University of California, Davis Health System
Department of Pathology and Laboratory Medicine
Hematology

DxH 600/800 Result Interpretation

and Rechecks Technical Procedure #1522.t

PURPOSE

This procedure establishes a standard format for evaluating and reporting blood count, differential, reticulocytes, and nucleated red cell results generated from the Beckman Coulter UniCell DxH 600/800 analyzers. Results will be reported by automated methods except when results exceed established flagging criteria, defined in this procedure. Exceptions may be made on a case by case basis. Smears are reviewed and correlated with automated results.

The analyzer flags abnormal specimens using a combination of instrument and user-defined flags. This flagging identifies abnormalities in the blood count, differential, reticulocyte, and nucleated red cell count. The presence of flags in any of these parameters mandates further investigation of abnormalities, which may include additional rechecks or examination of stained smears. User defined rules are incorporated in the Remisol middleware.

This procedure is to be used in conjunction with the following SOP's

SOP	Title
1521.t	DxH 600 800 and Workcell Operation
1524.t	DxH 600 800 Calibration
1525.t	DxH 600 800 Automated Body Fluids
1503.t	Beckman Coulter Remisol Middleware
1526.t	Peripheral Stem Cell and Bone Marrow Harvest

PROCEDURE

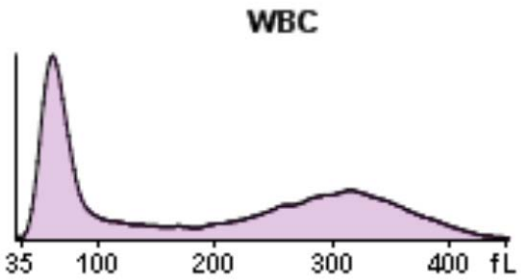
☐ Assay Specimens on UniCell DxH 600/800 analyzer per SOP *DxH 600 800 and Workcell Operation 1521.t.*

- Rerun specimens with suspected aspiration errors, which may be detected by low or high MCHC, delta checks, etc.
- Perform rechecks and interference correction procedures as needed. See *CBC Correction* below.

☐ Review Histogram and Dataplot. The reviewing CLS should be familiar with normal and abnormal histogram and dataplot patterns.

- WBC histograms are used to evaluate WBC interference. Particles present above the 35 fL threshold are usually WBC's and the first peak usually consists of lymphocytes. When non WBC particles (NRBC's, platelet

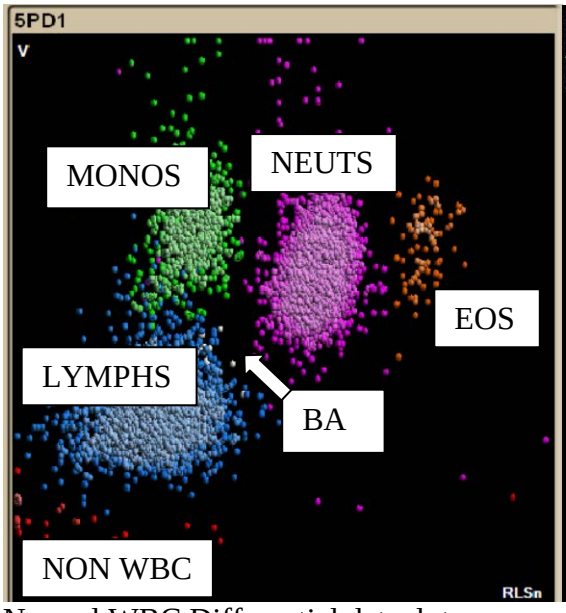
clumps, or cryoproteins) are present they frequently occur near the threshold, interfering with the typical pattern.



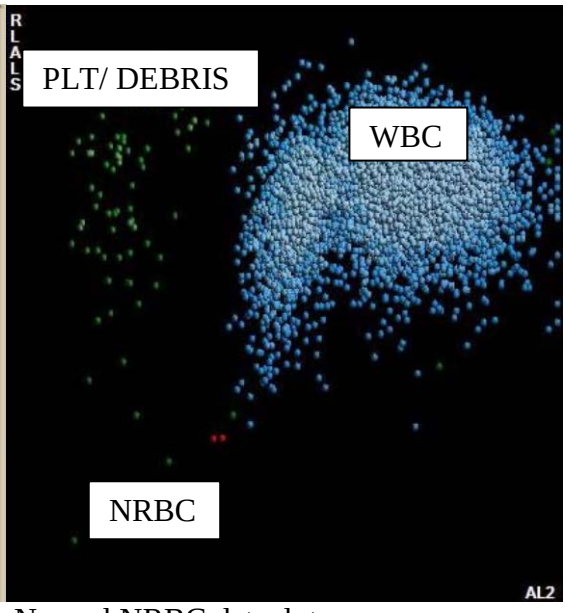
Normal WBC Histogram
The x axis is size in fL
The y axis is relative number of cells.

The first peak is lymphocytes,
The middle area is monocytes.
The end area is granulocytes.

- WBC dataplots show cell populations that are well separated and distinct, with little overlap between them. Particles scattering across the bottom of the dataplot represent non WBC (NRBC's. platelet clumps, unlysed RBC's.)



Normal WBC Differential dataplot



Normal NRBC dataplot

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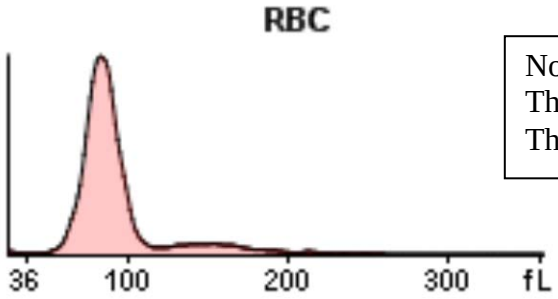
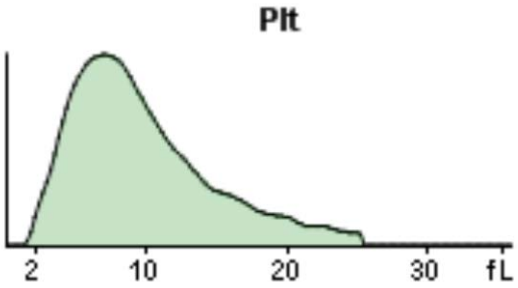
- RBC Histograms are Gaussian curves and reach the baseline on both sides. The peak is the Mode of the RBCs counted. Common interferences are from transfused RBC's and schistocytes.



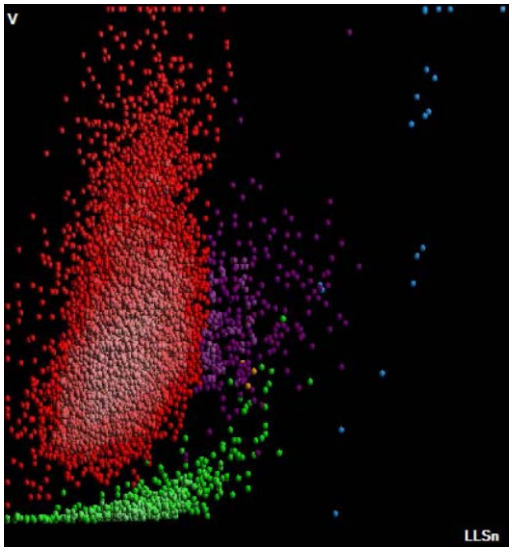
Normal RBC Histogram
The x axis is size in fL
The y axis is relative number of cells

- Platelet Histograms are two line curves between 0 and 25 fL. Schistocytes may falsely elevate the platelet count. Marked MCV of <50 fL may falsely increase the platelet count.



Normal Plt Histogram
The platelet histogram measure from 0 to 25 fL

- Reticulocyte Dataplots show a RBC population with a comma-shaped scattering of reticulocytes spreading up and to the right of the RBC's. RBC inclusions such as Howell-Jolly bodies and pappenheimers bodies may be counted as retics.



Normal Retic Dataplot
Red=Mature RBC's
Purple=Retics
Green=Plt/Debris

☐ CBC Results.

- Review Remisol middleware results screen for delta checks, comments, flags, codes, suspect messages and abnormal histograms or dataplots.
  - Platelets of 1 or 2 x10^3/ul will be reported as *'less than 3x10^3/ul'*
  - All WBC's less than 1.0 x10^3/ul must have a manual differential performed.
  - Neonates less than 8 days old must have a manual differential performed.

☐ Flags are single letter or symbols that appear to the right of the result.

  - E manual edit
  - R review
  - CL critical low
  - CH critical high

- aL low action limit
- aH high action limit
- P partial aspiration
- N non-blood samples

☐ Codes appear in place of results.

- = = = = = Analysis disabled
- xxxxx Parameter not enabled
- : : : : : Flow cell clog was detected
- - - - - Total vote out
- . . . . . Incomplete computation
- +++++ Result exceeds the operating range
- ? ? ? ? ? Result outside range of values that can be formatted for display
- ##### Results rejected

☐ Messages are displayed in the *Susp/Sys/Def Msgs* box below patient demographics.

WBC	RETIC
Abn WBC pattern	RET Inter: Debris
Cellular Inter	RET Inter: PLT
System Event: WBC	RET-RBC Overlap
WBC Carryover	System Event: R
RBC	PLT
Abn RBC Pattern	Platelet Clumps
System Event: RBC	PLT Carryover
HGB	PLT Inter: Debris
HGB Inter: WBC	RBC-PLT Overlap
HGB Blank Shift	System Event: PLT
System Event: HGB	NRBC
MCV	Abn NRBC Pattern
MCV Inter: PLT	AL2 Blank Volt: N
MCV Inter: WBC	Data Disc: N
DIFF	High Event Rate: N
Abn Diff Pattern	Low AL2 Events: N

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Aged Sample	Low DC Events: N
Data Disc: D	Low Event Rate: N
Excessive Debris: D	Low Event: N
High Event Rate: D	NRBC Inter
High OP Events: D	NRBC-LY Overlap
High RF Events: D	System Event: N
Low Event Rate: D	BF
Low Events: D	Abn TNC Pattern
Low OP Events: D	System Event: TNC
MO-NE Overlap	TNC Carryover
NE-EO Overlap	P
System Event: D	Bubbles
Undefined Pop: D	Carryover
RETIC	No Aspiration
Abn Retic Pattern	Non-blood Specimen
AL2 Blank Volt: R	Partial Aspiration
Data Disc: R	::::: Code
High Event: R	Flow Cell Clog: D
Low Event: R	Flow Cell Clog: N
Nucleated Cells	Flow Cell Clog: R

☐ Review and Report Results

- Results will be filed in Remisol middleware as detailed in SOP '*Beckman Coulter Remisol Middleware 1503.t'*
- Evaluate the printouts
- Highlight and thumbs up appropriate results to be verified in LIS

☐ Critical values are called to Nurse or Physician as detailed in SOP '*Critical Values 110.a'*

- Critical values
  - WBC >150 x10^3/ul
  - Hgb <5g/dL adult; Hgb <8g/dL neonate (less than 8 days old)
  - Hct <15% adults; Hct< 20% neonate (less than 8 days old)

- Plt <30 adult; Plt <20 neonate (less than 8 days old)

☐ Investigate Delta Checks

- Delta checks are set in the Remisol:
  - MCV ± 5fL
  - Hgb ±3g/dL within 48 hours
  - Plt -100 $x10^3$/ul
- Check specimen labels
- Compare previous results such as RBC indices, transfusion history, diagnosis, chemotherapy, chemistry results, etc. to determine if the change is clinically explainable.
- Communicate with phlebotomist, RN, or clinician regarding the method of blood draw and integrity of samples. Cancel only if instructed by clinician.

☐ All neonates less than 8 days old and all D5NU and D5 Neonatal patients need manual differentials.

☐ Review stained peripheral blood smear.

- Evaluate quality of stained peripheral blood smear.
  - Look at overall appearance of wedge smear. Look for acceptable RBC morphology area with ~200 RBCs per 100 x fields with RBCs not overlapping. Look for stain quality. Look for WBC integrity. Look for RBCs with '*moth eaten*' appearance that indicates water in methanol bath. Remake smear if necessary.
- Evaluate histogram and dataplot abnormalities.
  - For Cellular Interferences with WBC count perform a WBC estimate and compare to automated corrected WBC count. If the estimate does not agree with the corrected count, remake slide. A manual correction or manual WBC count may rarely be needed.
    - WBC estimate = # WBC's in 10 (50x) fields x 300
    - WBC correction for NRBC = WBC count/ (100 + # NRBC's)

- Perform platelet estimate. Compare estimate to automated count. Report as adequate, increased, decreased, marked increased, or marked decreased.
    - Report clumps if present as *platelet clumps present, numbers appear adequate, increased, or decreased.*
    - If the Platelet estimate does not agree with the automated count, remake slide or perform manual platelet count as detailed in *SOP Manual WBC and PLT counts (Thrombo-tic Method) 1125.t*
        - Plt estimate = # Plt in 5 (100x) fields x RBC count
- Evaluate RBC morphology. Report any clinically significant RBC morphology by type (i.e. sickle cells, schistocytes) and quantity (slight, moderate, marked).
- Report any clinically significant finding such as Plasmodium sp., blood parasites, yeast, or bacteria. Report any morphological abnormalities or inclusions seen.

☐ Perform WBC differential

☐ Correlate with automated differential. If automated differential is acceptable then evaluate for left shift. Report the automated differential with comment '*Left shift present*' or '*Left shift not present*'.

- Perform manual WBC differentials for:
    - CBCM, neonates less than 8 days old, WBC's less than 1.0 $x10^3$/ul, or if indicated by slide review.
    - Manual differentials include 100 WBC's. Cellavision differentials include 105 WBC's. Differentials for WBC's less than 1.0 $x10^3$/ul may include less than 100 WBC's.

☐ Submit for Pathology Review if applicable. Refer to SOP *Pathology Review of Abnormal Hematology Results 1077.t*

- If initial Path Review finding, submit by placing slide in path review box and reporting slot number in results *folder slot*.
- Submit all first time Immature Mononuclear Cells IMC results and call as a critical value.

- If repeat Path Review finding, do not submit slide. Report 'P' in the specimen *folder slot* results.

CBC Correction Methods:

☐ High WBC greater than 300 x10^3/ul

- When the WBC count exceeds linearity of 300 x10^3/ul the HGB is falsely increased due to increased turbidity and the RBC count and the MCV are falsely increased due to WBCs counted along with the RBCs. The HCT, MCH, and MCHC are incorrect because they are calculated from directly measured parameters that are incorrect. The platelet count may or may not be affected depending on whether or not there are significant numbers of WBC cytoplasmic fragments present.
- For WBC greater than 140 x 10^3/ul, no action is needed.
  - RBC is automatically corrected when WBC >140 x 10^3/ul
  - HGB is automatically corrected when UWBC >11 x 10^3/ul; unless System message '*HGB Inter:WBC'* is present.
  - MCV is automatically corrected when WBC >140 x 10^3/ul
  - RDW and RDW-SD are automatically corrected when WBC >140 x 10^3/ul
- WBC correction for WBC's above linearity of 300 x10^3/ul
  - Prepare a minimal dilution to bring the WBCs below linearity (300 x10^3/ul).
  - Cycle the diluted sample within 10 minutes of preparation.
  - Multiply the result by the dilution factor. This is the reported WBC count.
- Pump and Dump: Qualitative Saline Replacement for HGB, HCT, MCV, MCH, MCHC & RDW correction.
  - High WBC counts interfere with RBC, HGB, HCT, MCV, MCH, MCHC & RDW. The parameters have been corrected using the

incorrect WBC. Use the diluted WBC value (WBC x dilution factor) to correct the RBC

- Original RBC + Original WBC= uncorrected RBC
- Uncorrected RBC – Corrected WBC= Corrected RBC
  - Use this RBC count for all Pump & Dump calculations

- Pipette an aliquot of sample and spin in Stat Centrifuge.
- Using a Pasteur pipette, go beneath the buffy coat and remove red cells that are not mixed with WBCs.
- Place several drops of packed RBCs into a clean tube.
- Add saline; mix well.
- Cycle the sample within 10 minutes of dilution.
- The Pump and Dump WBC count should be less than 100 $x10^3$/ul to eliminate interference from the high WBC count.
- The MCV, MCH, and RDW results from this sample are correct as the high WBC interference has been removed.
- Use the following formula to calculate the correct HGB result:

$$\text{HGB g/dl} = \frac{\text{MCH (p\&d)} \times \text{RBC (corrected)}}{10}$$

- Use the following formula to calculate the correct HCT result:

$$\text{HCT \%} = \frac{\text{MCV (p\&d)} \times \text{RBC (corrected)}}{10}$$

- Calculate the MCHC with the correct HGB and HCT results and correlate to the P&D MCHC. Results should match.
  - MCHC %= (Hgb/ Hct) x 100
- If unable to perform this method due to small sample size or other technical problems, report the parameters that have been rechecked, omit the rest and place an explanation in the specimen comments section of the results.

☐ MCHC high or low

- Lipemia and icterus cause a falsely increased HGB and MCHC. Cold agglutinins cause a falsely decreased RBC and a falsely increased MCV resulting in a falsely increased MCHC. Spherocytes cause a truly increased MCHC. Hemolysis causes a high MCHC due to a decreased RBC count. Lyse-resistant RBCs (RBCs containing HGB F and HGB S) cause a falsely increased HGB and MCHC. Hyperglycemia causes a falsely increased MCV and HCT and a falsely decreased MCHC.

- Pump and Dump Qualitative saline replacement for **Lipemia/Icterus**

  - The HGB will be falsely high from the interference with lipemia or icterus with the methodology for HGB. The RBC, HCT and MCV are correct on the original results.
  - Pipette an aliquot of sample and spin in Stat Centrifuge. Lipemia or icterus should be evident. Remove plasma.
  - Add approximately the same amount of saline; mix well. Run on analyzer within 10 minutes of dilution.
  - The MCH results from the Pump and Dump sample are correct as the interference from lipemia or icterus has been removed.
  - **The MCV <u>must not</u> change more than <u>+</u> 2 fL. If it does a cold agglutinin may be present.**
  - Use the following formula to calculate the correct HGB result:

    $$\text{HGB g/dl} = \frac{\text{MCH (P\&D)} \times \text{RBC (original)}}{10}$$

  - Recalculate the MCHC using the corrected HGB.
    - MCHC% = (Hgb/ Hct) x 100

- Quantitative Saline Replacement Method for correction of Lipemia/Icterus

  - To an aliquot of sample add 2 ml of saline. Centrifuge the mixture in the Stat Centrifuge. Lipemia or icterus should be evident.
  - Quantitatively remove 2 ml of supernatant.
  - Mix specimen thoroughly and run within 10 minutes.

- **The MCV must not change more than ± 2fL. If it does a cold agglutinin may be present**.
- Verify the accuracy of the replacement by comparing the original and replacement RBC values which should agree ± 0.09 x 10^6/uL
- Report RBC, MCV, HGB, HCT, indices, and RDW obtained by this method. The WBC and PLT counts are not valid from this aliquot and are reported from the original run.

☐ Cold Agglutinins

- Cold agglutinins should be suspected whenever there is a high MCHC accompanied by a relatively low RBC count and relatively high MCV. As RBCs agglutinate, the RBC count is falsely decreased and the MCV is falsely increased. Since the HCT is computed by multiplying the RBC times the MCV, the HCT is falsely decreased and the MCH and MCHC are falsely increased. The HGB, WBC, and PLT are correct.

- Warming Method to Correct for Cold Agglutinins

  - Warm the sample to 37^0 C for 15 minutes.
  - Mix well and rerun immediately, before the tube has a chance to cool. Make a new slide with the warmed blood, if needed.
  - Correction is confirmed if the RBC count goes up, the MCV goes down and the MCHC is normalized. The HGB will be the same ±0.3 g/dL. **If this does NOT occur, the high MCHC is NOT due to a cold agglutinin and must be further investigated.**
  - Some specimens may require additional incubation time, warm saline replacement or maintenance of the collection tube at 37^0 degrees to correct for cold agglutinins.
  - Report the results from the warmed specimen with the canned text HCOLD in the LIS.

- Warm Saline Replacement Method to Correct for Cold Agglutinins

  - Warm the sample and 4 mL saline, separately, at 37^0 C for 15 minutes.
  - Take a warm aliquot of sample and add 2 ml warm saline. Mix, then spin in Stat Centrifuge.

- Quantitatively remove 2 ml of supernatant. Warm for another 5 minutes. Mix and run on analyzer rapidly.
- Verify the accuracy of the replacement by comparing the hemoglobin values which should agree ± 0.3 g/dL

☐ Low MCV less than 50 fL

- When MCV is less than 50 fl, cells less than 36 fl in size will not be included in any measurements making the RBC count inaccurate.
- Report all results with canned text HLMCV *"Reported RBC, MCV, HCT, MCH, MCHC, and PLT may be incorrect due to low MCV. Suggest monitoring HGB level, as low MCV does not interfere with its measurement."*

☐ Exceeds Linearity - Dilute and Rerun

- WBC >300
- RBC >8.00
- HGB >23.5
- PLT >4600

- Dilute specimen with diluent and rerun within 10 minutes.
- Multiply by the dilution factor.
- Evaluate the accuracy of the dilution
  - For WBC, HGB or PLT dilutions the diluted RBC count (corrected for the dilution factor) must match the original RBC count ± 0.09
  - For RBC dilutions the diluted HGB (corrected for the dilution factor) must match the original HGB ± 0.3
    - ◊ Results calculated with the original RBC (above linearity) must be recalculated. Use MCV from the dilution and calculate the Hct. Report corrected RBC,

original Hgb, calculated Hct, dilution MCV, calculated MCH, and MCHC.

- Do not report differentials from diluted specimens as they may give erroneous results.

☐ Spherocytosis

- Spherocytes have a lower surface to volume ratio than normal cells and thus have a true high MCHC. Spherocytes are detected by looking at a stained blood smear. When spherocytes are the cause of the high MCHC, report the high MCHC with the comment "*unable to correct due to spherocytes*".

☐ Hemolysis

- Hemolysis should be suspected when the MCHC is increased and the plasma has a pink to red color when the specimen is centrifuged.
- In-vitro hemolysis most commonly occurs during traumatic venipuncture but may also be caused by exposure of specimen to extremes in temperature (cold or heat) or other mechanical trauma (excessive vigorous mixing). If in-vitro hemolysis is present, the specimen should be rejected and cancelled according to laboratory policy.
- In-vivo hemolysis is rare but should be suspected when hemolysis is present in multiple venipuncture specimens and when other clinical findings support this hypothesis. In-vivo hemolysis is seen with patients on ECMO therapy. If in-vivo hemolysis is present, all results except the MCH and MCHC (which do not reflect the true RBC indices) are correct and may be reported.

☐ Lyse-Resistant Hemoglobins

- Lyse-resistant hemoglobins should be suspected when there is a high MCHC and no lipemia, icterus, cold agglutinins, spherocytosis or hemolysis detected. Hemoglobins F (neonates), S (sickle cells) and C (associated with marked target cells), are resistant to lysis in the short time that the diluted blood is present in the hemoglobin cuvette.
    - Correction Method
        - Dilute an aliquot of blood with an equal volume of water

- Wait for 10 minutes and rerun, and multiplying the HGB result by 2 and recalculating the indices. HGB, MCH and MCHC should decrease.

- The MCV must match ± 2 fL

☐ Cryoproteins are abnormal plasma proteins that precipitate at room temperature, causing falsely increased WBC and/or PLT values without affecting the red cell parameters. They may be suspected on specimens with a Cellular Interference Flag, or a ski slope WBC histogram. The WBC and/or PLT estimate may appear significantly lower than the automated count. The precipitate may be seen in the background of stained slides examined without immersion oil.

- This interference is sometimes reversible upon warming the specimen to 37^0C. If warming does not fully dissolve the cryoproteins, WBC results may be obtained by manual methods.

☐ Hi Glucose/Hypochromia is triggered when MCHC is <30g/dl and MCV >75 fl. This may be due to high glucose (greater than 600 mg/dl) which caused spurious elevation in MCV due to osmotic swelling

- Rule out specimen contamination by reviewing previous results, chemistry results from the same draw (sodium, chloride, glucose), or inquiring about the method of venipuncture. If contamination is established, the specimen must be rejected.

  - Correction Method

    - To an aliquot of blood, add a large volume of saline and let sit for 10 minutes.

    - Spin down and remove the supernatant mixture.

    - Add saline to aliquot and cycle the sample immediately. The MCV should go down and the MCHC should go up. If this does not occur, the low MCHC is not due to hyperglycemia.

    - The MCV, MCH, MCHC and RDW results from this sample are correct.

- The original HGB result is correct.
- Use the following formula to calculate the correct HCT result.

$$HCT = \frac{MCV \times RBC\ (original)}{10}$$

☐ Correcting WBC for NRBCs

- Only correct the WBC for the rare sample where the WBC estimate is markedly lower than the corrected WBC count due to a marked number of large NRBC's (greater than 35 fl) present.
  - $$WBC\ (corr) = \frac{WBC \times 100}{100 + NRBC}$$

☐ What is affected?

	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Cold Agglutinin		decreased		decreased	increased		increased	
Lipemia			increased			increased	increased	
Icterus			increased			increased	increased	
Cryoproteins	increased							
Platelet clumps	increased							decreased
High WBC		increased	increased	increased	increased			
Hemolysis in Vivo			increased			increased	increased	
Hemolysis in Vitro		decreased		decreased		increased	increased	
High Glucose				increased	increased			
Spherocytes							increased	
Lyse resistant HGB			increased				increased	
Schistocytes								increased

REFERENCES

Help Menu UniCel DxH Series with System Manager Software B26647AC July 2015

UniCel DxH Series 600/800 Training Guide Ver 1.1 October 2015

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PROCEDURE HISTORY

Date	Written/ Revised by	Revision	Approved Date	Approved By
8/2016	L Gandy	New	9/28/2016	L Howell MD
11/2016	L Gandy	Added high WBC		
2/2018	L Gandy	Clarified high WBC corr		
3/2018	L Gandy	Added D5NU manual diff		