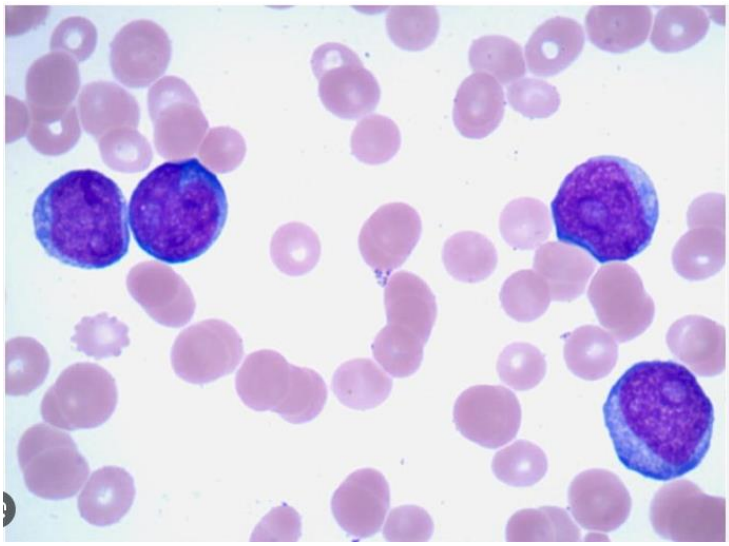
And one more that is a normal lymphocyte and some reactive in a severe case of Covid-19!



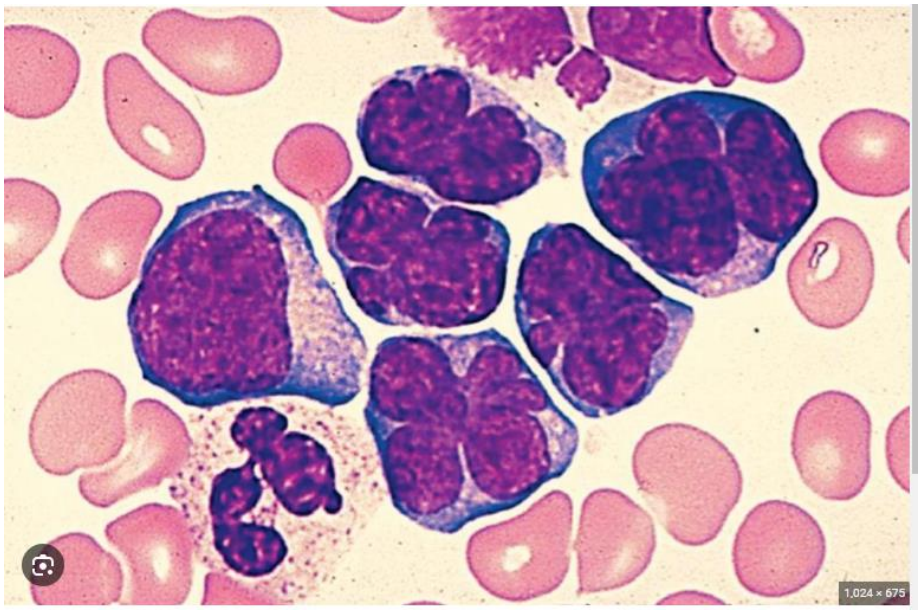
Let's compare those reactive lymphocytes to some lymphoma cells.

These are some lymphs from a B-cell lymphoma. Notice the great size of the whole cell and especially the nucleus, the very large present nucleoli, the apparent increased number of WBC's (especially lymphocytes), and the cytoplasm doesn't quite "pseudopod" or grab at red cells and get that dark blue edge quite as much.

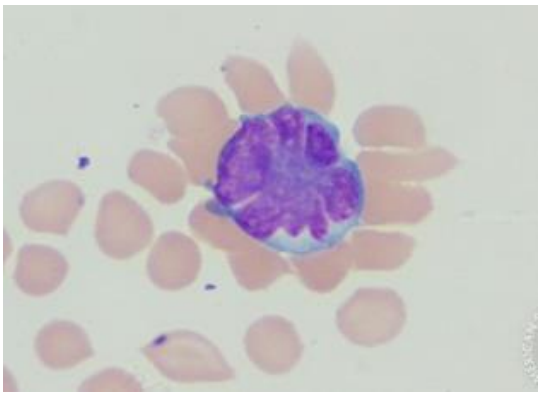
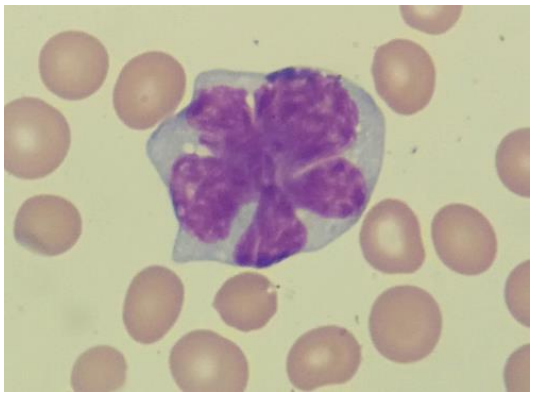




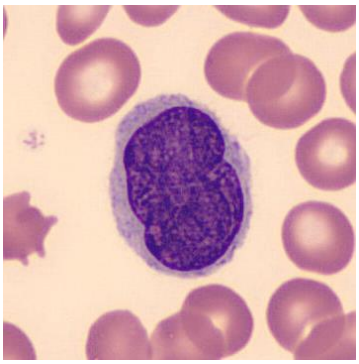
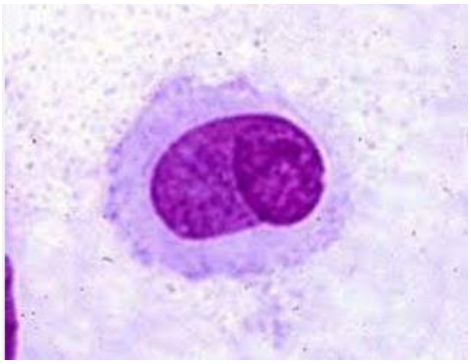
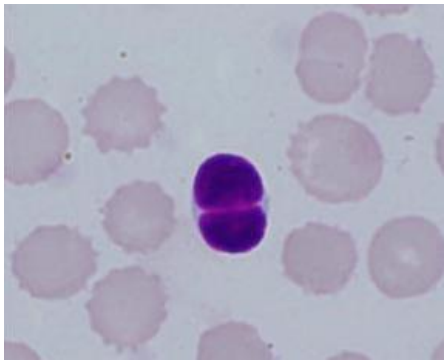
These are a little more obvious. These are Non-Hodgkin Lymphoma cells from a pediatric patient. They are quite obviously a different shape and size and the nucleus is a very odd shape with pinches and folds.



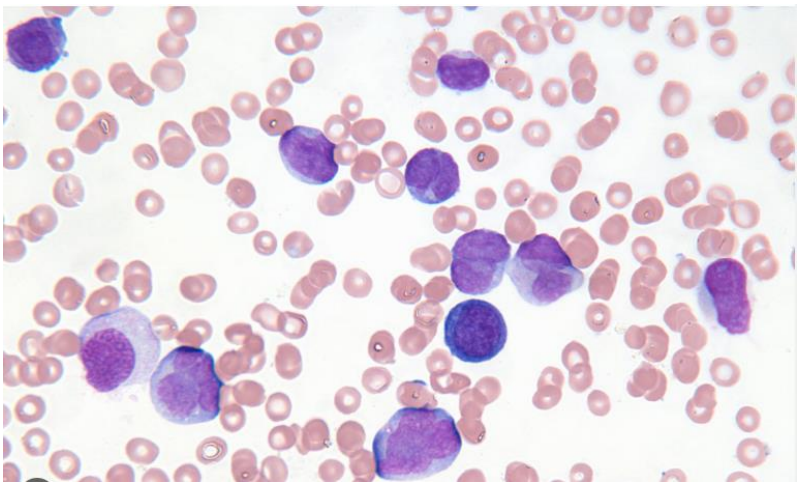
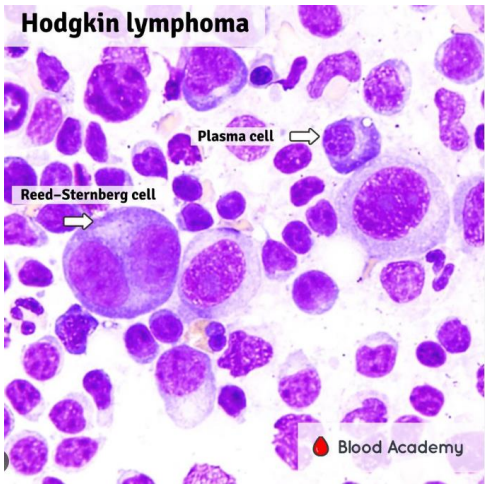
These are “flower cells” seen in a certain T-cell lymphoma. Again, the nucleus is an obvious clue to the abnormality of the cell.

These are “butt cells” seen in some lymphomas. Normal lymphs, and normal reactive lymphs, would not have the crease in the nucleus that makes it look like a butt.

Finally, here is a patient with Hodgkin’s Lymphoma. The left image is a peripheral smear, and the right is a bone marrow. Again, notice the increased “weirdness” of the lymphocytes with very large cells and very abnormal nuclei.

So, I realize that some of those lymphoma cells are obviously very different. Some of them look very similar to the reactive lymphs of the CAP sample (Downey Type III). That's where we must combine what we are seeing on the slide/image with the information given in the case history. For the CAP surveys, if they want lymphoma cells, they will almost always give a history of lymphoma or a patient presenting with a large mass somewhere. They will also almost always have elevated WBC counts. This sample was a good lesson to start simply, and then look toward something more complicated. The simplest answer was an atypical or reactive lymph.

If this were a real patient, and you classified these cells as "other" or "atypical lymphs," the slide would have gone for path review. Therefore, we would not totally be wrong. We would also likely have more information, like a Mono test, to give us some clues. As long as we know to classify these as some type of lymphocyte, and NOT a monocyte or PMN precursor, then we are helping the patient and physician. However, we want to be as accurate as possible when counting a differential. This miss was my fault for not putting all the pieces together, and I think we can all take this as a good example why one of our Sentara Safety Habits is to Pay Attention to Detail where we "STAR" or "Stop, Think, Act, and Review." See how I did that 😊? Trying to include that stuff they want us to have memorized.

Thanks for sticking with me. I hope you got a little something from this. As always, come to your senior tech with any questions or concerns. The following pages are the excerpt from the Participant Summary to give you exactly what CAP says about these cells.

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Surveys and Anatomic Pathology Education Programs

Virtual Peripheral Blood Smear VPBS-B 2023

Participant Summary

Self-Reported Training Available

This document is available online in e-LAB Solutions Suite.

VPBS-31

Case History for VPBS-31 – VPBS-36

This peripheral blood smear is from a 20-year-old woman presenting with cervical lymphadenopathy, sore throat, and fatigue. Laboratory data include: WBC = 7.3 x 10E9/L; RBC = 4.92 x 10E12/L; HGB = 14.3 g/dL; HCT = 43.4%; MCV = 89 fL; PLT = 151 x 10E9/L; and RDW = 13%.

(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=28fbdec4-36cb-4642-bc26-c2c52fe36173

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)	1300	28.2	4.1	14.5	28	16	40
Lymphocytes	1311	32.9	15.4	46.7	30	0	77
Lymphocytes, reactive	1134	27.7	16.3	58.7	29	0	73
Monocytes	1241	10.3	8.0	78.3	8	0	38
Eosinophils	601	0.1	0.3	*	0	0	1
Basophils	1253	2.0	0.9	47.8	2	0	4
Metamyelocytes	529	0.0	0.2	*	0	0	1
Myelocytes	536	0.0	0.2	*	0	0	1
Promyelocytes	531	0.0	0.0	0.0	0	0	0
Blasts	533	0.0	0.2	*	0	0	2
nRBC/100 WBC	569	0.0	0.0	0.0	0	0	0

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1213	2.062	0.320	15.5	2.04	0.20	3.72
Lymphocytes	1220	2.419	1.143	47.2	2.19	0.00	5.84
Lymphocytes, reactive	1048	1.992	1.187	59.6	2.04	0.00	5.33
Monocytes	1184	0.790	0.674	85.2	0.58	0.00	3.21
Eosinophils	564	0.008	0.024	*	0.00	0.00	0.14
Basophils	1158	0.144	0.070	48.8	0.15	0.00	0.37
Metamyelocytes	498	0.003	0.014	*	0.00	0.00	0.10
Myelocytes	505	0.002	0.011	*	0.00	0.00	0.07
Promyelocytes	502	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	501	0.002	0.018	*	0.00	0.00	0.20

*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.

VPBS-31, cont'd.

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

Cells not listed/differentiated	N = 90 Freq
Abnormal/atypical/reactive/variant lymphocyte	40
Immature lymphocyte	1
Large granular lymphocyte	1
Would refer for identification	48

Platelet Estimate

Intended Response: Adequate/normal platelets	N = 1307 Freq	%
Adequate/normal platelets	1245	95.3
Decreased platelets	57	4.4
Increased platelets	3	0.2
Unable to quantitate - platelet clumps present	2	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 = 2469 Freq	Total Responses N = 2469 % Total Response	Total Unique Kits N = 1112 % Unique Kits
Erythrocyte, normal	661	26.8	59.4
Stomatocyte	325	13.2	29.2
Polychromatophilic (non-nucleated) red blood cell	263	10.7	23.6
Teardrop cell (dacrocyte)	253	10.3	22.8
Erythrocyte with overlying platelet	217	8.8	19.5
Echinocyte (burr cell, crenated cell)	140	5.7	12.6
Microcyte (with increased central pallor)	139	5.6	12.5
Ovalocyte (elliptocyte)	111	4.5	10.0
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	87	3.5	7.8
Spherocyte	86	3.5	7.7
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	60	2.4	5.4
Rouleaux	44	1.8	4.0
Howell-Jolly body	35	1.4	3.1
Target cell (codocyte)	10	0.4	0.9
Bite cell (degmacyte)	8	0.3	0.7
Acanthocyte (spur cell)	7	0.3	0.6

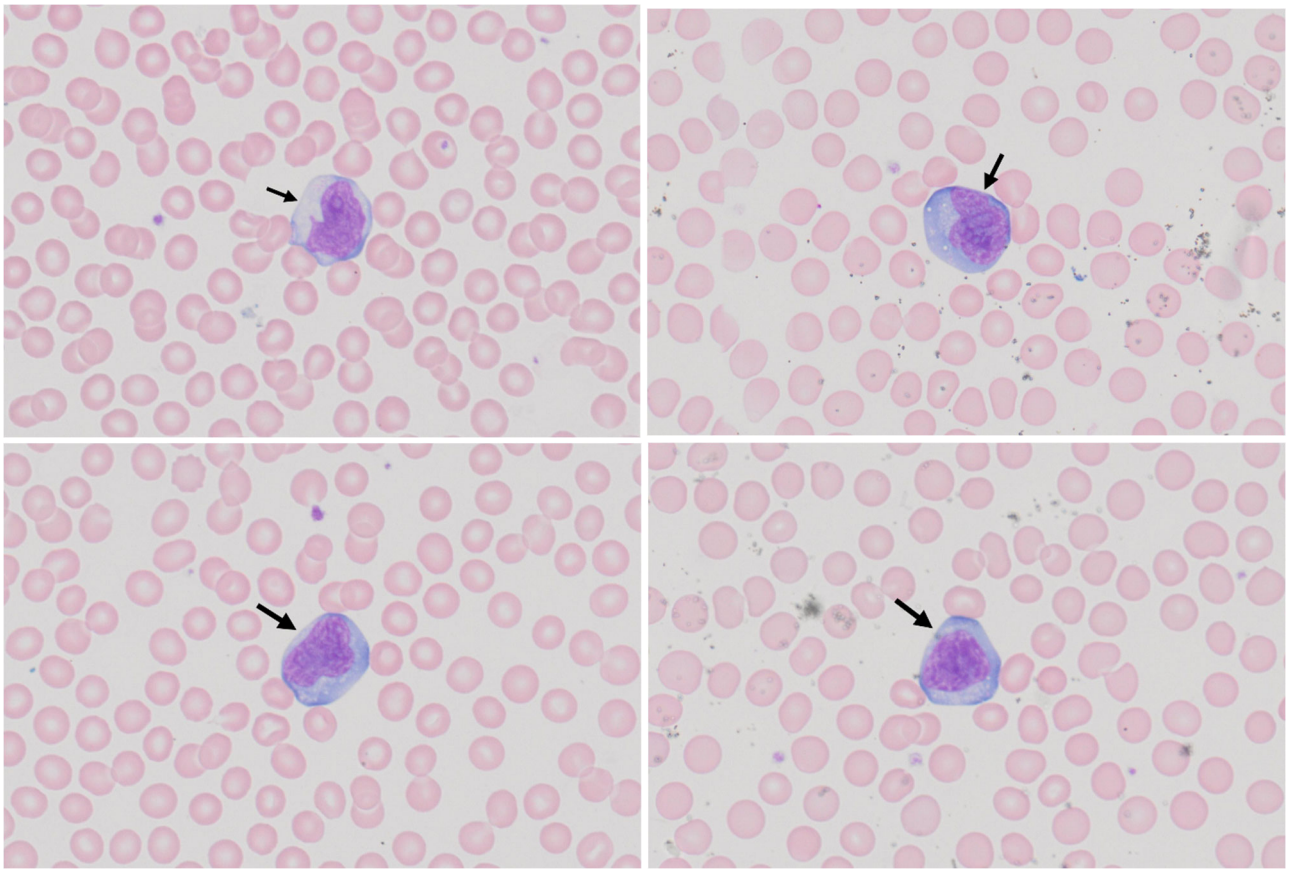
VPBS-31, cont'd.

Red Cell Morphology	Total Responses N = 2469 Freq	Total Responses N = 2469 % Total Response	Total Unique Kits N = 1112 % Unique Kits
Nucleated red blood cell, normal or abnormal morphology	7	0.3	0.6
Pappenheimer bodies (iron or Wright stain)	7	0.3	0.6
Basophilic stippling (coarse)	5	0.2	0.5
Red blood cell agglutinates	3	0.1	0.3
Blister cell/Prekeratocyte	1	0.0	0.1

Committee Comments on the CBC and Peripheral Blood Whole Slide Image

The provided CBC data reveal normal hemoglobin, WBC and platelet counts. On review of the peripheral blood smear, red blood cells are normochromic and normocytic. Platelets are adequate in number with normal morphology. Though the white blood cell count is within normal limits, there is a relative increase in lymphocytes, including numerous reactive lymphocytes. The reactive lymphocytes show some variability in size and shape, and have round to oval nuclei, moderately condensed chromatin, absent or indistinct nucleoli, and abundant gray-blue cytoplasm.

VPBS-34



Identification	Participants Freq	%	Evaluation
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1064	80.5	Educational
Monocyte	163	12.3	Educational
Monocyte, immature (promonocyte, monoblast)	38	2.9	Educational
Malignant lymphoid cell (other than blast)	19	1.4	Educational
Immature or abnormal cell, would refer for identification	13	1.0	Educational
Blast cell	12	0.9	Educational
Lymphocyte, large granular	6	0.5	Educational
Lymphocyte	4	0.3	Educational
Neutrophil, myelocyte	2	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational

The arrowed cells are lymphocytes, reactive, as correctly identified by 80.5% 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.

12.3% of participants incorrectly identified the arrowed cells as a monocyte. Monocytes are slightly larger than neutrophils, and have abundant gray or gray-blue cytoplasm that may contain vacuoles or fine, evenly distributed azurophilic granules. The nucleus is usually indented, but can also be folded or band-like. The chromatin is condensed but is usually less dense than that of a neutrophil or lymphocyte.

2.9% of participants incorrectly identified the arrowed cells as a monocyte, immature (promonocyte, monoblast). Monoblasts are large cells with round to oval nuclei, finely dispersed chromatin and distinct nucleoli. They have relatively more cytoplasm than a myeloblast with an N:C ratio ranging from 7:1 to 3:1. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, and their 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 may be present. For the purposes of proficiency testing, 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.

1.4% of participants incorrectly identified the arrowed cells as a malignant lymphoid cell (other than blast). 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.