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CBC Corrections

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A number of unusual factors present in patient samples may produce erroneous results on automated cell counters. This procedure explains how to correct for various factors that cause spurious values (e.g., high white blood cell (WBC) counts, cold agglutinins, lipemia, icteria, cryoglobulins, nucleated red blood cells (NRBC), clumped platelets, platelet satellitosis, giant platelets, microcytic red blood cells (RBCs), RBC fragments, hemolysis, and lyse resistant RBCs. 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.

II. SPECIMEN COLLECTION AND HANDLING:

A. Whole blood collected in a 4 mL vacutainer is the preferred sample. Capillary blood collected in a microtainer is also acceptable.
B. Specimens must be collected in Potassium Ethylenediaminetetraacetic acid (K_2EDTA).
C. The optimal sample size for whole blood is 4.0 ml. The minimum sample size is 2.0 mL.
D. The optimal sample size for capillary blood is 500 µL. The minimum sample size is 250 µL.
E. The specimen must be at room temperature and well mixed before being analyzed.
F. Check all microtainer specimens with wooden applicator sticks for clots.
G. Specimen is stable for 24 hours at room temperature.
H. Specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.

III. REAGENTS:

A. Sysmex CellPack DCL diluent is used to make dilutions due to numerical results or interpretive messages.

1. Store CellPack between 15-30ºC.
2. CellPack is stable for 60 days once opened.

IV. EQUIPMENT:

A. Heat block: 37ºC

V. PROCEDURE:

A. High WBC Counts (> 440.0 x 10^3/µL)

1. Parameters that exceed the upper limit of the analyzer measurement range (AMR) are flagged with "@" beside the result.
2. The sample must be diluted using the system diluent, reanalyzed and multiplied by the dilution factor. The suggested dilution ratio for high WBCs is 1:2. High numbers of WBCs can cause a false decrease in the WBC reported.
3. To correct a high WBC count:
    a. The Sysmex middleware holds the results. Request a "Rerun" in the middleware.
    b. Start out with the suggested dilution ratio per SYSMEX XN-L manual of a 1:2 dilution of the specimen (Add 200 µL of specimen to 200 µL of CellPack diluent.).
    c. Run on manual OPEN CAP mode of instrument within 20 minutes of making dilution.
    d. When the rerun test is complete, the instrument automatically sends the results to the middleware. The dilution factor (2) is entered on the appropriate rerun in the middleware and the results will be multiplied accordingly.
    e. Verify that the RBC count agrees within ± 0.1 of undiluted sample. If not, order a rerun in the middleware and rerun the sample.
    f. If the results are still not acceptable, order a rerun in the middleware and remake a higher dilution.
    g. A 1:5 dilution is made by adding 100 µL of specimen to 400 µL of CellPack diluent.
    h. Notify lead/supervisor if unable to resolve.
    i. If the specimen is run under the pre-dilution mode on the analyzer the results will automatically be multiplied x 7 in the middleware. If it is not run under the pre-dilution mode on the analyzer the dilution factor must be entered on the appropriate rerun in the middleware and the results will be

multiplied accordingly.

j. Accept the WBC count if the RBC count agrees ± 0.1 of original RBC count.

k. In the middleware, add internal comment "By dilution". The hemoglobin (HGB) and WBC are measured in different chambers on Sysmex analyzers, so there is no WBC interference with the HgB result.

l. See attachment A at end of procedure for step-by-step middleware instructions.

m. Store and Validate per procedure.

B. Cold Agglutinins

1. Cold agglutinins cause the spontaneous agglutination of RBCs at temperatures lower than 37ºC. The degree of agglutination is dependent on the cold agglutinin titer.
2. The Sample Rotor Valve (SRV) on the Sysmex analyzers is warmed to 37ºC, 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.
3. The mean cell volume (MCV) will be falsely elevated due to micro-agglutinates being sized as a single large cell.
4. The hemoglobin reading is usually correct. With a correct hemoglobin value and low hematocrit (HCT), the mean corpuscular hemoglobin concentration (MCHC) and the mean cell hematocrit (MCH) will be spuriously elevated.
5. Check and correct for cold agglutination on all bloods that exhibit the following:
    a. MCHC greater than 38%.
    b. When smear morphology and hematology analyzer MCV result do not agree (the MCV does not necessarily have to be macrocytic to be suspicious of a cold agglutinin).
    c. Smears with RBC agglutinates present.
    d. Any blood you are suspicious of because of a "lacy" appearance in the tube or on the slide.
    e. If b, c, or d is present, follow cold agglutinin procedure, regardless of MCHC value.
6. To correct for the effect of a cold agglutinin:
    a. The Sysmex middleware holds the results. Request a “Rerun” in the middleware.
    b. Place the blood in a 37ºC incubator (e.g., heating block or coagulation water bath) for at least 15 minutes.
    c. Mix specimen thoroughly and rerun the blood ASAP through the manual mode of the hematology analyzer, before the specimen cools down.
    d. Report the 37ºC results (for all parameters) if the MCHC is feasible.
    e. In the middleware, add “Possible Cold Agglutinin” comment by double

clicking on COM field next to MCHC result and selecting HE03, then SAVE.

7. For a cold agglutinin specimen that has instrument flags/lab values requiring microscopic review:
    a. If RBC agglutinates are present, make warmed smears to see if the agglutinates go away upon warming.
    b. Regardless of which smears (room temperature or warmed) exhibit agglutinates, report “RBC agglutinates present”.
    c. If case needs further review by a pathologist, submit both sets of clearly marked slides to the pathologist.
    d. Store and validate per procedure.
    e. Cold agglutinins must always be incubated at 37ºC before being reporting as such.

C. Lipemia

1. Lipemic plasma can cause a falsely elevated 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 bloods with:
    a. A lacy appearance of blood smear.
    b. MCHC greater than 38%.
3. To check for lipemia:
    a. Spin blood for approximately 5 minutes (or let settle for approximately 10-15 minutes).
    b. Visually check plasma layer for characteristic milky appearance.
4. To correct for lipemia, perform either of the following procedures:
    a. Plasma Replacement:
        i. Hold results in the middleware.
        ii. Spin down a portion of the blood specimen at 2000 rpm for 5 minutes.
        iii. Mark the top (meniscus) of the plasma level.
        iv. Carefully remove most, but not all, of the plasma.
        v. Replace the plasma with the same amount of Cell Pack diluent (add diluent up to the mark).
        vi. Mix the sample and cycle through the instrument.
        vii. Use the RBC result as a guide to verify proper re-dilution of the specimen.
        viii. If the RBC result is within ± 0.10 of the original RBC, accept the

new HGB, from the saline replacement run.

ix. Recalculate the MCH and MCHC using the new HGB and original MCV and HCT.

x. In the middleware, modify the results for HGB, MCH, and MCHC by double clicking on the result boxes and answering “Yes” to modify results. Enter the new HGB, calculated MCHC and MCH.

MCHC = HGB/HCT x 100
MCH = MCV x MCHC/100

xi. Use original WBC, RBC, HCT, MCV, PLT, MPV and RDW.

xii. In the middleware, add “Corrected for Lipemia” comment by double clicking on COM field next to MCHC result field and selecting HE02, then SAVE.

xiii. Store and Validate per procedure.

b. Plasma Blank:

i. Hold results in the Sysmex middleware.

ii. Spin a portion of the blood.

iii. Perform a hemoglobin on the plasma using the hematology analyzer open manual mode.

iv. Use the following formula to calculate the new correct hemoglobin:

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

v. Lipemic specimen example:
WBC: 5.1
RBC: 4.03
HGB: 16.1
HCT: 39.3
MCV: 97.5
MCH: 40.0
MCHC: 41.3
PLT: 250
Plasma Hgb: 4.5

vi. 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

vii. Corrected MCHC = 13.4/39.3 x 10
= 0.341 x 100
= 34.1

viii. Corrected MCH = 97.5 x (34.1/100)

= 97.5 x 0.341
= 33.2

ix. Corrected results:
WBC: 5.1
RBC: 4.03
HGB: 13.4 (New HGB Calculated from Plasma HGB above)
HCT: 39.3
MCV: 97.5
MCH: 33.2 (Calculated using new HGB)
MCHC: 34.1 (Calculated using new HGB)
PLT: 250

c. Corrected report:

i. In the middleware, modify the Results for HGB/MCH/MCHC by double clicking on the result boxes and answering “Yes” to modify results.

ii. Enter the new HGB, calculated MCH and MCHC.

iii. In the middleware, add “Corrected for Lipemia” comment by double clicking on COM field next to MCHC result field and selecting HE02, then SAVE.

iv. Store and validate per procedure.

v. To avoid any math errors, have second technologist verify calculations. Leave for Hematology Lead to perform a QA review.

D. Icteria (Hyperbilirubinemia)

1. If interference from bilirubin is suspected, a correct hemoglobin value may be obtained by performing one of the correction procedures described for lipemia and free texting "Corrected for Icteria" as an internal comment.

E. 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 * code for the WBC parameter.
2. The RBC count, hemoglobin, hematocrit and platelet count may be slightly increased along with a slightly decreased MCV.
3. Aggregates of blue staining amorphous material may be seen on Wright stained smears.
4. 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.
5. 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.

6. To correct for the effects of a cryoglobulin:
    a. The Sysmex middleware holds results. Request a Rerun in the middleware.
    b. Warm specimen to 37ºC for a minimum of 15 minutes.
    c. Rerun through the hematology analyzer manual mode.
    d. Report the 37ºC results (for all parameters) if the results are feasible and no parameter flags are present.
    e. In the middleware, free-text internal comment "Possible cryoglobulin".
    f. Store and validate per procedure.

F. Platelet Clumping

1. Platelet clumping can result in spurious low and widely fluctuating platelet counts on hematology instruments.
2. Platelet clumping may occur because of poor collection technique resulting in the presence of micro clots, delays in mixing the blood and anticoagulant, the presence of platelet agglutinins, or EDTA-induced clumping.
3. Examine a peripheral smear on all bloods with the following instrument flags or results:
    a. Platelet count less than 75 bill/L
    b. Platelet clump(s) flag
    c. Platelet delta check (decrease only)
4. To correct for platelet clumping:
    a. The Sysmex middleware holds the results for the original run.
    b. Check for platelet clumping by first checking for a clot. Document as an internal comment in the middleware.
    c. Make smear and stain.
    d. Examine Wright stained smear for clumping, particularly at the feathered and side (lateral) edges.
5. If significant platelet clumps are seen:
    a. Scan peripheral smear to determine if WBC estimate matches instrument count.
    b. If estimate does not match instrument count, add comment “WBC may be inaccurate due to WBC/PLT interference” to WBC comment field.
    c. Approximate platelet estimate from stained smear (e.g., ADQ, INC, DEC).
    d. In the middleware 'Result Validation' tab, remove platelet result by clicking on the Platelet Result field, then clicking Delete.
    e. Double click on the Platelet Result field. A window opens. Select the appropriate clumped platelet comment that correlates with the platelet

estimate:

i. "Platelets clumped in EDTA, appear adequate on smear."

ii. "Platelets clumped in EDTA, appear decreased on smear."

iii. "Platelets clumped in EDTA, appear increased on smear."

f. For EDTA-induced platelet clumping, if a blue top citrate tube is available from the same draw, a numerical platelet count may be attempted by running the PLT from a venous sample collected in a full 3.2% citrate tube (PLT count corrected x 1.1 for dilution) as long as the citrate smear does not exhibit clumping and the citrate platelet count is higher than the EDTA platelet count.

i. Obtain a full 3.2% citrate tube. Mix well.

ii. Run in manual mode for CBC.

iii. If the count is higher than the EDTA PLT count, then make a smear of the blue top and scan to make sure there is no clumping on the slide.

iv. Multiply the PLT count of the citrate tube by 1.1. Modify the result under the platelet column and enter the new calculated PLT count.

v. Comment in the result field COM box by typing “Performed on NA Citrate Tube”.

vi. If platelet clumps are present but are insignificant, report instrument count and add internal midleware comment: “Insignificant platelet clumps present”.

vii. Store and validate according to procedure.

viii. See Attachment B at end of procedure for middleware instructions.

G. Platelet Satellitosis

1. Platelet satellitosis is the adherence of platelets to neutrophils and is seen only in EDTA- anticoagulated blood. Platelet satellitosis can cause spurious low or fluctuating platelet counts on hematology instruments. In addition, spurious neutropenia can occur in white cell differentials performed on wedge blood smears.
2. Check for platelet satellitosis by examining feathered edges of a blood smear on all bloods with:
    a. Platelet counts less than 75 bill/L.
    b. Widely different platelet results from previously reported values.
3. To correct for platelet satellitosis:
    a. Results will be held in the Sysmex middleware.
    b. Approximate platelet estimate (ADQ, INC, DEC) from the stained smear.
    c. Delete PLT result from the middleware and replace it with the statement:

"Platelet satellitosis in EDTA - Appear (ADEQ, INC, DEC)".

d. Report hematology analyzer differential if scan agrees; otherwise perform manual diff.

e. A numerical platelet count may be attempted, if a sodium citrate tube is available from the same draw. See above 5. f.

f. If sodium citrate does not correct problem, remove PLT value from the middleware and add comment “Unable to report due to platelet satellitosis”.

g. See Attachment B at end of procedure for step-by-step middleware instructions.

H. Giant Platelets

1. The Sysmex hematology analyzers have floating discriminators so there should be no interferences in CBC results from giant platelets.
2. If there is WBC instrument/smear disagreement, add the following comment to the WBC field: “WBC may be inaccurate due to WBC/PLT interference.”
3. To correct for ‘Giant Platelets’ Flag:
    a. Examine stained smear for giant platelets.
    b. Scan peripheral smear to determine if WBC estimate matches instrument count.
    c. If estimate does not match instrument count, add “WBC may be inaccurate due to WBC/PLT interference” to WBC comment field.
    d. If Giant Platelets are present, there are 2 places in the middleware where they can be reported.
        i. For CBCND that needs a slide review, the comment can be used in the Result Validation field, in the PLT COM field: “PLT9: Giant Platelets Present”.
        ii. For CBCWD, Select the PLT9 comment found in the Morph tab DIFFC- Diff Comment result field.
        See Attachment B at end of procedure for step-by-step middleware instructions.

I. Hemolysis

1. In vitro hemolysis of red cells after sample collection may lead to a spuriously low RBC count and hematocrit. Cancel and recollect specimen.
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 (measured values for red cell count and hematocrit are still correct).
3. If in vivo hemolysis is suspected:
    a. The Sysmex middleware will hold the results.

b. Perform the plasma replacement procedure described under "Lipemia" above and recalculate the MCH and MCHC with the new corrected hemoglobin.

c. Verify platelet count on blood smear.

d. Free text the internal comment: "Corrected for hemolysis".

e. In some situations, a corrected value cannot be obtained. If a corrected value cannot be obtained by manual methods, add the comment “Unable to perform” to the affected parameter.
Instrument vote outs will cross into the middleware as “#nm”. This translates to “Not measured”. Add the comment “Unable to perform” to the affected parameter.

J. Hemoglobin S or C

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. A correct count is derived from the WBC channel, which has a stronger lysing reagent.

    a. To correct for the presence of RBC lyse resistance, verify hematology analyzer WBC against a WBC estimate from the peripheral smear.

K. Microcytic RBCs or RBC fragments

1. If Microcytic RBCs or RBC fragments are suspected, scan the peripheral smear for the presence of fragmented RBCs and other RBC abnormalities.

    a. If extremely microcytic RBC are present: verify the platelet count via platelet estimate on slide.

VI. REFERENCES:

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Attachments

Attachment A: Diluting Specimens for High WBC COUNTS / Above Linearity Parameter

Attachment B: Clumped Platelets/Giant Platelets/Platelet Satellitosis

Approval Signatures

Step Description	Approver	Date
Medical Director	Jeremy Powers: Chief, Pathology	5/27/2022
Medical Director	Muhammad Arshad: Physician	5/27/2022
Policy and Forms Steering Committee Approval (if needed)	Kristin Murphy: Medical Technologist Lead	5/27/2022
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	5/26/2022
	Kimberly Geck: Dir, Lab Operations B	5/26/2022
	Amy Conners: Dir, Lab Operations A	5/23/2022
	Katherine Persinger: Mgr Laboratory	5/23/2022
	Kristen Lafond: Mgr Laboratory	5/6/2022
	Ashley Dingess: Mgr Laboratory	4/27/2022
	Kristin Murphy: Medical Technologist Lead	4/27/2022