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CP Manual Peripheral Blood Differential and Morphology

SCOPE

A. Moorland Reserve Health Center 6/2020
B. Drexel Town Square Health Center 6/2020
C. Town Hall Health Center 6/2020
D. North Hills Health Center 6/2020
E. Tosa Health Center 6/2020
F. Mequon Health Center 6/2020
G. West Bend Health Center 6/2020

CLINICAL UTILITY

Valuable information can be obtained from the microscopic examination of the blood cells. A properly read differential is an important diagnostic tool in detecting malignant disorders, guiding therapeutic regimens, and indicating inflammatory processes.

TESTING PRINCIPLE

Differential count preparations require staining with Romanowsky stain. All laboratories use a version of the Romanowsky stain.The specific staining process and principle are detailed in separate procedures.

A. Drexel and Moorland Reserve: Wescor Aerospray Slide Stainer
B. Town Hall: Ames Hema-Tek Slide Stainer
C. North Hills, Tosa, West Bend Health Center: Hema-Fast 3 Step Differential Stain

Except in unusual situations, 100 WBCs are counted for each manual differential. Each identified cell is placed into one of the categories defined in this procedure.

A. When Caresphere is available, the differential count should be performed in Caresphere or in CellaVision.
B. During a Caresphere downtime, "Add Man Diff non Caresphere" button in Beaker may be used to perform a manual differential in Beaker.
C. During an Epic/Beaker downtime, the cells may be tallied using a manual cell counter with results documented on paper record until computer system is available.

DEFINITIONS:

Usable area of smear: The part of the smear where RBCs are just touching each other and display central pallor.

SPECIMEN

Anti-coagulated human whole blood in K2 EDTA

STABILITY:

Stability is based on stability studies performed using Sysmex analyzers.

TEMPERATURE:	TIME:
AMBIENT	8 hours
REFRIGERATED	24 hours

MATERIALS

REAGENTS:

A. Immersion Oil
B. See Stain Reagent details in procedures referenced in Testing Principle

EQUIPMENT:

A. Frosted glass slides for blood smear (See specific requirements in CellaVision DC-1 procedure when CellaVision is used.)
B. Optional regular glass slides or glass slides with clipped corners for spreading blood
C. Diff-Safe device or microhematocrit tube
D. Slide Stainer or Coplin jars as indicated in staining procedure
E. Microscope with 10X or 40X dry objectives plus 50X and 100X oil immersion objectives

QUALITY CONTROL (QC)

FREQUENCY:

A. Each blood smear is examined for overall quality of the smear:
    1. smear length is between 2/3 and 3/4 the length of the slide
    2. Straight or slightly rounded feathered edge
    3. No ridges, streaks or holes.
    4. Smear should be slightly narrow than slide so sides can be examined.
    5. Unacceptable smears are remade
    6. (See specific requirements in CellaVision DC-1 procedure when CellaVision is used.)

B. A stain check is performed each day with the first slide made. Document this quality check on site specific log.

ACCEPTABILITY:

Expected staining characteristics:

	NUCLEUS	GRANULES	CYTOPLASM
Erythrocytes			pink
Leukocytes:			
I. Granular:			
1. Polymorphonuclear neutrophils	purple	red-lilac	light pink
2. Eosinophils	blue	red-orange	
3. Basophils	dark blue	dark purple	
II. Non-Granular			
1. Monocytes	violet		light blue
2. Lymphocytes	violet		blue
3. Platelets		purple	lilac

Note: Basophil granules are water-soluble and therefore only vestiges of granules, sometimes apparently contained within small vacuoles, may be found.

PROCEDURE

Blood Smear Preparation

A. Prepare Blood Smear (See specific requirements in CellaVision DC-1 procedure when CellaVision is used.)
    1. If HemaPrep device is available in lab, refer to attached HemaPrep Blood Smear Preparation instructions. (Available at MR and DX 03/2022, TH and WBHC 03/2025.)
    2. Manual Smear Preparation
        a. Using Diff-Safe device or microhematocrit tube, dispense one drop of well-mixed blood near one end of the slide
        b. Hold a second spreader slide at approximately 45° angle to the first slide and draw into the drop of blood. Allow the blood to spread almost to the width of the slide. Then rapidly and smoothly push the spreader slide to the opposite end of the slide, pulling the blood behind it.
            i. Angle can be adjusted to obtain a smear that spans approximately 2/3 to 3/4 the length of the slide with a good feathered edge.
        c. Print the patient's name and container ID on the frosted end of the slide using a pencil or histology marking pen. Minimum labeling is 2 unique patient identifiers. If barcode labels are used, care must be taken not to get stain on the label that would obscure the patient name, specimen ID, or barcode.
    3. Refer to Buffy Coat Smear procedure if WBC is ≤0.5 x 10^3 /µL.
    4. Refer to Albumin Smear procedure if initial smear reveals greater than 15 smudge cells per 100-cell differential.
B. Stain according to site specific procedure.
    1. Stain the smear within one hour of preparation or fix within one hour with "water-free" (<3% water) methanol for later staining.

Smear Quality Checks

A. Macroscopically examine smear quality
B. Examine smear microscopically using 10X or 40X dry objective
    1. Check smear for stain quality
    2. Ensure there are not clumps of WBCs or Fibrin strands that would indicate a clotted specimen.
        a. Reject specimen if microscopic clots are present
    3. Examine for platelets clumps in the feathered edge. See separate Beckman Coulter Platelet Clumps procedure or Sysmex Hematology Abnormal Specimen Handling

procedure if platelet clumps are present.

4. Look for rouleaux or agglutination in the usable area of the slide. Report as "PRESENT" if found.
5. Scan smear for large abnormal cells that might be missed during the 100 cell differential

C. Correlate general concentration of WBC and Platelets with analyzer count. If analyzer count and smear do not appear to agree, make a new smear.

Verification of Unreliable WBC or Platelet Analyzer Results

Beckman Coulter Analyzer will flag unreliable results with an R flag. Sysmex analyzers will flag unreliable results with an asterisk (*).

Smear estimates are used to verify the unreliable result. (Refer also to Hematology Abnormal Specimen Handling procedure.)

A. Perform WBC Estimate in usable area of the smear using one of the methods below. Estimate should agree within 20% of the instrument's result. If the WBC and estimate do not agree, recheck by making a new slide and/or repeat the WBC on another instrument or review for NRBCs.

1. Using 10X objective: take the average number of WBC/field, Multiply by 1000 and divide by 4.
2. Using 40X objective: count the number of WBCs in 10 fields, divide this number by 10 and multiply this average time 2500 to obtain an estimate of total WBC.
3. <u>WBC Count corrections and NRBCs:</u>
    a. Do not include NRBCs in the WBC estimate
    b. If NRBCs were detected by the Beckman Coulter DxH, the printout will include an NRBC suspect flag. If the NRBCs were counted above the threshold to correct the WBC, then the analyzer will automatically correct the WBC count. This is indicated on the CBC results by a UWBC count that is higher than the WBC count.
        i. Look at the patient's WBC histogram. If the analyzer did not count enough NRBCs to meet the threshold for WBC count correction there will be a very little peak or no peak previous to the 35 fL line. No correction is needed because nRBCs were not sufficient to interfere with platelet count.
        ii. If there are NRBCs but the WBC count is not corrected, look at the WBC histogram. If the peak for the NRBCs falls to the left of the 35 fL line and not within the WBC counting range, then no correction is needed because the NRBCs were NOT counted as WBCs.
        iii. If several to many NRBCs are present and the count was not

corrected by the analyzer, look at the histogram. If the histogram does not show a peak before the 35 fL line, the peak for NRBCs is being hidden by the WBC peak within the WBC counting range. In this situation, the WBC count SHOULD be corrected. In this case, the NRBCs are the size of lymphocytes. This scenario is typically only seen in neonates. To correct the WBC Value count the number of NRBCs per 100 WBCs and calculate the corrected WBC value: (Total Uncorrected WBC x 100) / (100+ # of NRBC) = Corrected WBC.

iv. If the WBC count is corrected, verify the absolute values of differential calculated appropriately. absolute cell count = (%cells/100) x corrected WBC.

c. If NRBCs were detected by the Sysmex XN Analyzer, the analyzer will count the number of NRBCs and exclude them from the WBC count. No correction is required. If WBC or NRBC is flagged with (*), refer to Hematology Abnormal Specimen Handling procedure.

d. If NRBCs were suspected by the Sysmex XN550 analyzer, an "NRBC?" OP alert will be provided by the analyzer. Perform manual differential and count number of NRBCs present. Refer to Hematology Abnormal Specimen Handling procedure for instructions on how to handle "NRBC?" OP alert.

B. Perform Platelet Estimate

1. Add a drop of immersion oil and focus on 100X.
2. Select an area where the RBCs are close to touching, but none are overlapping.
3. Count the total number of platelets in 10 fields. Divide by 10. Multiply this average times 15,000 to determine a platelet estimate.
4. Platelet estimate should compare with the instrument result within 20%.
5. If platelet clumps are present the instrument count may be falsely decreased. If microcytic RBCs or cell fragments are present the instrument count may be falsely increased.
6. If the instrument's count and estimate do not agree, recheck by making a new slide and/or repeating the platelet count on another instrument. In the case of platelet clumping, see platelet clumping procedure.
7. Sysmex XN analyzers will perform a PLT-F (Fluorescent platelet count) when PLT count produces unreliable results. PLT-F will be default platelet count reported when present. Refer to Hematology Abnormal Specimen Handling procedure for more details.

Result Entry

Where available, manual differentials along with RBC and Platelet morphology may be performed using CellaVision. See separate Sysmex CellaVision DC-1 Operation and Maintenance procedure. CellaVision

differentials interface with Caresphere for final review and validation of results.

A. Differential

1. Manual differentials are performed in Caresphere when available. Caresphere rules will automatically add the required smear review type to the specimen order in Caresphere. Caresphere Smear types.png
2. If Caresphere is not available, use Result Entry function of Beaker. If neither Caresphere nor Beaker are available, use a manual counting device and document results on paper for entry into LIS when it become available.
    a. In Result Entry, Enter the accession number and select the manual differential portion of accession.
        i. If not present, add manual differential order in Beaker
            a. If specimen was originally run on Beckman Coulter analyzer, use [Add Manual Differential] button to add the manual differential.
            b. If specimen was originally run on Sysmex analyzer, use [Add Man Diff Non CareSphere] button to add the manual differential.
3. Hemogram results will display above the manual differential/morphology section. Hemogram may be reported prior to or simultaneously with the manual differential.
4. The count is automatically set to 100 cells. If WBC count is very high, the tech may choose to count more cells. In this case the number of cells to count will need to be changed prior to starting the count.
5. Perform the manual differential using the keys that are indicated in the column to the right of each type of cell.
6. Identify 100 WBCs and any nucleated RBCs seen using 50x or 100x. (See Description of WBCs section of this procedure. Pictorial reference manuals are available at the bench if needed.)
7. If any comments need to be added, use dot phrases or free text as a comment attached to the cell type or "Diff Comments" component.

Hover at end of the component result field to locate the comment icon. Click on the comment icon to open text box. See attached Standardized Tech Comments for available Beaker dot phrases.

B. RBC Morphology

1. Morphology of cells is evaluated along with each manual differential.
2. Evaluate the RBC morphology and report accordingly (See RBC Morphology section of this procedure)
    a. Examine multiple 50x and 100x fields and confirm observations with indices (not applicable to WBCD orders).

3. Evaluate WBC and platelet morphology and report accordingly (See WBC Morphology section of this procedure)
    a. Examine multiple 50x and 100x fields
4. Morphology is added in CellaVision or Caresphere when Caresphere is available.
    a. If WBC differential is done on an albumin smear, the RBC morphology should be done on the original smear without albumin, and the morphology should entered directly into Caresphere while the albumin smear is being run on the Cellavision.
5. If Caresphere is not available, document morphology in Beaker. Any comments in Beaker need to be added use dot phrases or free text as a comment attached to "RBC Morphology Comment" component.

Hover at end of the component result field to locate the comment icon. Click on the comment icon to open text box. See attached Standardized Tech Comments for available Beaker dot phrases.

Pictures of WBC and RBC morphology are available in the attached document or in pictorial reference books at bench. Descriptions are provided below.

DESCRIPTION of WBCs

A. Myeloid Cell

1. (Myelo)BLAST - Blast cells in normal myeloid maturation have a diameter of 12–20 µm and a relatively large round or oval nucleus with a fine chromatin pattern and one or more distinct nucleoli. The cytoplasm is basophilic with an absent Golgi zone and granules may or may not be present. May contain Auer rods.
    a. Leukemic myeloblasts vary in appearance. They can be large or small in size. Some may have a high N:C ratio, uncondensed chromatin and usually one or more prominent nucleoli. Others may have a lower N:C ratio and a few red-purple granules or Auer rods. Nuclear and cytoplasmic irregularities may be present, for example nuclear folding, cytoplasmic basophilia and cytoplasmic blebbing or pseudopods.
    b. **Initial identification of blasts needs to be sent for STAT Pathology Review-** blasts can be called for 3 months after initial Path Review identification.
2. PROMYELOCYTE - Normal promyelocytes are 15–25 µm in diameter, have an oval or round nucleus with fine/intermediate chromatin and a usually visible and prominent nucleolus. The cytoplasm is basophilic and contains blue-violet and red (primary) granules. A pale area equating to the Golgi zone is present adjacent to the nucleus.
    a. The promyelocytes in the hypergranular variant of APL (acute

promyelocytic leukemia) have nuclei that vary in size and shape and are often kidney shaped or bilobed. The cytoplasm is packed with large coalescent pink-purple granules and may contain Auer rods. These may be grouped in bundles or “faggots” within the cytoplasm. In the hypogranular or microgranular variant, the nuclear shape is usually bilobed but the cytoplasm contains few or no granules. Count these abnormal promyelocytes as blast equivalents and send for pathology review.

3. MYELOCYTE - The myelocyte is slightly smaller than the promyelocyte (10–18 µm) with a round or oval nucleus which may be eccentrically placed. The nuclear chromatin shows a moderate degree of coarse clumping and nucleoli are not seen. There is a moderate amount of blue-pink cytoplasm which contains numerous red-violet granules. As the myelocyte matures, the secondary granules develop definite neutrophilic, eosinophilic or basophilic characteristics.
4. METAMYELOCYTE - The metamyelocyte is smaller than the myelocyte with an indented or kidney-shaped nucleus. Nucleoli are not observed. The cytoplasm is usually clearly pink and contains granules that are clearly differentiated as neutrophilic, eosinophilic or basophilic.
   Immature granulocytes (promyelocytes, myelocytes and metamyelocytes) are not usually seen in normal peripheral blood.
5. BAND - Band neutrophils are 10–14 µm in diameter and have a nucleus that is nonsegmented or has rudimentary lobes that are connected by a thick band rather than a thread. Cytoplasm is abundant, pink and contains many small violet-pink neutrophilic or secondary granules distributed evenly throughout the cell. If in doubt as to whether a cell is a band or a seg, classify it as a segmented neutrophil.
6. SEGMENTED NEUTROPHIL - A granulocyte that is 10–14 µm in diameter with a lobulated nucleus (usually 3–4 lobes, but small numbers of 2 and 5 lobed neutrophils may also be seen) connected by a thin thread of chromatin. The chromatin is coarse, stains violet and is arranged in clumps. Small nuclear appendages may be seen. There is abundant pink cytoplasm with many small secondary granules.
7. EOSINOPHIL - The diameter of the eosinophil is 12–17 µm. The nucleus usually only has 2 lobes with coarsely clumped, violet-staining chromatin. There is abundant cytoplasm containing many eosinophilic (orange) secondary granules that are larger than neutrophil granules and more uniform in size.
8. BASOPHIL - A basophil is 10–16 µm in diameter with pale blue cytoplasm containing purple-black secondary granules. These granules are water soluble and may dissolve on staining leaving clear areas in the cytoplasm. The nucleus is segmented but is often obscured by basophilic granules which may vary in number, size and shape.

B. Lymphocytes

1. (Lympho)BLAST - The lymphoblast has a diameter of 8–20 µm. The nucleus is round or oval with fine granular chromatin and one or more indistinct nucleoli. The cytoplasm is scanty and basophilic, and cytoplasmic granules are absent. It cannot be reliably distinguished from some types of undifferentiated or minimally

differentiated myeloblasts and therefore should be counted as a blast cell. **Initial identification needs pathology review.**

2. PROLYMPHOCYTE - Cell is intermediate-sized with a greater amount of cytoplasm than the mature lymph (grey to pale blue), a round nucleus with somewhat condensed chromatin, and usually a single prominent nucleolus. Count as a lymphocyte with a comment added that prolymphocytes are present. Lymphoblasts and prolymphocytes are not usually seen in the normal peripheral blood. Prolymphocytes are considered blast equivalents and are subject to pathologist smear review. Refer to Pathologist Smear Review procedure for more details.
3. LYMPHOCYTE - Lymphocytes seen in the peripheral blood are predominantly small (10–12 µm), or, less frequently large (12–16 µm).
    a. Small lymphocytes are usually round in outline, and the nucleus is round with coarse, densely staining chromatin. Cytoplasm is scanty.
    b. Large lymphocytes are usually irregular in outline, and the nuclear chromatin is not as coarse as in small lymphocytes. Cytoplasm is abundant and tends to be light sky blue in colour.
    c. Large granular lymphocytes (LGLs) are of the same appearance as large lymphocytes but the cytoplasm contains prominent small red-violet granules. These cells can comprise up to 10–20% of the peripheral blood lymphocytes in normal subjects. LGLs are not counted as a separate lymphocyte population. A comment may be added if LGLs are present in increased numbers (>20%). This may prompt further investigations such as flow cytometry.
    d. Lymphocytes predominate in the blood films of infants and children until 4 years of age. These lymphocytes are more pleomorphic than those seen in normal adult blood films.

C. REACTIVE LYMPHOCYTE - Include in lymphocyte count and comment if present.
The reactive lymphocyte must have 2 of the 3 following characteristics. (Usually has all three)
    1. Overall size is usually increased due to nuclear or cytoplasmic involvement or both.
    2. Nuclear chromatin is very smooth, chromatin strands are fine, few chromatin clumps present. Usually round, may be slightly indented. Nucleoli may be present.
    3. Cytoplasm usually increased in amount; stains pale to deep blue and darker at the periphery; may be partially indented by adjacent cells; few lavender granules and or vacuoles can be present.
        a. Plasmacytoid Lymphocytes: The plasmacytoid lymphocyte is also recognized as a reactive lymph. It is a B lymphocyte, which will eventually become a plasma cell. Overall size is usually normal. The nucleus is centrally to slightly eccentrically located, indented or oval, with a developing perinuclear halo (HOF); chromatin strands are heavy or in dense blocks; cytoplasm is intensely basophilic and may contain vacuoles.

D. LYMPHOMA CELLS - Present in peripheral blood in cases of lymphomas that has metastasized to the bone marrow or in chronic lymphocytic leukemia. Overall cell size is

usually larger than normal. Nucleus is large; the chromatin pattern is smooth; chromatin strands may be thick. The nucleus may have a cleft or be indented. A single large round light blue staining nucleolus is present. The cytoplasm stains moderately basophilic and usually normal to slightly increased in amount. May contain small vacuoles. Examples are lymphoma cell descriptions are provided below. Do not attempt to classify lymphoma cells based on cell morphology. **Count as lymphocytes initially and send for pathology review.** Lymphoma cells must be confirmed by manual microscopy and can only be called if previously confirmed by pathologist.

1. Follicular lymphoma – These lymphoma cells are often small with scanty, weakly basophilic cytoplasm and have nuclei with notches or deep narrow clefts. Sometimes the cells are larger and more pleomorphic with small but distinct nucleoli and nuclear clefts or notches.
2. Mantle cell lymphoma – These lymphoma cells are pleomorphic varying in size and N:C ratio. Chromatin condensation is less than in CLL lymphocytes and some cells may appear blastic with cleft or irregular nuclei and a prominent nucleolus.
3. Burkitt lymphoma – These lymphoma cells are large with dispersed nuclear chromatin, one or more prominent nucleoli and moderately abundant, deeply basophilic and vacuolated cytoplasm.
4. Sézary syndrome – Sézary syndrome is a mature T-cell lymphoma with neoplastic T lymphocytes in the peripheral blood. The cells are present in variable numbers ranging from a few cells to a frankly leukemic picture with a marked leucocytosis. The cells may be large or small but the nuclear morphology, classically described as cerebriform, is the characteristic cytological feature of both cell types. The nucleus has deep narrow clefts with superimposed and folded lobes giving it a very convoluted appearance.
5. Adult T-cell leukemia/lymphoma (ATLL) – ATLL is characterized by a broad spectrum of cytological features but the characteristic ATLL cells have been described as ‘flower cells’ with many nuclear convolutions and lobules.

E. Hairy cells
Hairy cell leukemia is a chronic B cell lineage leukemia with morphologically distinctive neoplastic cells. Hairy cells are larger than normal lymphocytes and have abundant pale blue-grey cytoplasm with fine hair-like projections. The nucleus varies in shape and may be round, oval, bean-shaped or bilobed. **Count as lymphocytes and initially send for pathology review. Once confirmed by pathologist, count as lymphocytes and comment on presence of hairy cells.**

F. Plasma Cells - Plasma cells vary in size from 15-25mm. A plasma cell is larger than a normal small lymphocyte, has deeply basophilic cytoplasm, an eccentric round or oval nucleus, coarsely clumped chromatin and a pale Golgi zone or perinuclear halo adjacent to the nucleus. **Count as Plasma Cells. Initial identification of >2% Plasma cells needs pathology review.**

G. Monocytes

1. (Mono)BLASTS- Monoblasts are larger than myeloblasts (20-30 µm), with a round/oval nucleus, fine chromatin and one or two prominent nucleoli. The cytoplasm is basophilic and usually lacks granules. Count as "Other" and send for pathology review on initial finding. If confirmed, count as blasts on subsequent finding.

2. PROMONOCYTES (count as blast) - Promonocytes may be rarely seen in the peripheral blood in reactive conditions as well as in some leukaemias. They are large cells with a nucleus that is convoluted/indented with a delicate, lace-like chromatin pattern and prominent nucleolus. The cytoplasm is blue-grey and may contain a small number of fine red-violet granules. Handle promonocytes in same manner as blasts; count as "Other" and send for pathology review on initial finding. If confirmed, count as blasts on subsequent finding.
3. MONOCYTE - Monocytes are the largest cell in the peripheral blood, variable in size but usually 15–22 μm in diameter. The nucleus is irregular in outline (often kidney shaped), and the chromatin is arranged in fine strands with sharply defined margins. The cytoplasm is light blue-grey and contains numerous fine dust-like granules. Some cells may contain a small number of red-violet granules. Vacuolation and pseudopods may be present. Others
4. Abnormal monocytes -Monocytes produced under conditions of bone marrow stress or stimulation, for example infections, growth factor (GM-CSF) administration, show an increased N:C ratio, a more delicate chromatin pattern, nucleoli and increased numbers of vacuoles. Granulation and cytoplasmic basophilia may also be increased.
   Abnormal monocytes can be seen in a number of haematological neoplasms. In contrast to monoblasts and promonocytes, the abnormal monocytes are larger, have irregular nuclei and increased cytoplasm. Count as monocytes. Send for pathology review if this is a new, unexplained finding.

H. UNCLASSIFIED - Cell is in most cases blast-like in appearance, but cannot be identified by tech. Slide should be reviewed by pathologist as soon as possible.

WBC MORPHOLOGY:

GRANULOCYTES:

A. **Toxic Granulation:**
   Coarse, purple staining primary (azurophilic) neutrophil cytoplasmic granules which occur as a response to infection and inflammation. A non-specific reactive change, it is a result of abnormal primary granule maturation with retention of their azurophilic staining properties. May very in intensity from a few, small in size to many present and large in size. Toxic granulation should be seen in the majority of neutrophils to be significant. Report as **PRESENT.**

B. **Vacuolated PMNs:**
   Neutrophil cytoplasmic vacuolation in infection is due to granule fusion with a phagocytic vacuole and release of lysosomal contents to kill bacteria. This vacuolation may appear as 'pin-hole' vacuolation – small, discrete vacuoles, but the vacuoles may be larger. Other causes of neutrophil vacuolation include alcohol toxicity and prolonged exposure to EDTA anticoagulant (storage artifact).
   Report as **PRESENT.**

C. **Döehle Bodies:**
   Pale light blue or grey, single or multiple, cytoplasmic inclusions found near the periphery of the neutrophil. Döhle bodies are a non-specific reactive change but may also indicate May-Hegglin anomaly if associated with thrombocytopenia and giant platelets. Döhle bodies

may also be seen in patients on growth factor therapy such as granulocyte colony-stimulating factor (G-CSF). Report as **PRESENT.**

D. **Hypersegmented neutrophils**:
Neutrophil hypersegmentation is defined as any neutrophil having 6 or more lobes or more than 3% of neutrophils having 5 lobes, when 100 neutrophils are examined. May be seen in B12 or Folate deficiencies or patients on chemotherapy. Report as **PRESENT**.

E. **Hyposegmented neutrophils:**
A neutrophil whose nucleus has no more than 2 lobes. Chromatin may be very clumped. Majority of neutrophils should have this characteristic as well as eosinophils and basophils. Include cells in segmented neutrophil count. When two nuclear lobes are connected by a thin filament, this is described as a "pince-nez" appearance. By definition this is a segmented neutrophil, but when the majority of PMN's have this appearance, Pelger-Huet or pseudo Pelger-Huet is suspected. It is important that these hyposegmented neutrophils not be confused with myelocytes, metamyelocytes or band neutrophils. They are mature neutrophils and can be differentiated by their smaller nucleus and lower nuclear: cytoplasmic ratio (N:C ratio) and condensed nuclear chromatin.**Confirm with pathology review**.

F. **Hypogranulated PMNs:** Reduced or absent neutrophil granulation causing the cytoplasm of mature neutrophils to appear blue-grey.

RED BLOOD CELL MORPHOLOGY

To examine Red Cell morphology, the area of the smear should contain approximately 100-150 cells per oil immersion field. The red cells should overlap, but should be close together. The central pallor should be present in normal cells.

Examine red blood cells for the following findings that correlate with indices. These morphologic findings are not reported.

1. Microcytosis or microcytes correlates with MCV less than reference range
2. Macrocytosis or macrocytes correlates with MCV greater than reference range
3. Hypochromia correlates with MCH and/or MCHC less than reference range
4. Anisocytosis – Variability in size among the RBCs. Correlates with RDW.

Report the following RBC morphology findings as indicated based on attached Morphology Grading Chart

1. Polycytosis is reported as 1+, 2+, 3+
2. Poikilocytosis – Overall variability in shape of RBCs. 1+, 2+, 3+
3. Schistocytes and Spherocytes are reported as number of cells per oil field. (choose range from drop/down in Result Entry)
4. Identify the abnormal shapes and inclusions present. See attached Peripheral Blood Morphology Standardization Images.
    1. Burr cells
    2. Ovalocytes

3. Crenated cells
4. Target cells
5. Stomatocytes
6. Blister Cells
7. Bite Cells
8. Acanthocytes
9. Tear Drops
10. Helmet Cells
11. Pappenheimer Bodies,
12. Howell Jolly Bodies
13. Hemoglobin Crystals
14. Sickle Cells
15. Döhle Bodies
16. Cabot Rings
17. Malaria parasites

5. Rouleaux – Rouleaux appears as cells stacked in a line like stacked coins. This should be recognized when first scanning the slide using 10x. It is important to evaluate rouleaux only on well prepared smears with a feathered edge. Rouleaux is reported as **PRESENT**.
6. Agglutination - Agglutination appears as clustering of cells like grapes. This should be recognized when first scanning the slide using 10x. It is important to evaluate agglutination only on well prepared smears with a feathered edge. Agglutination is reported as **PRESENT**.
7. **NUCLEATED RBC**: RBC contains a nucleus which has dark and condensed chromatin and pink to gray-pink cytoplasm, seen in newborns and in patients responding to hemolytic crisis. Count as a NRBC on keyboard while counting your manual differential. NRBCs are reported in unit /100WBCs.

Report the following platelet morphology as "Present" based on attached Morphology Grading Chart.

1. Giant Platelets (larger than RBCs)
2. Hypogranular platelets
3. Platelet satellitosis

SMEARS TO BE EXAMINED BY PATHOLOGIST

See separate Pathology Smear Review procedure

EXPECTED RESULTS and CRITICAL VALUES:

See separate Hematology Reference Ranges and Critical Values procedure

CALCULATED DATA - APPLICABLE FORMULAS:

Absolute counts on manual differentials are calculated by LIS.

Absolute Neutrophils = [(%bands + %segs)/100] x WBC

Absolute Lymphocytes = (%lymphocytes/100) x WBC

Absolute Monocytes = (%Monocytes /100) x WBC

Absolute Eosinophils = (%Eosinophils/100) x WBC

Absolute Basophils = (%Basophil/100) x WBC

LIMITATIONS

A. In some cases of anemia, polycythemia or abnormal proteins, blood smears produced may be suboptimal.

B. What is observed on a smear should be reported, providing it is not an artifact. Clinical significance is left to the clinician.

REFERENCES

A. National Committee for Clinical Laboratory Standards. Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods. NCCLS Document H20-A, March 1992. NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085, 1992

B. Palmer, L. et al; ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. International Journal of Laboratory Hematology. December 2014. accessed 5/15/2020 from: https://onlinelibrary.wiley.com/doi/epdf/10.1111/ijlh.12327

Attachments

- Caresphere Smear types.png
- HemaPrep blood smear preparation.docx
- Morphology Grading Chart
- peripheral blood morphology standardization images - ISCH.docx
- Standardized Tech Comments 10-18-2021.docx

Approval Signatures

Step Description	Approver	Date
Technical Specialists	Colleen Turtenwald: Technical Specialist	Pending
Technical Specialists	Carolyn Webb: Heme Technical Specialist	03/2025
Policy Owner	Carolyn Webb: Heme Technical Specialist	03/2025

Applicability

Community Physician

Standards

No standards are associated with this document