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| **Purpose** | This procedure provides technical instruction for understanding and performing an acceptable outcome for the “Differential Protocol”. |
| **Scope** | This procedure applies to testing personnel authorized to perform testing using the Sysmex HST-5000 System, Bayer Hema-tek slide stainer, and Olympus Microscopes. |
| **Responsibility** | All authorized personnel are responsible for following procedural guidelines and insuring good laboratory practice is followed. |
| **Related Documents** | CORE 6505 F Submission of Peripheral Smear for Pathologist Review CORE 6510 R Hema-Tek stainer Procedure CORE 6501 R Sysmex XE-5000 Procedure CORE 6504 R Sysmex SP 1000i Slide Maker Stainer Olympus Microscope user manual Hematology Glossary |

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If a CBC and differential is needed on a patient, the most efficient utilization of laboratory resources will occur if a CBC with differential (CBCD) is ordered. When a CBCD is ordered and the results violate certain Meditech-defined limits, reflex testing consisting of a smear review or manual differential will be performed. This is done automatically by the LASC computer system when the XE-5000 sends the appropriate data needed to reflexively order a smear.

Section IV of this procedure applies to samples that generate a smear review but in some way the slide failed to be pulled by the HST-5000 system.

If all parameters fall within acceptable limits the electronic differential will be reported as the differential for the patient; this is handled automatically by the Meditech computer system. (See procedures for Hematology Instruments and electronic differential).

**I. PRINCIPLE**

A peripheral blood smear is stained with a Romanowsky stain and examined microscopically. A number of leukocytes, usually 100, are counted and the different cell types identified. The results are reported as a percent of each cell type seen. The slide is also scanned and remarks are made concerning abnormal leukocytes, RBC morphology and platelet information.

**II. SPECIMEN**

A properly collected 4ml or 2.0 ml EDTA lavender vacutainer is required. For pediatric patients and for difficult draw patients a lavender EDTA microtainer may be used. The vacutainer tube should be filled; a tube filled at least 1/3 full is acceptable. A microtainer must be filled between the 250 and 500 marks on the side of the container. Specimens that are clotted or do not contain the minimum amounts listed above should be rejected and a new specimen requested. The specimen should be transported to the laboratory immediately following collection. The blood smear should be made within 3 hours of being received in the laboratory. After 3 hours at room temperature, degenerative changes may be observed upon examination of blood smears. Samples stored at 2 – 8º may have smears added up to 24 hours.

**EQUIPMENT AND REAGENTS**

Sysmex HST-5000 System Bayer Hema-tek Slide Stainer 75 X 25mm   
Frosted glass slides

Capillary tubes Olympus

Microscope

**III. PROCEDURE – Automated 5 Cell (Electronic) Differential**

A. If all 5 cell parameters fall within acceptable limits and no significant flags are noted the electronic differential will be reported as the differential for the patient. This is handled automatically by the Meditech computer system. (See procedures for Hematology Instruments and electronic differential). When this occurs no additional steps are required.

**IV. PROCEDURE - Manual Smear Review (HSR)**

A. Prepare a blood smear if the LASC failed to order a slide on the SP 1000i.

1. Use the capillary tubes to transfer a small drop of blood to a pre-cleaned slide, frosted side up. The blood is placed in the centerline of the slide just past the frosting.

**NOTE**: Specimens from the nursery with a HCT >55 should have a 1:1 dilution made after running the specimen using equal parts of blood and cell pack. This greatly improves the quality of the white blood cells and also allows for better review of the red blood cell morphology.

2. A second slide, especially configured slide, is used as a spreading slide. The “pusher” slide is placed at an angle of about 45 degrees to the slide containing the blood drop. is moved back to make contact with the drop.

3. The drop should spread out quickly along the line of contact of the spreader with the slide.

4. The moment this occurs, the film should be spread by a rapid, smooth, forward movement of the spreader.

5. The drop of blood should be of such a size that the film is about 30mm in length. If necessary repeat this process until an acceptable slide is obtained.

Label the slide using a sharp pencil. On the frosting, record the patient's last name, or as much as will fit, along with the accession or barcode number. Allow the slide to air dry. Load the slide on the Sysmex SP1000i slide maker stainer. See CORE 6504 R for detailed instructions. For alternate staining methods load slides on the Hema-Tek 1000 stainer or the Aerospray stainer. Consult procedure CORE 6510 R or CORE 6512 R respectively for detailed operating instructions.

After staining***, label the slide with a Meditech footnote label.***

accession number of the smear to be reviewed.

C. After entering the accession number **Hematology Smear Review** screen will appear. The blue flashing curser *should* appear in the ***Hematology Smear Review Comment*** text box. If the curser defaults to the ***Add Manual Differential YES/NO*** text box with the word NO entered this means that a smear review was done within the last 24 hours. If this is the case move the cursor down to the last text box – ***Add Smear Review (DO NOT EDIT)*** and change the YES to NO and verify. The Auto diff will be transmitted to the patients report.

**NOTE:** Review the 5 cell electronic diff displayed on the ***Hematology Smear Review*** Screen to make sure all cell parameters have been transmitted by the Sysmex analyzer. If any parameter is missing you must order a manual differential. **T*he XE-5000 counts a 6 part differential but only transmits a 5 part differential. It counts GR% and IG% separately but only transmits the GR%. If the 5 cell electronic differential does not = 100%, check to see if the analyzer counted any immature granulocytes (IG%). If so, add the IG% to the GR% and the 5 cell differential should equal 100%.***

D. For samples showing all 5 cell parameters on the electronic differential and requiring a smear review use the 50x oil immersion objective and examine the smear microscopically. Check the smear to see that it is well made, the distribution of the cells is uniform and the staining is satisfactory. Criteria for a well-stained smear include:

1. No precipitated stain should be seen.
2. The erythrocytes are orange or pink.
3. The nuclei of the leukocytes are purplish-blue.
4. Neutrophilic granules are reddish to pink-Iilac.
5. Eosinophilic granules are red to orange.
6. Basophilic granules are very dark bluish-purple.
7. Platelets stain dark blue-purple.

E. Scan the slide and look for abnormal or suspicious cells that may be in disproportionately low numbers. Look for nucleated red cells, immature cells, atypical lymphocytes and platelet clumps or large platelets. Estimate the white cell count and the 5 cell electronic differential to see if there is any gross error in the instrument count. This could also detect a clotted specimen or perhaps a mix-up in blood specimens.

F. If no significant abnormalities are noted the comment "*Smear review agrees with automated differential. No significant red cell or platelet abnormalities are noted"*

should be entered. In the **Hematology Smear Review** screen, with the blue flashing curser in the ***Hematology Smear Review Comment*** text box press key **F9**.

This will prompt you to a **LOOKUP** screen showing the following message: **CHECKED Smear Reviewed; AGREE.** Pressing the **F12** key will display the comment and pressing F12 again will file the comment and return you to the **Hematology Smear Review** main screen. The curser will now be in the ***Add Manual Differential YES/NO*** text box with the text **NO** inserted**.** Press F12, place a check in the verify results text box, and verify results.

G. If a smear review shows a discrepancy with the automated differential or reveals any significant abnormalities or problems for which a manual differential is deemed medically necessary, a manual differential will be performed.

**NOTE: Errors noted in the 5 cell differential may cause inaccurate counts for the If the absolute neutrophil count. If this occurs, count the manual differential, recalculate the absolute neutrophil count and enter in meditech. Notify the appropriate nurse or physician and document the the result was called.**

**WBC x Neutrophil % = Absolute neutrophil count**

**Example: WBC = 8.0 Neutrophil % = 72 %**

**8000 x 0.72 = 5760**

**V. PROCEDURE - Manual Differential (DIF) *or* (D)**

A. The Meditech computer system has a real time differential cell counting program. The cell counter is accessed through the **Result Entry** program using **Enter Results (Entry Screen)**. At the result entry screen enter the Mnemonic **DIF**. It will then prompt you to **IDENTIFY SPECIMEN TO RESULT** screen and at this point enter the accession or barcode number of the differential to be counted.

**NOTE:** *Using the* ***DIF*** *Mnemonic generates separate screens -one for the* ***differential count*** *and one for* ***morphology****. The DIF mnemonic also shows the previous counts and morphology. Using the* ***D*** *mnemonic utilizes only one screen and shows no previous data.*

B. After entering the accession or barcode number the **WBC Differential and Morphology** screen will appear. This is the Meditech online cell counter screen. The last option will be the question to change the number of cells counted, it defaults to 100. If the WBC is markedly decreased you can enter a number less than 100.

C. Scan the area to be counted and look for abnormal or suspicious cells that may be in disproportionately low numbers. Look for nucleated red cells, immature cells, atypical lymphocytes and platelet clumps or large platelets. Estimate the white cell count to see if there is any gross error in the instrument count. This could also detect a clotted specimen or perhaps a mix-up in blood specimens.

D. Once in **WBC Differential and Morphology** screen count the differential and identify WBC’s seen. This program displays the keys assigned to each cell type and morphology comment. Using these keys enter the cells as you count them on the slide. Differentials should be counted using the **50x** oil immersion objective. If any doubt exists as to the identity of a cell, the magnification should be increased to 1000X. Use the morphology comment keys to enter any red cell morphology. Smears may be scanned in any direction, in the middle third of the smear. A wedge-type smear should be scanned across the width of the slide just behind the feathered edge. The red cells should be separated, but occasionally touching, and the central pallor should be easily seen.

E. Every diff must include a comment regarding morphology. If the red cells appear normal enter the comment RBCN, which translates into **RBC Morphology Normal.** You must also include a platelet estimate. With the Olympus microscopes at 1000 power, count the platelets seen in a field and multiply by 6. This will give you an approximate platelet count for that specimen. The actual platelet count can be seen on the **DIFF** portion of the **WBC Differential and Morphology** screen. Certain blood samples may exhibit platelet abnormalities such as giant platelets or platelet satellitosis or clumped platelets. See procedure CORE 6520 R titled “CLUMPED, GIANT PLATELETS AND PLATELET SATELLITOSIS” for detailed response.

**RBC MORPHOLOGY GRADING**

**Grade 1+2++ 3+++**

Anisocytosis RDW 14.6-18 RDW 18-22 RDW>22

Poikilocytosis 3-5% 5-25% >25%

Microcytosis MCV 70-80 MCV 60-69 MCV <60

Macrocytosis MCV 100-115 MCV 115-125 MCV >125

Hypochromasia MCH 23-26 MCH 21-23 MCH <20

Polychromasia 1-5% 6-20% >20%

Acanthocytes 1-10% 11-30% >30%

AggRBC 1-25% 25-50% 50+%

Burr Cells 1-25% 25-50% 50+%

Crenated 1-25% 25-50% 50+%

Dohle bodies 1-25% 25-50% 50+%

**RBC MORPHOLOGY GRADING (Continued)**

**Grade 1+2++ 3+++**

Giant platelets 1-25% 25-50% 50+%

Helmet cells 1-25% 25-50% 50+%

H-J bodies 1-6% 6-12% 12+%

Hypersegmentation 1-20% 20-50% 50+%

Ovaloyctes 1-20% 20-50% 50+%

Rouleaux 1-25% 25-50% 50+%

Schistocytes 1-5% 5-15% >15%

Sickle 1-30% 30-60% 60+%

Smudge cells 1-25% 25-50% 50+%

Spherocytes 1-5% 6-20% 20+%

Basophilic stippling 1-6% 6-12% 12+%

Stomatocytes 1-30% 30-60% 60+%

Target cells 1-30% 30-60% 60+%

Tear drop cells 1-6% 6-12% 12+%

Toxic granulation 1-50% 50-75% 75+%

Toxic vacuolation 1-50% 50-75% 75+%

**MORPHOLOGY GRADING**

F. After the differential is complete the slide should be filed in the slide cabinets next to the differential counting area. Slides are filed by the day of the week in which they are counted. Slides are retained for a period of three weeks and are then discarded. The overnight charge tech is responsible for the correct storage and discarding of slides.

**VI. Procedural Notes: Reflexive Orders for Smear Review and Manual Differential**

The following information summarizes what tests will be reflexively ordered, either electronically or by action of a technologist, and at what limits. Please note that in addition to these limits a manual differential will be reflexively ordered if the automated differential is not reported by the laboratory instrumentation.

Blood films will be examined microscopically (**manual differential or smear review**) by technologists whenever:

A. A physician orders a manual differential as part of the complete blood count or as a stand-alone test. When ordered along with the CBC, the manual diff is performed by a laboratory technologist. The ordering physician can also specifically request that a pathologist examine the blood film.

B. A laboratory technician has reason to consider a specific disease that can be diagnosed by a smear review (malaria, candida, sepsis, etc.). Any slide found to be positive or suspicious for these conditions will be referred to a pathologist for confirmation or refutation. The technician may also do a manual differential if he/she feels it is beneficial.

C. There is a presence of the following results on a CBCD. A smear review will be done when:

1. the leukocyte count is <2.5 or >25 K/ul

2. platelet count is <50 or > 1000 K/ul

3. when the RDW >20%

4. when the MCV >105fl.

5. marked flagging (>250) for Blast, Imm Gran, Left Shift, Atypical Ly, Abn Ly/L\_Bl, or PLT Clumps.

6. when the automated differential yields the following results:.

* 1. a. lymphocyte count is <10% or >55%**\***
  2. b. eosinophils >20%
  3. c. basophils >5%
  4. d. monocytes >25%

D. Microscopic examinations (manual differential or smear review) **will not** be provided, unless specifically ordered noted by physician, when:

1. A microscopic examination has already been carried out within 24 hours
2. When the cell counter data is entirely normal.

E. A manual differential **will** be performed whenever a request is made for a pediatric patient (up to 16 years) or when the blood film review (scan) has revealed any of the following abnormalities:

1. Neutrophils less mature than the myelocyte stage
2. Abnormal leukocyte morphology
3. Leukocytes with nuclei that have open chromatin or nucleoli (blast –like).
4. Marked red cell abnormalities (includes NRBCs, 3+ aniso, 3+ poikilocytosis)
5. If a discrepancy exists between the automated differential and the visual review (HSR).

F. **Criteria for pathologist review of the blood film follows:**

A *Peripheral Blood Smear Review Submission Form* (CORE 6505 F) is to accompany the slide

\_\_\_\_ Clinical physician request/order

\_\_\_\_ Review for parasites

\_\_\_\_ Meets criteria for smear review (circle criteria met below)

1. Leukocyte count >50 K/ul.
2. Normal leukocyte count but automated diff showing granulocytes <15%, lymphs

>75% (except newborns), atypical lymphocytes >20%, monocytes >30%

1. Platelet count >999K/ul.
2. Suspected micro-organism or atypical intracellular inclusions
3. Possible basal cells, plasma cells, smudge cells, or cells that cannot be routinely classified by a technician.

***NOTE*: ALL PATH SLIDES MUST BE STORED IN THE SMALL PLASTIC SLIDE BOXES KEPT IN THE DRAWER JUST BELOW THE LARGE WOODEN SLIDE BOXES. THE NAME, DATE, AND ACCESSION OR THE BARCODE NUMBER MUST BE WRITTEN ON THE SLIDE AND ON THE LID OF THE BOX. THE DRAWER IS LABELED *PATHOLOGIST REVIEW SLIDES***.

A comment will be appended to each differential indicating whether it is an automated or manual differential. The Meditech system handles this task automatically. ***Please note*** that while the Meditech computer system automates and simplifies the differential algorithm it does not take the place of the judgment of the human operator. If, in the operator's opinion, a smear review or differential should be performed on a specimen when one is not ordered by the computer system the operator has the option of ordering a smear review or differential. *Please note that the operator should not override a computer decision that a smear review was ordered unless approved by the Lead Technologist.*