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| Dignity Health Logo.jpg  Arroyo Grande Community Hospital  French Hospital Medical Center  Marian Regional Medical Center | Title: **Differential Criteria and Pathology Review** | | | |
| Document Number:  7500.H.CC.11 | | Page 1 of 6 | |
| Department:  Clinical Laboratory | Implementation Date: | Review Date: | | Revision Date(s):  10/2014 |
| Distribution:  Departmental | Author:  Jamie Benjamin, MT(ASCP) | | Approved By: | |

**Purpose:** Establish criteria for review of CBC and differential results, manual differentials, and pathology reviews based on clinically important results and/or results flagged as abnormal by the automated hematology analyzer.

**Principle:** This procedure aims to define guideline or “rules” which can be applied to criteria for the review of CBC and differential results from automated hematology analyzers. The automated hematology analyzers are configured with these defined rules and are set to alert users when manual reviews and/or manual differentials are needed. These rules are necessary to standardize laboratory practices and to allow for the identification of abnormal/immature/atypical cells, recognize clinically significant morphological abnormalities, and verify the results generated by the automated analyzers.

**Specimen Collection:**

1. Whole blood collected in Potassium EDTA
2. Specimen rejection criteria

* Clotted samples, samples with microclots, and samples containing fibrin
* Improperly labeled specimens
* Specimens exceeding stability
* Hemolyzed samples should be recollected if possible.
* Follow SOP 7500.H.CC.31 Spurious Results Protocol for handling hemolyzed samples that cannot be recollected.
* Results released on sub-optimal specimens are accompanied by a comment noting the condition of the sample.

1. All samples are thoroughly mixed immediately after collection to ensure adequate mixing of the whole blood sample with the EDTA anticoagulant. Samples are also mixed immediately before being sampled to ensure homogeneous dispersion of cells before analysis. The DxH rocking platform has been validated to be sufficient to mix samples with up to four hours of settling.
2. Specimens that have been allowed to settle more than four hours are mixed by rocker or manual inversion to ensure homogeneous dispersion of cells before analysis.
3. Specimen stability: Automated CBC with differential

* <24 hours at 18-26 °C
* <48 hours at 2-8 °C

1. Specimen stability: Manual Differential/Smear Review/RBC or PLT Morphology

* Optimal slide preparation is <4 hours from collection stored at 18-26 °C
* Acceptable extended storage is <24 hours at 2-26 °C

1. Allow refrigerated samples to come to room temperature before analysis
2. Peripheral smears are retained for a minimum of 7 days

**Reagents/Supplies:**

1. Equipment

* Automated hematology analyzer
* Automated slide stainer
* Mechanical slide maker
* CellaVision
* Microscope
* Zebra 2D label printer

1. Supplies

* Glass slide
* Applicator sticks
* Blood drop dispensing device

**Calibration:** N/A

**Quality Control:**

All stained slides are to be reviewed for acceptable staining. A properly stained slide will display the following characteristics.

1. RBCs will be red to pink in color.

2. WBC nucleus coloration.

a. Lymphocyte and Neutrophil nucleus are a dark purple in color.

b. Monocyte nucleus is light blue to purple.

3. White blood cells and platelets appear:

a. Neutrophil cytoplasm will be light pink with lilac colored granules.

b. Lymphocyte cytoplasm varies from light to dark blue.

c. Monocyte cytoplasm is gray-blue with a few reddish granules.

d. Eosinophil cytoplasm is filled with large red to orange granules.

e. Platelets will appear as small round or oval blue-gray fragments.

f. Basophil cytoplasm is filled with large purple to blue granules.

4. Slight variations may appear in the colors described above, but if the various morphological structures are distinct, the stain is satisfactory.

5. Corrective Action must be taken if staining is unacceptable. This may include:

a. Decolorizing smear and restaining

b. Remaking smear and restaining

c. Maintenance of Slide Stainer

1. Perform and record all instrument quality control, maintenance and function checks in accordance with each corresponding operating procedure.
2. Do not report patient results if quality control is not within an acceptable range.

**Procedure:**

1. Run the EDTA whole blood sample through the automated hematology analyzer according to procedure.
2. The analyzers are configured with rules and flags that reflect the criteria in 7500.H.CC.11 Attachments 1 through 3 for performing smear reviews, manual differentials, and/or pathology reviews. Results that trigger these rules or flags are set to automatically print on the analyzer’s corresponding printer.
3. CBC W/ MANUAL DIFF: Specimens with an order for a CBC with Manual Differential will only run a Hemogram on the automated analyzers and may or not have printed results. If a CBC with Manual Differential is ordered, it is useful to obtain the instrument differential and corresponding flags before a manual differential is performed.
   * Orders may be modified on the instrument to include a differential prior to running the specimen.
   * Alternately, if hemogram results have been previously released from an analyzer but a differential is desired, the specimen can be rerun on the same analyzer for default testing (CBC with differential).
4. RDIF: Orders for Pathology Review (RDIF) may or may not print based on flags or messages.
   * Perform a Manual Differential on all specimens sent for pathology review regardless of the automated analyzer results.
   * Print CBC with differential results from the analyzer and if necessary, place an order for a Manual Differential in Cerner.
5. HEMOGRAM: Hemogram orders with printed results containing a Suspect or Definitive Message may be release without further action. Hemogram orders with a System Message must be resolved before results are released. Refer to 7500.H.CC.31 Spurious Results Protocol for instruction on resolving System Messages.
6. Any printed result on an order for a CBC w/ Automated Differential must be investigated. Review the printed results for Suspect Messages, System Messages, and Definitive Messages.
   1. SUSPECT MESSAGES (displayed in RED) are generated by internal algorithms to convey that a clinical condition may exist with a specimen based on an abnormal cell distribution or population.
   2. SYSTEM MESSAGES (displayed in GREEN) indicate an event occurrence that may affect the operation of the system and are accompanied by R (review), P (aspiration), or N (non-blood) flags.
   3. DEFINITIVE MESSAGES (displayed in BLUE) appear for results based on exceeded limits or triggered rules.
7. For System Messages, identify the affected parameter(s). Refer to 7500.H.CC.31 Spurious Results Protocol for instruction on resolving System Messages. Do not accept results with “P” or “N” results. Results with R flags may be accepted only after verification by smear review, manual estimate, or manual differential.
8. Instructions for handling each triggered Suspect/Definitive Message appear in the Comment section on the bottom of each printed patient result. These instructions correspond to the action criteria in Attachments 1 through 3. If comment instructions are incomplete on the printout due to limited space, full comment instructions can be viewed on the DxH analyzer by viewing the patient results on the screen, selecting MORE on the bottom right of the screen and selecting RULES TRIGGERED.
9. Unusual, inconsistent, or System Flag results are not released until results are verified by smear review, estimate, manual differential, or until the result can be validated/explained after speaking with a nurse or physician. If results can be explained after clinical investigation (i.e. transfusion, surgery, blood loss) and smear review/differential is determined to be unnecessary, attach a comment explaining all pertinent information to the corresponding results.
10. Prepare a slide for any specimens requiring a smear review, manual differential, or RDIF according to procedure. Each peripheral blood film should have a gradual transition in thickness, without any grainy streaks, troughs, ridges, holes, or bubbles.

The blood film must be at least 30 mm in length, terminating 5-15 mm from the edge. The smear must cover approximately ⅔ to ¾ of the slide.

1. Label each slide with full accession number and patient last name. A 2D accession number barcode label is used for slides processed on the CellaVision. Use the barcode scanner attached to the Zebra 2D barcode label printer to scan the accession number. A 2D accession number barcode label will print. Attach the printed label to the frosted end of the slide. Write the patient’s last name above the barcode for a second identifier.
2. Stain the slides according to procedure.
3. It is preferable to perform a smear review, differential, platelet estimate, and/or red blood cell morphology using the CellaVision. Follow SOP 7500.H.07, CellaVision Smear Review and Differential for instructions on performing a smear review using the CellaVision. Alternately, if the CellaVision is unavailable, a manual microscopic procedure can be used, see SOP 7500.H.08 Manual Microscopic Smear Review and Differential.

**Interpretation:**

1. Specimens flagged for WBC smear review that do not meet criteria defined in 7500.H.CC.11 Attachment 2 for performing a manual differential require no further action. Follow the SOP 7500.H.07, CellaVision Smear Review and Differential to confirm the automated differential using the CellaVision, and release the DxH auto differential results. If the smear review is performed using the manual microscopic method, retain the stained smear for a minimum of 7 days and release the DxH auto differential results.
2. A manual differential is performed on any specimen flagged for WBC smear review meeting criteria in 7500.H.CC.11 Attachment 2 or 3 for a manual differential and/or pathology review. Perform a CellaVision or manual microscopic differential, including an evaluation of RBC morphology and platelet estimate/morphology, according to procedure.
3. Specimens flagged for RBC/Platelet Smear Review must have a red blood cell and platelet estimate/morphology performed. Perform a CellaVision or manual microscopic RBC morphology and platelet estimate/morphology according to procedure.
4. Perform a manual platelet estimate on any specimen with an automated platelet flag in order to confirm the platelet count.
5. Specimens that meet criteria in 7500.H.CC.11 Attachment 3 for pathology review (or physician requests for pathology review), require a manual differential. Perform a CellaVision or manual microscopic differential according to procedure as well as an evaluation of RBC morphology and platelet estimate/morphology. Print the completed results from Cerner and attach the original DxH printout. Ensure that an RDIF order has been placed. Submit all printouts, a Cerner label, and a stained slide to pathology. Log the patient information into the Pathology Smear Review logbook located in Hematology.

**Result Reporting:**

1. For specimens requiring a smear review due to WBC flags/messages, view automated DxH results in Cerner by entering the patient accession number in Accession Result Entry.
2. In the SCAN field, open the dropdown box and select SCAN. The SCAN selection will allow both release of the automated differential or an order for a manual differential (and cancelation of the auto differential) once the Smear Review is competed.
3. Ensure that a checkmark appears next to all valid results and select PERFORM.
4. Once results are performed, all results which do not require a smear review or manual estimate to confirm are verified by placing a checkmark next to each result and selecting VERIFY. DO NOT VERIFY THE “SCAN” RESULT. In most instances, hemogram results can be verified before a WBC smear review is performed. Any automated result in question should remain in PERFORMED status until confirmed by smear review.
5. Refer to corresponding CellaVision or Manual Differential Procedures for information on entering Manual Differential and RBC/Platelet morphology results.
6. For specimens with a RBC and/or Platelet flag requiring a smear review, access the DxH results in Cerner by entering the patient accession number in Accession Result Entry.
7. In the SCAN field, open the dropdown box and select SCAN/MORPH.
8. Ensure that a checkmark appears next to all valid results and select PERFORM.
9. Once results are performed, all results which do not require a smear review or manual estimate to confirm are verified by placing a checkmark next to each result and selecting VERIFY. In most instances, hemogram and automated differential results (including the SCAN/MORPH result) can be verified before RBC/Platelet smear review is performed. The exception to this would be any result with a System (R, N, or P) Flag or any flag/message which may indicate an inaccurate result (i.e. platelet clumps). Any automated result in question should remain in PERFORMED status until confirmed by smear review.
10. Refer to corresponding CellaVision or Manual Differential Procedures for information on entering RBC/Platelet Morphology results.
11. Specimens requiring a smear review that do NOT meet criteria defined in 7500.H.CC.11 Attachments 2-3 for manual differential or pathology review are released with the original auto differential and a smear review comment. Access the original automated DxH results in Cerner by entering the patient accession number in Accession Result Entry.
    1. Ensure that a checkmark appears next to the SCAN result.
    2. Right click and select COMMENT, or click the COMMENT icon.
    3. The Comment box appears. Select EDIT and enter the result comment “Confirmed by Smear Review.” Add the date and time by hitting F5 and type the performer information.
    4. Once the comment has been entered, verify the automated differential results. Ensure that all results (including the SCAN result) have a checkmark and select VERIFY.
12. After a smear review has been performed and it is determined that a manual differential is indicated, access automated DxH results in Cerner by entering the patient accession number in Accession Result Entry.
13. In the SCAN field, open the dropdown box, change the SCAN result to DIFM and select PERFORM.
14. Ensure that a checkmark appears next to the DIFM result, UNCHECK ALL AUTOMATED DIFFERENTIAL RESULTS and select VERIFY. Once verified, the DIFM order will automatically cancel the automated differential in Cerner and place an order for a Manual Differential and RBC Morphology and PLT estimate.
15. Refer to corresponding CellaVision or Manual Differential Procedure for information on entering manual differential results.

**Limitations:**

1. Smear review, manual differential, and pathology review criteria are meant to be used as guidelines to standardize practices. CLS personnel may perform smear reviews, manual differentials, or send slides for pathology review at their discretion if results do not meet stated criteria but are deemed significant in their professional opinion.
2. CLS personnel may use professional judgment in determining the significance of flagged results/messages based on clinical information, prior results, or other mitigating circumstances. All information used in determining action contrary to this procedure will be documented as a comment in Cerner.
3. Results released on suboptimal specimens are accompanied by a comment noting the condition of the sample and if possible, the effects that the specimen’s condition will have on the reported results.

**References:**

1. Collage of American Pathologists, Hematology and Coagulation Checklist. Northfield, IL, Current Addition.
2. Instructions for Use: UniCel DxH 800 Coulter Cellular Analysis System. Beckman coulter, Inc. Brea, CA. December 2012.
3. Suggested Criteria for Action Following automated CBC and WBC Differential Analysis. International Consensus Group for Hematology Review, 2004.