TITLE: COMPLETE BLOOD COUNT (CBC) WITH AUTO DIFFERENTIAL

And RETICULOCYTE COUNT COULTER DxH800

PRINCIPLE / PURPOSE:

The complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells and platelets. The WBC differential, also a fundamental test, identifies and enumerates the percent and absolute number (% & #) of the white bloods cells and nucleated red blood cells present in whole blood. The complete Reticulocyte count, an analytical test evaluates red cell production and maturity. The DxH 800 CBC is based on the Coulter aperture electrical impedance Principle. The WBC differential, NRBC and Reticulocyte analyses use VCS technology. The CBC with WBC differential, NRBC, and Reticulocyte counts are for in vitro diagnostic use in screening patient populations.

Refer to the Coulter Operator’s Manual for detailed methodology information.

Directly measured parameters are: WBC, RBC, PLT, NRBC, HGB, MCV, DIFF %.

Calculated parameters are: HCT, MCH, MCHC, DIFF#.

SCOPE:

This procedure provides the instructions necessary for performing a complete blood count (CBC), white blood cell (WBC) differential, nucleated red blood cell (NRBC) count, and reticulocyte count (Retic) on the UniCel DxH 800 Coulter Cellular Analysis System.

SPECIMEN:

Patient Preparation: None Required

Type: Anti-coagulated human whole blood in K2 or K3 EDTA

Handling Conditions:

All non-refrigerated whole blood samples should be analyzed within 24 hours.

Before analysis, check each sample for clots using wooden applicator sticks.

Refrigerated (2-8° C) may be tested up to 48 hours.

Differential should not be reported on specimens > 48 hours old.

CBC results may be reported from specimens >48 hours old with comment:

“Specimen age exceeds recommended testing interval, interpret results with caution”

Causes for rejection: Clotted, severe hemolysis, QNS

Microtainer must contain minimum of 500 microliters for 2 aspirations.

Venipuncture tube must contain a minimum of 1ml.

EQUIPMENT AND MATERIALS:

Equipment:

Beckman Coulter DxH800 Cellular Analysis System

Materials:

The following are the recommended reagents:

COULTER DxH Diluent PN 628017 (10 L)

COULTER DxH Diluent is a cyanide-free, isotonic buffered saline solution. COULTER DxH Diluent dilutes the specimen, is used for rinsing module components between sample analyses, and provides a sheath stream to transport the sample through the flow cell.

COULTER DxH Cell Lyse PN 628019 (5L)

COULTER DxH Cell Lyse is a cyanide-free CBC lytic reagent that lyses red blood cells for the white blood cell and total nucleated cell counts, and works in conjunction with COULTER DxH Diluent to generate a stable hemoglobin measurement. COULTER DxH Cell Lyse is also used to lyse the red blood cells and discriminates nucleated red blood cells from white blood cells.

COULTER DxH Cleaner PN 628023 (10 L)

DxH Cleaner is a cyanide-free, aldehyde-free cleaning agent that degrades residual materials so that they may be flushed from the system with the diluent.

COULTER DxH Diff Pack PN 628020

The COULTER DxH Diff Pack consists of the Erythrolyse Lytic Reagent and Stabilyse Preservative Reagent. The Erythrolyse Lytic Reagent is a cyanide-free lytic reagent that dilutes the blood sample, and lyses red blood cells in preparation for white blood cell measurement in the flow cell. The StabiLyse Preservative Reagent neutralizes the Diff lytic reagent and preserves the white blood cells for measurement in the flow cell. Together, Erythrolyse and StabiLyse provide a dilution for the five-part differential.

COULTER DxH Retic Pack PN 628021

The DxH Retic Pack consists of a reticulocyte stain reagent and a reticulocyte-clearing reagent.The reticulocyte stain reagent is a cyanide-free reagent that uses a dye to stain reticulocytes. The reticulocyte-clearing reagent is a cyanide-free reagent that stabilizes the dye-reticulum complex to enhance discrimination of reticulocytes from mature red blood cells utilizing the VCSn technology.

For additional information, please refer to the package inserts available at: https://www.beckmancoulter.com

Preparation:

COULTER DxH Diluent is ready to use.

COULTER DxH Cell Lyse is ready to use.

COULTER DxH Cleaner is ready to use.

COULTER DxH Diff Pack is ready for use.

COULTER DxH Retic Packis ready to use.

To avoid cross contamination when replacing the DxHDiff Pack and DxH Retic

Pack, transfer each reagent pick up tube one at a time.

Performance Parameters:

FOR ALL DxH REAGENTS:

Do not use product past expiration date. Use product at temperatures stated in the instrument product manuals and/or online help. Opened containers are stable for 60 days when stored as recommended. Dispose of waste product, unused product, and contaminated packaging in compliance with federal, state, and local regulations

IMPORTANT: If product has been partially or completely frozen, allow product to warm to room temperature. Invert the container 16 times to ensure complete mixing prior to placement on the instrument. Install and prime the diluent as directed in your instrument product manuals and/or online help. Verify background counts are acceptable before analyzing patient samples

Storage Requirements:

COULTER DxH DILUENT: Store at 2-40° C.

COULTER DxH CELL LYSE: Store at 2-40°C.

COULTER DxH CLEANER: Store at 2-25°C.

COULTER DxH DIFF PAK: Store at 2-25° C.

COULTER DxH RETIC PAK: Store at 2-30° C.

CALIBRATION:

Verify Calibration:

• Every 6 months

• When controls begin to show evidence of unusual trends

• When controls exceed the manufacturer’s defined acceptable limits

• If the average ambient room temperature changes more than 10°F or 12° C from the calibrating temperature.

Calibrate:

• At installation

• After the replacement of any component that involves dilution characteristics (such as the BSV) or the

primary measurements (such as the apertures)

• When advised to do so by your Beckman Coulter Representative.

• If you fail verify calibration procedure.

REFER TO SEPARATE “CALIBRATION” PROCEDURE SOP# HEME-501

QUALITY CONTROL:

Coulter 6C Cell Control:

3 levels run every 12 hours that analyzer is used for patient testing.

ReticX Cell Control:

3 levels run every 8 hours that analyzer is used for patient testing.

REFER TO SEPARATE “RUNNING AND EVALUATING QC” PROCEDURE SOP#HEME-101

PROCEDURE:

CBC with Auto Differential:

CASSETTE PRESENTATION MODE:

1. Place instrument online. Refer to Job Aids as needed.
2. Place barcoded tube in cassette. NOTE: Only barcoded tubes will run in cassette mode. Non-barcoded tubes and microtainers must be run in single tube presentation mode.
3. Place in input buffer on right side of instrument
4. Pull cassette forward to front of buffer.
5. Instrument will automatically move cassette into sampling station and process.

SINGLE TUBE PRESENTATION MODE:

1. Press single tube presentation icon top left of screen (hand w/tube)
2. Enter specimen ID either by using handheld barcode reader, barcode reader on instrument or typing in ID and enter.
3. If order is not present in worklist, analyzer assigns default test (CD) and allows you to edit order after pressing OK.
4. Order may be edited by removing default order and adding another choice or default order may be left.
5. Submit
6. After mixing well: Place capped or uncapped full size tube in left side of single tube cassette or uncapped microtainer in right side of cassette.
7. Analyzer will automatically retract the cassette and process specimen.
8. When processing is complete, remove tube and click “Exit” to return to cassette mode.

Reticulocyte Count:

Change default test on System Status screen to CDR and follow above instructions.

Analyzer aspirates 165 microliters no matter what tests are ordered.

CALCULATIONS:

The following parameters are calculated by the analyzer using directly measured parameters:

HCT% = MCV X RBC

10

MCH (pg) = HGB X 10

RBC

MCHC(g/dl) = HGB X 10

HCT

INTERPRETATION & REPORTING RESULTS:

All unflagged results will be auto-released.

Reference Ranges:

REFERENCE LIMIT

NEONATE 0 - 29 DAYS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 9.0 | 30.0 | 9.0 | 30.0 |
| RBC | 4.00 | 6.60 | 4.00 | 6.60 |
| HGB | 14.5 | 21.0 | 14.5 | 21.0 |
| HCT | 45.0 | 67.0 | 45.0 | 67.0 |
| MCV | 95.0 | 121.0 | 95.0 | 121.0 |
| MCH | 31.0 | 37.0 | 31.0 | 37.0 |
| MCHC | 29.0 | 36.0 | 29.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 6.0 | 26.0 | 6.0 | 26.0 |
| LY# | 2.0 | 11.0 | 2.0 | 11.0 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

30 - 60 DAYS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 5.0 | 19.5 | 5.0 | 19.5 |
| RBC | 3.00 | 5.40 | 3.00 | 5.40 |
| HGB | 10.0 | 18.0 | 10.0 | 18.0 |
| HCT | 31.0 | 55.0 | 31.0 | 55.0 |
| MCV | 85.0 | 123.0 | 85.0 | 123.0 |
| MCH | 28.0 | 40.0 | 28.0 | 40.0 |
| MCHC | 29.0 | 36.0 | 29.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.0 | 9.0 | 1.0 | 9.0 |
| LY# | 2.5 | 16.5 | 2.5 | 16.5 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

61 - 90 DAYS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 5.0 | 19.5 | 5.0 | 19.5 |
| RBC | 2.70 | 4.90 | 2.70 | 4.90 |
| HGB | 9.0 | 14.0 | 9.0 | 14.0 |
| HCT | 28.0 | 42.0 | 28.0 | 42.0 |
| MCV | 77.0 | 115.0 | 77.0 | 115.0 |
| MCH | 26.0 | 34.0 | 26.0 | 34.0 |
| MCHC | 29.0 | 36.0 | 29.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.0 | 9.0 | 1.0 | 9.0 |
| LY# | 2.5 | 16.5 | 2.5 | 16.5 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.12 | 0.0 | 0.1 |

REFERENCE LIMIT

3 - 6 MONTHS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 6.0 | 17.5 | 6.0 | 17.5 |
| RBC | 3.10 | 4.50 | 3.10 | 4.50 |
| HGB | 9.5 | 13.5 | 9.5 | 13.5 |
| HCT | 29.0 | 41.0 | 29.0 | 41.0 |
| MCV | 74.0 | 108.0 | 74.0 | 108.0 |
| MCH | 25.0 | 35.0 | 25.0 | 35.0 |
| MCHC | 29.0 | 36.0 | 29.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.0 | 8.5 | 1.0 | 8.5 |
| LY# | 4.0 | 13.5 | 4.0 | 13.5 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

6MO - 2 YEARS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 6.0 | 17.5 | 6.0 | 17.5 |
| RBC | 3.70 | 5.40 | 3.70 | 5.40 |
| HGB | 10.5 | 13.5 | 10.5 | 13.5 |
| HCT | 33.0 | 39.0 | 33.0 | 39.0 |
| MCV | 70.0 | 86.0 | 70.0 | 86.0 |
| MCH | 23.0 | 31.0 | 23.0 | 31.0 |
| MCHC | 29.0 | 36.0 | 29.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.0 | 8.5 | 1.0 | 8.5 |
| LY# | 3.0 | 13.5 | 3.0 | 13.5 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

3 - 5 YEARS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 5.0 | 17.0 | 5.0 | 17.0 |
| RBC | 3.90 | 5.30 | 3.90 | 5.30 |
| HGB | 11.5 | 13.5 | 11.5 | 13.5 |
| HCT | 34.0 | 40.0 | 34.0 | 40.0 |
| MCV | 75.0 | 87.0 | 75.0 | 87.0 |
| MCH | 24.0 | 30.0 | 24.0 | 30.0 |
| MCHC | 32.0 | 36.0 | 32.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.5 | 8.5 | 1.5 | 8.5 |
| LY# | 1.5 | 9.5 | 1.5 | 9.5 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

6 - 12 YEARS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 4.5 | 14.5 | 4.5 | 14.5 |
| RBC | 4.00 | 5.20 | 4.00 | 5.20 |
| HGB | 11.5 | 15.5 | 11.5 | 15.5 |
| HCT | 35.0 | 45.0 | 35.0 | 45.0 |
| MCV | 77.0 | 95.0 | 77.0 | 95.0 |
| MCH | 25.0 | 33.0 | 25.0 | 33.0 |
| MCHC | 32.0 | 36.0 | 32.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.5 | 8.0 | 1.5 | 8.0 |
| LY# | 1.5 | 7.0 | 1.5 | 7.0 |
| MONO# | 0.0 | 1.0 | 0.0 | 1.0 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO# | 0.0 | 0.1 | 0.0 | 0.1 |

REFERENCE LIMIT

12 YEARS - ADULT (150 YEARS)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TEST | MALE LOW | MALE HIGH | FEMALE LOW | FEMALE HIGH |
| WBC | 3.8 | 10.6 | 3.6 | 11.0 |
| RBC | 4.40 | 5.90 | 3.80 | 5.20 |
| HGB | 13.0 | 18.0 | 12.0 | 16.0 |
| HCT | 40.0 | 52.0 | 35.0 | 47.0 |
| MCV | 80.0 | 100.0 | 80.0 | 100.0 |
| MCH | 26.0 | 34.0 | 26.0 | 34.0 |
| MCHC | 32.0 | 36.0 | 32.0 | 36.0 |
| RDW | 11.5 | 14.5 | 11.5 | 14.5 |
| PLT | 150 | 440 | 150 | 440 |
| NE# | 1.4 | 6.5 | 1.4 | 6.5 |
| LY# | 1.0 | 3.6 | 1.0 | 3.6 |
| MONO# | 0.2 | 1.0 | 0.2 | 0.9 |
| EO# | 0.0 | 0.7 | 0.0 | 0.7 |
| BASO | 0.0 | 0.1 | 0.0 | 0.1 |

DXH800 LINEARITY

|  |  |  |
| --- | --- | --- |
| PARAMETER | LOWER LIMIT | UPPER LIMIT |
| WBC | 0.02 | 395.9 |
| RBC | 0.00 | 8.23 |
| HGB | 0.0 | 24.5 |
| PLT | 1 | 4918 |

Procedures for Abnormal Results:

Refer to separate procedure “Unexpected Result Protocols” SOP#HEME 702 for . evaluation of results and actions needed to verify flagged results prior to release

Reporting Format:

Results are finalized per Lab LIS protocol.

LIMITATIONS OF THE PROCEDURE:

CBC, Diff, NRBC and Reticulocyte parameters may be affected by interferences (severe lipemia, rbc agglutiniation, large amount of sickle cells, nrbc’s,megakaryocytes) that will generate instrument messages and flags. Confirmatory testing within the laboratory should be performed to determine the source of unexpected abnormal results.

Retics—There are interferences that may, in some cases, cause an artifactual elevation in the automated retic count. According to Beckman Coulter, there are no documented interferences that cause a falsely low retic%. Erythrocyte inclusions stain with New Methylene Blue if sufficiently numerous within a sample, and some hemoglobinopathies (SS, SC) might affect the accuracy of the reticulocyte enumeration. Other erythrocyte inclusions that do not stain with New Methylene Blue (malaria, babesia) may also affect the accuracy of the reticulocyte enumeration by changing the conductivity and /or light scatter properties of the RBCs and falsely elevate the reticulocyte result. Heinz bodies are not visible with Wright’s Stain and our laboratory staff does not routinely review smears for parasites; therefore, all of these samples are sent for pathologist review and further comments.

REPORTING RESULTS:

Refer to the following chart for reporting or verification of results:

|  |  |  |  |
| --- | --- | --- | --- |
| DxH 800 Retic Result | ACTION | NOTES | REPORT |
| + | Repeat | Result above instrument reportable range | Report >27.4%  (>) |
| >27.4% | Repeat | Result above ARMC reportable range | Report >27.4%  (>) |
| < 0.14 % | Repeat | Result below ARMC reportable range | Report <0.14%  (<) |
| 0.14% -1.5%  Flagged abnormal retic pattern or verify reitc or not flagged | Repeat | See note below if RBC is not reportable | Report |
| 3.0% - 27.41%  Flagged abnormal retic patern or verify retic or not flagged | Repeat | See note below if RBC is not reportable | Report |
| >1.5% but <3.0%  (not flagged) |  |  | Report |
| >1.5% but < 3.0%  Flagged—abnormal retic pattern or verify retic | Scan the smear for HJ bodies, baso stippling, sickle cells, or pappenheimer bodies | This flagged result requires a smear to loof for interference | If not interference seen, report. Leave for path review.  Interference seen—add appropriate comment below and send for path review  Morphology comments:  HJB SICK PAB  STIP |
| If the RBC is unreportable as when there are severe cold agglutinins or other interferences, look to see if the retic result flags abnormal retic pattern or verify retic—if it does flag, then follow this chart—> | No accurate retic count (% or #) can be obtained with this specimen. | The RBC cannot be used to calculate the Retic#. Also the Retic% may be incorrect. | Cancel the retic, credit, and comment “Unable to report valid retic count due to \_\_\_\_\_\_\_.”  Notify the physician/nurse as appropriate. |
| If the RBC is unreportable as when there are severe cold agglutinins or other interferences AND there is NO abnormal retic pattern or verify retic flag, follow this chart —> | No accurate Retic # can be calculated. | The RBC cannot be used to calculate the Retic #. | Report the Retic% only.  Enter “Unable to report valid retic # due to\_\_\_\_\_.” |

REFERENCES:

Unicel DxH 800 Coulter Cellular Analysis System

Instruction For Use

PN 629743 AG (November 2010)

|  |  |  |  |
| --- | --- | --- | --- |
| Review Date | Signature | Mgmt. | Director |
| 6/11/15 | Quanisha Dyson | x |  |
| 11/3/16 | Quanisha Dyson | x |  |
| 3/21/17 | Quanisha Dyson | x |  |
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SOP HISTORY PAGE

SOP Number: HEME – 701

SOP Title: COMPLETE BLOOD COUNT WITH AUTO DIFFERENTIAL AND RETICULOCYTE COUNT COULTER DXH800

Written By: Kate Pruitt, MLS

Manual in which Hard Copy of this SOP is located: Hematology Procedure Manual

Distribution: Cancer Center Hematology Manual

Supersedes Procedure:

SOP CHANGE CONTROL

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Approvals | |  | Action | In |
| Mgmt. | Date | Director | Date |  | Effect |
|  |  |  | 12/5/14 | Added check samples for clot before analysis |  |
|  |  |  |  |  |  |
|  |  |  | 11/3/16 | Updated neonatal hemoglobin normal range upper limit to reflect new critical values | 5/1/16 |
|  |  |  |  |  |  |
| Q.Dyson | 3/21/17 |  |  | Updated information on Retic interference | 3/21/17 |
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| Date archived: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | |  |
| Reason: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Initials:\_\_\_\_\_\_\_\_\_\_ | | | | |  |
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