PROCEDURE
Corewell Health East - XN CBC Corrections

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Grosse Pointe Hospital, Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital, Corewell Health Farmington Hills Hospital, Corewell Health Taylor Hospital, Corewell Health Trenton Hospital, Corewell Health Wayne Hospital, Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Hematology

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1. Principle

A. This procedure explains how to correct for various factors (e.g., high WBC counts, cold agglutinins, lipemia, icteria, cryoglobulins, nucleated RBCs, giant platelets, microcytic RBCs, RBC fragments, hemolysis, lyse resistant RBCs, and marked changes in plasma constituents) that may cause spurious values on automated Hematology cell counters.

B. Unknown interferences may also adversely affect results obtained from the instruments. It may be necessary to use a combination of correction procedures to obtain valid results.

2. Responsibility

Personnel who have completed the competency requirements will perform this testing.

3. Definitions

A. Laboratory Information System (LIS)
B. White Blood Cell (WBC)
C. Red Blood Cell (RBC)
D. Hemoglobin (HGB)
E. Hematocrit (HCT)
F. Mean Corpuscular Hemoglobin Concentration (MCHC)
G. Platelet (PLT)
H. Mean Cell Volume (MCV)
I. Mean Corpuscular Hemoglobin (MCH)
J. Nucleated Red blood Cell (NRBC)

4. Reagent/Equipment Needed

A. Heat block: 37°C.
B. XN Series Analyzers
C. HemataStat II microhematocrit centrifuge (if available).
D. CellPack DCL
   1. Store at 2º-35ºC, away from direct sunlight.
   2. If frozen, thaw and mix thoroughly before using.

3. CellPack DCL is clear and colorless.
4. If showing signs of contamination or instability such as cloudiness or discoloration, replace.
5. Unopened, it is stable until the expiration date printed on the container.
6. Once opened, it is stable for 60 Days.

5. Quality Control

A. Since most corrections are performed via visual, temperature or mathematical means, no control materials are available. The manual microhematocrit is the only procedure where a control is run (see site specific Corewell Health East - Microhematocrit - Royal Oak).

6. Procedure

A. **HIGH WBC COUNTS - 440.0 10*9/L or higher:**

1. WBCs counts above instrument linearity can cause a false decrease in the WBC reported. To correct a high WBC count:
   a. Middleware holds the result and an "@" will appear beside the parameter on the analyzer.
   b. Perform a dilution of the well-mixed specimen (1:2, 1:3, 1:5, etc.).
      1) Run in manual mode on the instrument within 30 minutes of setup. As a QC check, verify that the RBC count agrees within +/- 0.20 of undiluted sample. If not, rerun sample. If it is still not acceptable, remake dilution. Notify supervisor if unable to resolve.
      2) NOTE: **Do NOT use the Pre-dilute mode on the XN.**
   c. If diluted rerun was added in the middleware, the middleware will automatically calculate the results. (Refer to the Hematology Corewell Health East - Caresphere Resulting procedure).
   d. Accept the WBC count if the dilution is within linearity and the RBC count agrees +/- 0.20 of original RBC count.
   e. In the middleware, add internal comment *"Verified by dilution".*
      1) **NOTE:** HGB is not affected by high WBC counts as these are measured in different chambers on Sysmex analyzers.
      2) **NOTE**: Perform manual differential.

B. **HIGH RBC, HGB, PLT/PLT-F, or Hematocrit**

1. Linearity Values
   a. RBC: 8.60 10*12/L or higher
   b. HGB: 26.0g/dL or higher
   c. HCT: 75.0% or higher
   d. PLT/PLT-F: 5000 10*9/L or higher
2. Parameters that exceed the manufacturer's limits are flagged with an "@" beside the result on the analyzer.
3. The sample must be diluted and rerun in manual mode as described in the WBC section of this procedure.
   a. NOTE: Do NOT use the Pre-dilute mode on the XN.
4. In the middleware, add internal comment *"Verified by dilution."*

C. **COLD AGGLUTININS:**

1. Cold agglutinins cause the spontaneous agglutination of RBCs at temperatures lower than 37°C. The degree of agglutination is dependent on the cold agglutinin titer.
2. Because the Heat Reaction Chamber on the Sysmex XN analyzers is warmed to 37°C, only strong cold agglutinins will be apparent. The strong cold agglutinins will cause spurious low RBC counts due to counting micro-agglutinates as single cells. Also, the MCV will be falsely elevated due to micro-agglutinates being sized as a single large cell. The hemoglobin reading is usually correct. Also, with a correct hemoglobin value and low hematocrit, the MCHC and the MCH will be spuriously elevated.
3. Check for cold agglutination on all bloods with:
   a. MCHC greater than 37.5 g/dL

b. Smear and hematology analyzer MCV do not agree
   1) NOTE: MCV does not necessarily have to be macrocytic to be suspicious of a cold agglutinin.
c. Smears with RBC agglutinates
d. Whole blood with a "lacy" appearance in the tube or on the slide.
e. NOTE: If b, c, or d is present, follow cold agglutinin procedure, regardless of MCHC value.

4. To correct the effect of a cold agglutinin:
   a. Place the blood in a 37°C incubator (e.g., heating block or water bath) for **at least 15 minutes.**
   b. Mix specimen thoroughly and rerun the blood through the manual mode of the hematology analyzer as quickly as possible so the specimen does not cool down.
   c. Report the 37°C results (for all parameters) if the MCHC corrects to <37.5 g/dL.
   d. In the middleware, add *"Possible Cold Agglutinin"* comment next to MCHC result, then SAVE.
   e. For a cold agglutinin specimen that has instrument flags/lab values requiring microscopic review: If RBC agglutinates are present, make warmed smears to see if the agglutinates go away upon warming. Regardless of which smears (room temp or warmed) exhibit agglutinates, report "RBC agglutinates present". If the case needs further review by a pathologist, submit both sets of clearly marked slides to the pathologist.
   f. **NOTE:** Cold agglutinins must **always** be incubated at 37°C before being reported as such.
5. If the MCHC does not correct upon warming, some parameters may be reported:
   a. Determine if there is **optical interference with the HGB** by spinning an aliquot of the sample. Evaluate for interference due to lipemia, icteria, or hemolysis. If plasma is free from optical interference proceed as follows:
      1) Sites able to perform a spun HCT: The HGB may be reported from the warmed sample provided there is no optical interference. Perform a spun hematocrit and the recalculated MCHC.
         a) Ensure the HGB and HCT values are consistent with each other and review patient history. The warmed HGB, spun HCT, and recalculated MCHC may then be reported.
         b) Verify the WBC, PLT, and differential on smear for the first occurrence and on future occurrences if any delta checks or flags pertaining to these parameters are present. WBC, PLT, and differential may be reported (review smear and perform differential as instrument flags require).
         c) Remove the RBC, MCV and MCH by entering "#NM" in the middleware.
         d) Refer to the calculations and interpretations section for the calculation formulas. The MCV and MCH cannot be reported, as these calculations require the RBC, which cannot be obtained if the effect from the cold agglutinin has not been resolved.
      2) Sites unable to perform a spun HCT: Report the warmed HGB. WBC, PLT, and differential may be reported.
         a) Verify the WBC, PLT, and differential on smear for the first occurrence and on future occurrences if any delta checks or flags pertaining to these parameters are present.
         b) For all parameters that cannot be reported (e.g., RBC, MCH, MCHC, HCT, MCV), remove the result from the middleware by entering "#NM" in the result field.
6. **NOTE: Do not release reticulocyte results if a cold agglutinin does not resolve with warming.** When the RBC result is unable to be obtained due to a strong cold agglutinin, "#NM" the retic parameters if a reticulocyte is ordered.
7. NOTE: See the following section and **Attachment A- MCHC >37.5 Workflow** for samples with optical interference.

D. **LIPEMIA**

1. Lipemic plasma can falsely elevate hemoglobin due to a cloudy SLS-hemoglobin solution which decreases the amount of transmitted light through the solution to the photocell. (Lipemia usually occurs in patients with hyperchylomicronemia whose triglycerides are greater than 1,000 mg/dL.)
2. Lipemia should be suspected on all samples with: (a) a lacy appearance of blood smear or (b) an MCHC over 37.5 g/dL. Action must be taken on MCHC's greater than 37.5 g/dL.
   a. NOTE: See **Attachment A – MCHC > 37.5 Workflow** (if applicable).
3. To check for lipemia, spin down a portion of the blood for approximately 5 minutes at 2000 rpm (or let settle for approximately 10-15 minutes) and visually check plasma layer for characteristic milky appearance.
4. To correct for lipemia perform either of the following procedures:
   a. Plasma Replacement:
      1) Spin down a **PORTION** of a well-mixed blood specimen at 2000 rpm for 5 minutes.
      2) Mark the top (meniscus) of the plasma level.
      3) Carefully remove most**, but not all**, of the plasma.
      4) Replace the plasma with the same amount of Cell Pack DCL diluent (add diluent up to the mark) and mix.
      5) If the aliquot still appears lipemic after plasma replacement, repeat the plasma replacement steps on the aliquot until it no longer appears lipemic. This may need to be performed multiple times to correct for interference.
      6) Place XN in manual mode.
      7) Enter the sample# manually or use a barcoded label.
      8) Check **Cap Open**.
      9) Mix sample then **REMOVE** the cap and run the sample in manual mode.
      10) Use the RBC result as a guide to verify proper re-dilution of the specimen.
         a) If the RBC result is within +/- 0.20 of the original RBC, report the HGB from the re-diluted sample.
      11) Recalculate the MCH and MCHC using the new HGB and the original RBC and HCT.
      12) In the middleware, add *"Corrected for Lipemia"* comment next to the MCHC result field, then SAVE.
         a) NOTE: To avoid any math errors, have a second technologist verify calculations.
   b. Plasma Blank:
      1) Spin down a **PORTION** of a well-mixed blood specimen for 5 minutes at 2000 rpm.
      2) Perform a hemoglobin on the **PLASMA** using the hematology analyzer manual mode.
      3) Place XN in manual mode.
      4) Barcode sample into the XN or manually enter order ID.
      5) Check **Cap Open.**
      6) **REMOVE** the cap and run in manual mode.
      7) Use the following formula to calculate the corrected HGB:

Corrected HGB = Original HGB - [(1-{Original HCT/100}) x Plasma HGB]

a) Example: Results from Original Run:

WBC	5.1
RBC	4.03
HGB	16.1
HCT	39.3

MCV	97.5
MCH	40.0
MCHC	41.3
PLT	250

Calculation for Corrected Hemoglobin (HGB)

Plasma HGB	4.5
Corrected HGB	= 16.1 - [(1-[39.3/100]) x 4.5] = 16.1 - [(1-0.393) x 4.5] = 16.1 - [0.607 x 4.5] = 16.1 - 2.7315 = 13.4

i. Recalculate the MCH and MCHC using the **new** HGB, **original** HCT and **original** RBC.

Corrected Values

WBC	5.1
RBC	4.03
HGB	13.4
HCT	39.3
MCV	97.5
MCH	33.3
MCHC	34.1
PLT	250

8) In the middleware, add *"Corrected for Lipemia"* in the comment box next to the MCHC result, then SAVE.
9) **NOTE:** To avoid any math errors, have a second technologist verify calculations.

E. **ICTERIA (HYPERBILIRUBINEMIA):**
1. If interference from bilirubin is suspected, a corrected hemoglobin value may be obtained by performing one of the correction procedures described above for LIPEMIA (i.e. plasma replacement or plasma blank) and adding *"Corrected for icterus"* in the comment box next to the MCHC result.
   a. NOTE: See **Attachment A – MCHC > 37.5 Workflow** (if applicable).

F. **HEMOLYSIS:**
1. *In vitro* hemolysis of red cells after sample collection may lead to a spuriously low RBC count and hematocrit. If possible, recollect the sample.
2. Extensive *in vivo* hemolysis may lead to a falsely elevated hemoglobin value that represents both plasma and red cell hemoglobin. Thus, the MCH and MCHC may be spuriously elevated. (The measured values for red cell count and hematocrit are still correct.) If *in vivo* hemolysis is suspected:
   a. Perform the plasma replacement procedure described under section D. LIPEMIA above and recalculate the MCH and MCHC with the new corrected hemoglobin.
   b. Verify the platelet count on blood smear.

c. Free text the internal comment: *"Corrected for hemolysis".*
d. From the middleware morphology tab, add the diff comment *"Sample is markedly hemolyzed. Suggest clinical correlation to assess whether due to in-vitro or in-vivo hemolysis."*
1) This comment can be added if only a CBC is ordered.

G. **CRYOGLOBULINS:**
1. Cold-precipitated plasma immunoglobulins (cryoglobulin) or fibrinogen (cryofibrinogen) in a blood sample can cause a falsely increased WBC count with excessively high takeoff at 35 fL resulting in a vote out (-----) and/or an asterisk (*) code for the WBC parameter. Also, the RBC count, hemoglobin, hematocrit and platelet count may be slightly increased along with a slightly decreased MCV.
2. Aggregates of blue staining amorphous material may be seen on Wright-stained smears. Increased levels of cryoglobulin may be associated with myeloma, macroglobulinemia, lymphoproliferative disorders (e.g. CLL), metastatic tumors, autoimmune disorders, infection, and as an idiopathic disease. Cryofibrinogen has been observed in association with many disorders including myeloma, carcinoma, leukemia, aneurysm, pregnancy, the use of oral contraceptives, thromboembolic phenomena, diabetes, and as an essential disease.
3. To correct for the effects of cryoglobulin:
a. Warm specimen to 37°C for a **minimum of 15 minutes**.
b. Mix the sample thoroughly. Rerun the sample in manual mode.
c. Report the 37°C results (for all parameters) if the results are feasible and no parameter flags are present.
4. In the middleware, free-text internal comment "Possible cryoglobulin".

H. **NUCLEATED RBCS:**
1. NRBCs are a reportable parameter from the Sysmex XN-Series analyzers. The XN analyzer provides a "corrected WBC" taking into account the NRBCs recognized by the analyzer. XN-Series NRBC counts are linear to 600 per 100 WBC, however discrepancies from the analyzer NRBC count and what is seen upon smear review may arise.
2. If performing a scan and a significant discrepancy of NRBCs on the slide versus the analyzer count (+/- 10) is noted, a WBC correction must be performed.
a. **For Sysmex XN-450 (XN-L) analyzers, refer to Attachment B** and **the Corewell Health East - Caresphere Resulting**
b. **For Sysmex XN-10 analyzers, refer to the Corewell Health East - Caresphere Resulting**
c. Add comment *"WBC corrected or adjusted for the presence of nRBCs"* to the WBC field.
d. If a differential is ordered, a manual differential must be performed after the corrected WBC count is entered into the middleware. The middleware will calculate the corrected absolute values.
e. If an erroneous WBC count was released prior to discovering the discrepancy, call the corrected WBC to physician or nursing unit as applicable.
3. The general equations for a WBC count and differential corrected for the presence of NRBCs are as follows:
a. Corrected WBC Count = (Uncorrected WBC Count × 100) / (NRBCs + 100)
b. Corrected WBC * (% cell type / 100) = Corrected diff parameter

I. **MICROCYTIC RBC OR RBC FRAGMENTS:**
1. Scan the peripheral smear for the presence of fragmented RBCs and other RBC abnormalities.
2. If extremely microcytic RBC are present:
a. Estimate the PLT count.
b. Utilize the fluorescent platelet (PLT-F) if needed. (Not applicable to the XN-L sites)

J. **PLATELET CLUMPS OR SATELLITOSIS:**
1. See the Corewell Health East - Caresphere Resulting procedure for instructions.

K. **GIANT PLATELETS SEEN (With PLT Abnormal Distribution Flag or with PLT clumps):**

1. Giant Platelets and clumped platelets may falsely elevate the WBC and falsely decrease the platelet count. The PLT will be asterisked.
   a. For XN-10 sites: perform PLT-F.
   b. For XN-L sites; perform a rerun on the second analyzer.
2. A visual estimate of the WBC count must be performed. If there is a discrepancy between the analyzer and WBC estimate, add the comment, *"WBC may be inaccurate due to WBC/PLT interference".*

L. **RBC LYSE RESISTANCE:**
1. Red blood cells containing large amounts of abnormal hemoglobin S or C may be more resistant to lysis. Due to RBC lyse resistance, the WBC count from the DIFF scattergram may be suspect. However, a correct count is derived from the WBC channel, which has a stronger lysing reagent.
2. Verify the hematology analyzer WBC against a WBC estimate from the peripheral smear.
   a. WBC count estimate (average # WBC @ 20X x 0.5 or average # WBC @ 50X x 3= WBC bil/L ± 25%)
3. Perform a manual differential if there is disagreement between the smear and analyzer results.

M. **MARKED CHANGES IN PLASMA CONSTITUENTS:**
1. Marked changes in plasma constituents (i.e. very low sodium or very high glucose) may cause cells to swell or shrink. When it is determined the interference is not due to cold agglutinin, lipemia, icteria, hemolysis, or RBC lyse resistance, review the sodium and glucose results if testing is ordered for the patient.
2. The middleware holds results for MCHC >37.5.
   a. (Site specific) If applicable, perform a spun hematocrit. If the spun hematocrit is greater than the value obtained from the analyzer, report the spun hematocrit if the recalculated MCHC is less than or equal to 37.5. Recalculate the MCV and MCHC. Refer to the calculations and interpretations section for the calculation formulas. See site specific microhematocrit procedure.
   b. Alternatively, a dilution may be performed.
      1) Perform a dilution of the specimen (1:2, 1:3, 1:5, etc.) Allow to equilibrate for 5 minutes before analysis.
      2) Run in manual mode on the instrument within 20 minutes of setup.
      3) As a QC check, verify that the RBC count agrees within +/- 0.20 of undiluted sample.
      4) If diluted rerun was added in the middleware, the middleware will automatically calculate the results. (Refer to Corewell Health East - Caresphere Resulting procedure.)
      5) Document the steps taken in the middleware as an internal comment, i.e.., "Run 2 performed by dilution."
   c. NOTE: Do not use differential results from a diluted specimen.

N. **RBC INDICIES CALCULATIONS AND INTERPRETATIONS:**
1. **Mean corpuscular volume (MCV)** is the volume of the average RBC of a given sample of blood. Results are expressed in femtoliters (fL), formerly known as cubic microns or μ^3.

   a. MCV = (HCT% x 10) / RBC

2. **Mean corpuscular hemoglobin (MCH)** is the average weight of hemoglobin contained in an average erythrocyte. Results are expressed as picograms (pg) which is the same as micromicrograms (μμg).

   a. MCH = (HGB x 10) / RBC

3. **Mean corpuscular hemoglobin concentration (MCHC)** is the average concentration of hemoglobin in a given volume of packed red cells. Results expressed as % or gm/dL.

a. **MCHC = (HGB x 100) / HCT**

O. **NOTES:**

1. In some situations, a corrected value may be unable to be obtained. If a corrected value cannot be obtained by manual methods, add the comment *"Unable to perform due to unknown interference"* to the affected parameter in the middleware or LIS. Consult supervisor if further direction is needed.
2. Instrument voteouts (----) will come across into the middleware as "#nm". This translates to "Unable to perform" in the LIS.
3. Abnormal paraproteins found in multiple myeloma patients can falsely increase the HGB. To correct HGB, perform plasma replacement or plasma blank procedure.
4. MCHCs over 37.0 gm/dL may be a result of **falsely high** HGBs or **falsely low** HCTs.
   a. Falsely high HGB possible causes:
      1) Lipemia
      2) Increased bilirubin
      3) Abnormal proteins
   b. Falsely low HCT possible causes:
      1) Cold agglutinin
      2) Gross hemolysis
      3) Instrument failure
      4) Incorrect RBC count

P. REFERENCES:

1. Cornbleet J. Spurious results from automated hematology cell counters. Lab Med, 1983; 14(8): 509-514.
2. Gagne C, et al. Effect of hyperchylomicronemia on the measurement of hemoglobin. Am J Clin Path, 1977; 68:584-586.
3. Nelson DA. Basic methodology. In: Davidson I, Henry JB (eds). Clinical diagnosis and management by laboratory methods. 17th ed, Philadelphia: WB Saunders, 1984: 592.
4. Koepke JA. Tips on Technology. MLO, July 1995.
5. Flagging Interpretation Guide, Sysmex, Document Number MKT-40-1010, December 2008.
6. Hematology Applications Specialist, Sysmex Diagnostics. Personal communication, 2000.
7. XN Flagging Guide, Sysmex, January 2015, Document -1166-LSS

7. Procedure Development and Approval

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8. Keywords

Not Set