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| **SOP Number:** | CH-050 | **Creation Date:** |  |
| **Department:** | Hematology | **Effective Date:** |  |
| **Author:** | K. Clark MT (ASCP),  R. Bernshausen (ASCP) | **Version:** | 1.0 |

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| Applicable Standards | | | |  | Version History | | | |
| Standard | | Organization | |  | Version | Effective Date | | Deactivation Date |
| HEM.34400 | | CAP | |  |  |  | |  |
| HEM.34500 | | CAP | |  |  |  | |  |
| HEM.34600 | | CAP | |  |  |  | |  |
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| Related Documents | | | |  |  |  | |  |
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| Review History (Up to the Last 15 Occurrences) | | | | | | | | |
| Date | Version | | Revision Type | | | | Review By | |
|  |  | | Director Review | | | | System Laboratory Medical Director, Joe A. Lewis, M.D., F.C.A.P. | |
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| Distribution |
| CSHCC-Shoreline Lab |
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**Principle:**

Quantitative determinations of total leukocytes, platelets, hemoglobin, hematocrit, erythrocytes and their associated indices (MCV, MCH, MCHC and RDW) will provide the physician with information that can indicate a number of disease states; however, a significant number of diseases involving both erythrocytes and leukocytes may not be detected by these studies but are suggested from examination of stained blood smears.

Staining of peripheral blood smears using a polychromatic stain allows for the identification and quantifying of the various leukocyte cell types and an evaluation of erythrocyte morphology. Polychromatic stains are mixtures of methylene blue which have been altered either by heating in sodium bicarbonate, or in acid bichromate and eosin. Methylene azure is blue-violet in color and stains acidic cell components such as nuclei and cytoplasmic RNA. Eosin is red in color and stains basic cellular components such as hemoglobin. Some cellular components stain with the methylene azure and the eosin.

The purpose of the differential is to establish relative frequency of each cell type and identification of any abnormal forms, which might be present. The differential is also incorporated into the overall smear exam, which includes evaluation of erythrocyte morphology and platelets. The presence of any deviations from normal (increased or decreased levels) in one or more cell types may be helpful in providing information for diagnosis (sepsis, etc.), provide data for selection of further pertinent diagnostic tests, and to monitor harmful effects of chemotherapy or radiotherapy.

**Specimen:**

Wright stained peripheral blood smear. (All smears are retained for one week by the laboratory). See procedures for blood smear slide preparation and for blood smear Wright staining.

**Equipment and Materials:**

* Binocular microscope with 10x, 40x, 50x oil, and 100x oil immersion lenses.
* Microscope Oil (High and Low Viscosity)

**Calibration:**

No calibration is required for this procedure.

**Quality Control:**

* Once each day, review a stained slide for acceptability. If signs of stain contamination or deterioration are evident (i.e. retractile bodies present in erythrocytes, precipitated stain, bacteria) perform cleaning and troubleshooting procedures on the Wescor Stainer.

**Procedure-Stepwise for WBC Differential:**

1. Inspect the smear under low dry power (10x) and observe the distribution of leukocytes and adequacy of the stain. Select the best area for morphological evaluation where the erythrocytes are not quite touching each other, devoid of broken areas and any flattening or distortion of erythrocytes causing spurious macrocytosis.
2. Move to 40x high dry power lens. Correlate the WBC count obtained from the instrument with the numbers on the blood smear. On counts that do not correlate within ±25%, rerun the specimen on the instrument and remake the peripheral blood smear. Use the following criteria for performing WBC estimation. At least 10 HPF’s should be examined.

**Table: CH050-01A**

|  |  |
| --- | --- |
| **Average Leukocytes/HPF** | **Estimated Total Leukocyte Count** |
| 2-4 | 4,000-7,000/cumm |
| 4-6 | 7,000-10,000/cumm |
| 6-10 | 10,000-13,000/cumm |
| 10-20 | 13,000-18,000/cumm |

1. Shift to the 50x oil immersion objective to perform the actual 100 cell differential. To perform the 100-cell leukocyte differential, move the slide from the extreme lower edge, counting and classifying each leukocyte in the successive fields until 100 leukocytes are counted. Use the LIS Result Entry Screen option (Laboratory; Results Entry; Results Entry Screen) option to perform count and release results.

**Note:** If the total WBC count is <1000/cumm, the technologist may count less than 100 cells for the differential. The results are given as a percentage of each cell type. In the LIS differential part, change the total number of cells counted from 100 to the actual number of cells counted.

1. Shift to the 100x oil immersion objective for the RBC morphology, platelet evaluation and the study of the cytoplasm features of leukocytes. Technologists will grade the presence of specific characteristics (see below) as 1, 2, 3 or 4+. Use the LIS Result Entry Screen option (Laboratory; Results Entry; Results Entry Screen) option to perform count and release results. See tables 50-03 through 50-20 for definitions of the grading system for RBC and tables 50-21 through 50-24 for definitions of the grading system for WBC cytoplasm features.
2. The platelet estimate is performed under 100x oil immersion in an area of the slide where the RBC’s barely touch. Count the number of platelets present in a total of 10 fields, then divide by 10 to get an average count. Multiply the average count by 15,000 for microscopes using 10x20 mm eyepieces or by 12,000 for microscopes using 10x22 mm eyepieces.
3. You may also use the table below to determine the estimated count based on the average number of platelets seen in 10 fields.

Table CH50-01B

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Average of 10 fields** | **Estimated Plt Count** | **Average of 10 fields** | **Estimated Plt Count** | **Average of 10 fields** | **Estimated Plt Count** |
| 0.1 | 1,200 | 15 | 188,000 | 38 | 475,000 |
| 0.2 | 2,500 | 16 | 200,000 | 39 | 488,000 |
| 0.3 | 3,800 | 17 | 212,000 | 40 | 500,000 |
| 0.4 | 5,000 | 18 | 225,000 | 41 | 513,000 |
| 0.5 | 6,300 | 19 | 238,000 | 42 | 525,000 |
| 0.6 | 7,500 | 20 | 250,000 | 43 | 538,000 |
| 0.7 | 8,200 | 21 | 264,000 | 44 | 550;000 |
| 0.8 | 8,800 | 22 | 275,000 | 46 | 575,000 |
| 0.9 | 10,400 | 23 | 289,000 | 48 | 600,000 |
| 1 | 12,000 | 24 | 300,000 | 50 | 625,000 |
| 2 | 25,000 | 25 | 312,000 | 55 | 688,000 |
| 3 | 37,000 | 26 | 325,000 | 60 | 750,000 |
| 4 | 50,000 | 27 | 338,000 | 65 | 815,000 |
| 5 | 63,000 | 28 | 350,000 |  |  |
| 6 | 75,000 | 29 | 363,000 |  |  |
| 7 | 88,000 | 30 | 375,000 |  |  |
| 8 | 100,000 | 31 | 388,000 |  |  |
| 9 | 111,000 | 32 | 400,000 |  |  |
| 10 | 123,000 | 33 | 412,000 |  |  |
| 11 | 138,000 | 34 | 425,000 |  |  |
| 12 | 150,000 | 35 | 438,000 |  |  |
| 13 | 162;000 | 36 | 450,000 |  |  |
| 14 | 176,000 | 37 | 463,000 |  |  |

**Reference Ranges:**

Normal ranges for the manual leukocyte differential and erythrocyte morphology are:

**Table CH50-02**

|  |  |  |  |
| --- | --- | --- | --- |
| **ANALYTE** | **AGE** | **NORMAL RANGE** | **UNITS** |
|  |  |  |  |
| ***SEGS*** | 0D-1MO | 32-65 | % |
|  | 1MO-18YR | 28-56 | % |
|  | 18YR-ADULT | 37-80 | % |
|  |  |  |  |
| ***BANDS*** | ALL | 0-10 | % |
|  |  |  |  |
| ***LYMPHS*** | 0-1DAY | 28-May | % |
|  | 1D-ADULT | 19-48 | % |
|  |  |  |  |
| ***MONOS*** | ALL | 0-10 | % |
|  |  |  |  |
| ***EOS*** | ALL | 0-7 | % |
|  |  |  |  |
| ***BASOS*** | ALL | 0-2 | % |
|  |  |  |  |
| ***METAS*** | ALL | 0-1 | % |
|  |  |  |  |
| ***MYELOS*** | ALL | 0 | % |
|  |  |  |  |
| ***PROMYELOS*** | ALL | 0 | % |
| . |  |  |  |
| ***BLASTS*** | ALL | 0 | % |
|  |  |  |  |
| ***NRBC*** | 0-4 DAYS | 0-5 | % |
|  | 4D-ADULT | 0 | % |
|  |  |  |  |
| ***PLASMA CELLS*** | ALL | 0 | % |
|  |  |  |  |
| ***ATYPLYMPHS*** | ALL | 0-10 | % |
|  |  |  |  |
| ***RBC MORPH*** | ALL | NORMAL | % |
|  | Schistocytes | Up to 1% | % |

**Reporting Format:**

All leukocyte cell types are reported in % (whole numbers, no decimals). All erythrocytes and leukocyte morphology is to be graded as 1, 2, 3, or 4+. Cell descriptions and specifics on reporting are as follows:

* **Segmented Neutrophil (SEGS):** Round cells ranging from 10-15 microns in diameter. The nucleus is lobulated with two to five lobes connected by a thin chromatin thread and contains no nucleolus. The chromatin stains purple and is coarse and arranged in clumps. The cytoplasm is abundant, clear and contains many small, fine lilac neutrophilic (secondary) granules distributed evenly throughout the cell. The nucleus to cytoplasm ratio (N: C) is 1:3. Result as % present.
* **Band Neutrophil (BANDS):** These cells are 10-15 microns in diameter. The nucleus may be either centrally or eccentrically located and is indented to more than half the distance from the farthest nuclear margin. The nucleus may appear in the shape of a band or sausage, may be "C" or "U" shaped or may be lobulated. If the nucleus is lobulated, the bridge between the lobes must be wide enough to have two distinct parallel dark margins with light nuclear chromatin material in between. The chromatin is coarse and clumpy. Nucleoli are absent. The cytoplasm is abundant and pale pink or colorless. It may or may not contain a few reddish-purple azurophilic (primary) granules but does contain a large number of the fine, lilac, neutrophilic (secondary) granules. The N: C ratio is from 1:1.5 to 1:2. Report as % present.
* **Lymphocyte (LYMPHS):** Normal mature lymphocytes range in size from 7 to 15 microns in diameter. The cells are round or ovoid but occasionally may be notched or slightly indented. The chromatin is diffusely dense and nucleoli are not normally visible. A perinuclear clear zone surrounding the nucleus as a halo is visible in some cells. The cytoplasm stains light blue and ranges from sparse to moderately abundant in amount. Granules are usually not present but occasionally a few, unevenly distributed; pink azurophilic granules may be present. The N: C ratio ranges from 5:1 to 2:1. Report as % present.
* **Monocytes (MONOS):** Mature monocytes range in size from 12 to 24 microns in diameter. Most are round with smooth margins, but some may have one or more pseudopod-like protrusions. The nucleus may be round, oval, indented, bank-shaped, folded, lobulated, or rarely segmented. The chromatin may be moderately clumped but is relatively less dense compared to that of neutrophils or lymphocytes. Nucleoli are absent. The cytoplasm is abundant, stains gray or grayish-blue (ground-glass appearance), and may contain fine, evenly distributed, pink azurophilic granules. Vacuoles and phagocytized particles may be present in the cytoplasm. The N:C ratio ranges from 4:1 to 2:1. Report as % present.
* **Eosinophils (EOS):** Eosinophils are granulocytes which are slightly larger than neutrophils and contain bright orange-red spherical granules, each of which is approximately the same size. These granules are evenly distributed and fill the cytoplasm but rarely overlay the nucleus. Report as % present.
* **Basophil (BASOS):** Basophils are characterized by the presence of a small to moderate number of coarse, densely stained granules of different sizes and shapes. Basophilic granules (specific or secondary granules) are generally larger than the neutrophilic granules and often approach the size of eosinophilic granules. These granules are roughly spherical in shape but may be irregular. They stain predominantly bluish-black but some may stain purple or purple-red. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Report as % present.
* **Metamyelocyte (METAS):** these cells range in size from 10-16 microns in diameter. They are similar in shape to myelocytes. The nucleus is centrally or eccentrically located and usually indented to less than half the distance from the farthest nuclear margin and thus appears kidney or bean shaped. Occasionally the nucleus may be flattened. The chromatin is usually clumped and there is no nucleolus. The cytoplasm is abundant and appears pink or colorless. The cytoplasm may contain a few reddish-purple azurophilic (primary) granules and/or many fine, lilac, neutrophilic (secondary) granules. The N:C ratio ranges from 1.5:1 to 1:1. Report as % present.
* **Myelocyte (MYELOS):** Myelocytes range in size from 10-18 microns in diameter. They are round or oval in shape and are usually smaller than promyelocytes. The nucleus is centrally or eccentrically placed and may be round, oval or occasionally either flattened or very slightly indented on one side and generally lacks a nucleolus. There is a variable degree of chromatin clumping. The cytoplasm is abundant and stains bluish-pink, light pink, or may appear colorless and contains some reddish-purple azurophilic (primary) granules and/or numerous fine, lilac, neutrophilic (secondary) granules. The N:C ratio is 2:1 to 1:1. Report as % present.
* **Promyelocyte (PROMYELO):** These cells are round or oval and measure 12-24 microns in diameter. The nucleus is large, round or oval and is often centrally located but may be occasionally eccentric. The nucleus may contain one or more nucleoli. The chromatin is fine, with little or no clumping. The cytoplasm is sparse and stains pale blue. It contains a few or many coarse, reddish-purple, azurophilic (primary) granules, which may or may not overlay the nucleus. The N:C ratio ranges from 5:1 to 3:1. Report as % present.
* **Lymphocyte, Reactive (REACTLYMPH)** This category of cells includes reactive, stimulated and plasmacytoid lymphocytes. The main characteristic of these cell types is the variability of cellular size and shape as well as nuclear size, shape and chromatin pattern. See more detailed descriptions below. Report as % present.

Different morphological types of reactive lymphocytes re often seen concurrently in many viral illnesses (e.g. Infectious Mononucleosis). The cells measure 10-25 microns in diameter and may be round, ovoid or irregular. The nucleus may be round, oval, notched, indented, folded or lobated. One or more nucleoli may be present. The chromatin may be fine, medium, or coarse. The amount of cytoplasm is abundant, but may vary in staining characteristics from gray through pale blue to deep blue and is generally darker at the periphery and lighter near the nucleus. The cytoplasm may contain a few pink azurophilic granules and/or vacuoles. The N:C ratio ranges from 3:1 to 1:2

Downey type I 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 present.

Downey type II cells resemble a large lymphocyte and have a round to oval nucleus, moderately clumped chromatin (giving it a “smeared” appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm which appears granular. These cells may frequently 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.

Plasmacytoid lymphocytes are also a form of reactive lymphocyte. They range in size from 10 to 20 microns in diameter and are round or ovoid in shape. The nucleus may be centrally or slightly eccentrically located. The nucleus is round in shape with slightly too moderately coarse chromatin but no distinct nucleoli. These cells have a moderate amount of homogeneously deep blue cytoplasm. The N:C ratio is 3:1 to 1:1.

Immunoblasts are stimulated lymphocytes which range in size from 15 to 20 microns in diameter. They are round or ovoid in shape. The nucleus has one or more prominent nucleoli. The chromatin is finely dispersed. The cytoplasm is moderately abundant and stains deep blue due to high RNA content. The N:C ratio ranges from 3:1 to 2:1.

* **Plasma Cell (PLASMA):** Mature plasma cells are oval of ovoid in shape and range from 10 o 20 microns in diameter. The nucleus is usually eccentric and almost always round or ovoid. The chromatin is coarse, clumped and may be arranged in a clock-face or wheel-like arrangement. Nucleoli are absent. The cytoplasm is abundant and deep blue in color. There is a prominent, pale or lightly stained area in the cytoplasm touching the nucleus that corresponds to the Golgi zone. The N:C ratio is 1:2. Report as % present.
* **Nucleated Red Blood Cell (NRBC):** The term nucleated red blood cell is used to record the presence of any normoblasts in the peripheral blood regardless of the stage of maturation (i.e. pronormoblast, basophilic normoblast, polychromatophilic normoblast and orthochromic normoblast). In most instances, this term is used to enumerate the orthochromic normoblast (metarubricyte). This cell ranges in size from 8-12 microns in diameter. It has very small nuclei which is often pyknotic, and sometimes appears as a homogeneous mass of dense chromatin. The nucleus is often eccentrically located and at times may be extruding or fragmented. The cytoplasm stains pinkish-orange with little basophilia. Report as number present/100 WBC. **Note:** Please review the analyzer print-out carefully to determine if the Automated WBC count has already removed the nRBC’s from the total leukocyte count. If the Automated count does not have nRBC’s added to it, please do not let the LIS system correct the WBC count again. (The ADVIA 2120*i* tends to eliminate the nRBC’s from the total leukocyte count better than the ADVIA 120).
* **RBC Morphology (MORPHCOMM):** A normal, mature erythrocyte ranges in size from 6.7-7.8 microns in diameter. It is a biconcave disc, appearing as round or slightly ovoid. Due to its hemoglobin content, the erythrocyte stains pink-red. A zone of central pallor is present due to the biconcavity of the cell. This zone occupies one-third of the cell (2-3 microns) diameter. The RBC population should be judged on size, shape, color, degree of central pallor and the presence of inclusions. If the RBC population is normal, enter “N” at the MORPHCOMM prompt in the LIS.
* **Anisocytosis (ANISO):** This term describes variations in erythrocyte size. This is determined by examining the smear and correlating the degree of size variation with the RDW (Red Cell Distribution Width) value obtained from the CBC hemogram. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-03**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **No size**  **variation**  **(NP Default)** | **Occasional size variation/field** | **Slight**  **size variation/field** | **Moderate**  **size variation/field** | **Marked size variation/**  **field** |

* **Poikilocytosis (POIK):** Poikilocytosis is used to describe variations in the shape of erythrocytes. Grading levels depend on the percentage or fraction of abnormally shaped red cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-04**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **No shape**  **Variation**  **(NP Default)** | **<25%**  **of all red**  **cells** | **25-50%**  **of all red**  **cells** | **50-75%**  **of all red**  **cells** | **>75%**  **of all red**  **cells** |

* **Microcytosis (MICRO):** Microcytes are smaller than normal erythrocytes. They measure less than 6.5 microns in diameter (smaller than the nucleus of a small lymphocyte) and less than 80 fL in volume. They may be normochromic or hypochromic. Microcytes are commonly seen in iron deficiency anemia, thalassemia and lead poisoning. Grading level depends upon the relative fraction or percentage of microcytic red cells and the relative size of microcytic cells compared to normal cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-05**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<25%**  **of all red**  **cells** | **25-50%**  **of all red**  **cells** | **50-75%**  **of all red**  **cells** | **>75%**  **of all red**  **cells** |
| **Normal MCV** | **70-80 fl** | **60-70 fl** | **50-60 fl** | **<50 fl** |

* **Macrocytosis (MACRO):** Macrocytes are larger than normal erythrocytes. They measure more than 8 microns in diameter. And are usually normochromic but may occasionally exhibit hypochromia. Macrocytes are associated with liver disease, and vitamin B12 or folate deficiency. Unlike reticulocytes, the cytoplasm is pink-red in color instead of bluish-gray. Grading levels depends upon the relative percentage of macrocytic red cells and the relative size of macrocytic red cells compared to normal cells.

Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-06**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<25% of all red cells** | **25-50% of all red cells** | **50-75% off all red cells** | **>75% of all red cells** |
| **Normal MCV** | **95-110 fl** | **110-125 fl** | **125-140 fl** | **>140fl** |

**NOTE:** Newborn babies, infants and toddlers MCV is not in the same range as an adult. The normal MCV for the specific age group being examined should be used to determine the degree of microcytic and/or macrocytic cells observed. Please refer to the chart below:

**Table CH050-07**

|  |  |
| --- | --- |
| **AGE** | **MCV** |
| 0-3 Days | 95-118 fl |
| 3-7 Days | 95-121 fl |
| 7-14 Days | 88-126 fl |
| 14Days-1 Month | 86-124 fl |
| 1Month-2 Month | 77-115 fl |
| 2 Month-6 Month | 77-87 fl |
| 6 Month-2 Year | 77-95 fl |

* **Hypochromia (HYPO):** This is described as less than the normal amount of hemoglobin (MCH) and less than the normal concentration of hemoglobin in the red cells (MCHC). This is identified morphologically by the expansion of the zone of central pallor to more than one-third (greater than 3 microns in diameter) of the cell. Hypochromia is often associated with iron deficiency anemia. Grading level depends upon the relative size of the central pallor as well as the relative percentage of hypochromic red cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-08**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<25% of all red cells** | **25-50% of all red cells** | **50-75% off all red cells** | **>75% of all red cells** |
| **Normal MCHC** | **30-32 pg/dL** | **28-30 pg/dL** | **26-28 pg/dL** | **<26 pg/dL** |

* **Polychromasia (POLY):** Polychromatic red cells are non-nucleated erythrocytes ranging in size from 8-10 microns in diameter. They are larger than a mature erythrocyte and lack a central pallor. The stain homogeneously pink-gray or pale purple. Grading depends on the percentage of red cells that are polychromatophilic. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-09**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1-5%** | **5-10%** | **10-20%** | **>20%** |
| **Reticulocyte**  **Correlation** | **1-5%** | **5-10%** | **10-20%** | **>20%** |

**\***A fair correlation between the grading level of Polychromasia and the Reticulocyte, although not always achieved, exists.

* **Acanthocyte (ACANTHO):** Densely staining red cells with multiple, irregularly spaced thorn-like projections (usually less than 10) of variable size and shape. Their presence is associated with hereditary abetalipoproteinemia, end stage liver disease and hepatorenal failure. Grading depends on the percentage of acanthocytes. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-10**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<5%** | **5-10%** | **10-30%** | **30-60%** | **>60%** |

* **Basophilic Stippling (BASOSTIP):** Fine, medium or coarse blue granules uniformly distributed through the red cell. These granules represent ribosomal RNA precipitated during staining. It is most commonly seen in polychromatophilic red cells. When seen in both polychromatophilic RBC’s and non-polychromatophilic RBC’s, it is usually associated with such pathological conditions as thalassemias, hemoglobinopathies, sideroblastic anemias or heavy metal poisoning. Grading levels depend on the percentage of red cells with basophilic stippling. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-11**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<1%** | **1-3%** | **3-6%** | **6-12%** | **>12%** |

* **Burr Cell or Echinocyte (BURR):** RBC’s with many (10-30) short, blunt or pointed, uniformly distributed projections. These cells still show a central pallor. They are commonly seen in uremia, chronic renal disease and pyruvate kinase deficiency. Grading depends on percentage of burr cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-12**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<10%** | **10-25%** | **25-50%** | **50-75%** | **>75%** |

* **Howell-Jolly Bodies (HJBODIES):** Usually single, almost perfectly round purple nuclear fragments approximately 0.5 microns in diameter seen within non-nucleated red cells but occasionally in nucleated RBC’s. These are seen following splenectomy and in megaloblastic anemias, hemoglobinopathies, and severe hemolytic anemia. They may be seen in neonates, especially premature neonates. Grading depends on the percentage of red cells containing Howell-Jolly bodies. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-13**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1-3%** | **3-6%** | **6-12%** | **>12%** |

* **Ovalocyte or Elliptocyte (OVALO):** Erythrocytes appearing in the shape of a pencil or thin cigar are called Elliptocytes. Those which have the shape of an egg or a wide cigar are called ovalocytes. A small number of these cells may be present on smears from normal patients whereas a moderate to marked number are seen in hereditary elliptocytosis, megaloblastic anemias, thalassemias, sideroblastic anemias and severe iron deficiency anemia. Grading depends on the percentage of oval red cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-14**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<6%** | **6-20%** | **20-50%** | **50-75%** | **>75%** |

* **Schistocytes (SCHISTO):** Red cell fragments that may be of any size or shape. These cells are seen in severe burns, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura and other microangiopathic hemolytic anemias. A normal PBS can contain up to 1% schistocytes. Grading depends on the percentage of fragmented red cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-15**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1-3%** | **3-6%** | **6-12%** | **>12%** |

* **Sickle Cell or Drepanocyte (SICKLE):** Red cells appearing in the shape of a sickle with two pointed ends. This is caused by the polymerization/gelation of deoxygenated hemoglobin S. Grading depends on the percentage of sickle cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-16**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<5%** | **5-10%** | **10-30%** | **30-60%** | **>60%** |

* **Spherocyte (SPHERO):** Densely staining, spherical or globular red cells in which the central pallor is absent. Their diameter is usually less than 6.5 microns. They are found in patients with hereditary spherocytosis, severe burns and immune hemolytic anemias. Grading depends on the percentage of spherocytes. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-17**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Normal** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1-3%** | **3-6%** | **6-12%** | **>12%** |

* **Stomatocyte (STOMATO):** Red cells in which the central pallor is straight or appears as a curved rod-shaped slit. They are found in hereditary stomatocytosis, liver disease and acute alcoholism. Grading depends on the percentage of stomatocytes. Grading depends on percentage of stomatocytes. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-18**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<5%** | **5-10%** | **10-30%** | **30-60%** | **>60%** |

* **Target Cell (TARGET):** These are thin red cells with greater than normal surface membrane to volume ratio. They are flattened-out on the smear to reveal a greater than normal diameter. They are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone resulting in a “bull’s-eye” appearance. They are usually found post splenectomy or in jaundiced patients or in those who have liver disease. Grading depends on the percentage of target cells. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-19**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **<5%** | **5-10%** | **10-30%** | **30-60%** | **>60%** |

* **Rouleaux (ROULEAU):** This term refers to the appearance of four or more red blood cells organized in a linear arrangement that simulates a stack of coins. The length of this arrangement (18 microns or more) will exceed its width (7-8 microns), which is the diameter of a single red cells. True rouleaux formation is present when this artifact is seen in the thin area of a blood film. It is usually associated with a proteinaceous, blue staining background. This is due to increased amounts of plasma proteins, primarily fibrinogen and globulins. It is seen in a variety of infectious and inflammatory disorders associated with polyclonal increases in globulins and/or increased levels of fibrinogen. It is associated with multiple myeloma and malignant lymphomas such as Waldenstrom’s macroglobulinemia. Grading depends on the percentage of red cells involved in rouleaux formation. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-20**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | <10 | 10-25% | 25-50% | 50-75% | >75% |

* **Toxic Granulation (TOXGRAN):** Toxic granulation is the presence of large purple or dark blue cytoplasmic granules resembling primary granules in the cytoplasm of neutrophils, bands, and metamyelocytes. Toxic changes result from the actions of cytokines released in response to infection, burns, or trauma and indicate a shortened maturation time and activation of post-mitotic neutrophil precursors. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-21**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **Small Granules** | **Small Granules** | **Medium Granules** | **Large Granules** | **Large Granules** |
|  | **<5%** | **5-25%** | **25-50%** | **50-75%** | **>75%** |

* **Toxic Vacuolation (TOXICVAC):** Vacuoles within the cytoplasm of neutrophils and their precursors constitutes toxic vacuolation. Toxic changes result from the actions of cytokines released in response to infection, burns, or trauma and indicate a shortened maturation time and activation of post-mitotic neutrophil precursors. Grading depends on the average number of vacuoles per cell plus the percent of cells containing vacuoles. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-22**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1-2/cell** | **2-4/cell** | **5-7/cell** | **7-10/cell** | **>10/cell** |
|  | **<5%** | **5-25%** | **25-50%** | **50-75%** | **>75%** |

* **Döhle Bodies (DOHLE):** Döhle Bodies appear as single or multiple blue, gray-blue, or green gray inclusions of variable size (0.1 to 2.0 microns) and shape (round, elongated or triangular) in the cytoplasm of neutrophils, bands or metamyelocytes. They are often found in the periphery of the cytoplasm near the cell membrane. These inclusions represent parallel strands or rough endoplasmic reticulum. Toxic changes result from the actions of cytokines released in response to infection, burns, or trauma and indicate a shortened maturation time and activation of post-mitotic neutrophil precursors. Grading depends on the average number of Döhle Bodies per cell and the percentage of cells containing Döhle Bodies. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-23**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1/cell** | **1-2/cell** | **3-5/cell** | **5-7/cell** | **>7/cell** |
|  | **<5%** | **5-25%** | **25-50%** | **50-75%** | **>75%** |

* **Auer Rods (AUERRODS):** These are pink or red, round or rod-shaped cytoplasmic inclusions that are seen mostly in immature granulocytes and occasionally in monocyte precursors in patients with acute non-lymphocytic leukemia’s. These inclusions represent agglomeration of azurophilic granules. Grading depends on the average number of Auer Rods per cell and the percentage of cells containing Auer Rods. Report as 1, 2, 3 or 4+ using the chart below:

**Table CH050-24**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Normal** | **Trace** | **1+** | **2+** | **3+** | **4+** |
| **(NP Default)** | **1/cell** | **1-2/cell** | **3-5/cell** | **5-7/cell** | **>7/cell** |
|  | **<5%** | **5-25%** | **25-50%** | **50-75%** | **>75%** |

***PROCEDURE NOTES:***

1. When the nucleus of a neutrophil is folded or twisted so that the connecting band filaments are difficult to visualize, use the following to determine if the cell is a ban or segmented neutrophil (please refer to the illustrations)
2. Assume a hidden filament is present and classify the cell as a segmented neutrophil if:

* The margins of two adjacent lobes are completely separated.
* The width of either of the two adjacent lobes markedly narrows or converges toward the junction of the lobes, making it possible that a thin filament could be hidden.
* The nucleus is so extensively folded that it cannot be determined if a filament is present.

1. Assume that the cell is a folded band if:

* An elongated band form crosses over itself without evidence of constriction to a filament.
* Only the distal tip of the nucleus is slightly bent back upon itself.
* The hidden area in the fold between two superimposed lobes is so small, and the lobe width is so large that it is unlikely that a thin filament could be hidden behind the fold.



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