Resolving Pre-analytical CBC Sample Problems

Principle

The presence of cold agglutinins, lipemia, icterus, platelet clumping, hemolysis in the sample can cause erroneous results. These results must be corrected before being released.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing scheduled maintenance. Refer to: Policy and Procedures Safety Manual Infection Control and Procedures 11-085-01.

Materials and Reagents

37 ° C Dry bath and timer
CELLPACK DCL
MLA pipette, 500 uL/300 uL
12X75 Tubes
MLA tips
Sysmex Analyzer

Procedure

Samples Issues:

A. **Cold Agglutinin:** The presence cold agglutinin can adversely affect the results of the CBC, i.e. inaccurate results. The parameters affected are **RBC, HCT, MCV, MCH** and **MCHC**. This is due to the agglutination of the RBC's in the sample as it cools to room temperature.

Step	Action
1	Incubate the CBC sample by putting it in a 37 ° C Dry bath for at least 30 minutes.
2	Mix well and rerun the specimen ***immediately*** after pre-warming.
3	If MCHC is corrected, then the other indices have been corrected for cold agglutinin. Proceed to result the corrected values.
4	In cases of very strong cold agglutinins where the MCHC does not correct after warming the sample for more than 1 hour, manually prepare a 1:5 dilution with CELLPACK DCL and run CBC on XN in Manual Analysis mode.
5	If results are valid, correct results for dilution factor for, RBC, HGB and HCT. Replace WBC and PLT from the original run then validate in WAM. **NOTE:** RBC Indices results are unaffected by dilution and do not require correction.
6	If dilution does not correct problem, then perform plasma replacement procedure.
7	If a manual diff is needed, prewarm several slides in the dry bath for 15-30 minutes before making the smear.

Procedure, continued

B. **Severe Cold Agglutinin:** In cases with high cold agglutinin titers, a plasma replacement using warm CELLPACK DCL may be necessary to reduce the interference from the antibody.

Step	Action
1	Pre-Warm CELLPACK DCL at 37° C dry bath for at least 15 minutes
2	Aliquot 1 mL of well mixed pre-warmed sample of specimen in a 12 mm tube
3	Centrifuge the 1 mL aliquot for 2000 RPM for 10 minutes to separate plasma from red cells.
4	Using either a 500 μL MLA pipette or 300μL MLA pipette, carefully remove plasma without disturbing the buffy coat.
5	Change tip on MLA pipette and add back the same amount of removed plasma with Pre-warmed CELLPACK DCL diluent.
6	Mix the sample, order a rerun and cycle through the analyzer in manual mode
7	Use the original WBC and RBC results obtained as a guide to verify proper re-dilution of the specimen. If the RBC results are within ±5% of original result results, report the RBC, HGB, HCT and Indices from the re-diluted sample
8	If plasma replacement corrected the symptom, replace the WBC and Platelet with the original values since centrifugation will alter these parameters. Document on WAM "Severe Cold Agglutinin, RBC and indices corrected by Plasma Replacement."
9	If plasma replacement does not correct the symptom, request for a redraw where sample is kept at 37° C at all time.

C. **Lipemic Samples:** Lipemia falsely elevates HGB and MCHC. MCHC will be ≥ 37.0. Follow the steps below to process a lipemic sample.

Step	Action
1.	Process blood through the XN analyzer. Order rerun if indicated.
2.	Manually prepare a 1:5 dilution with CELLPACK DCL and run on XN in Manual Analysis mode. If results are valid then validate in WAM. If dilution does not correct problem, then go to next step.
3.	Take a portion of the specimen and transfer into another tube.
4.	Centrifuge the aliquot at 2000 RPM for 10 minutes to thoroughly separate the cells from the plasma.
5.	Using either a 500 μL MLA pipette or 300μL MLA pipette, carefully remove plasma.
6.	Change tip on MLA pipette and replace removed plasma with CELLPACK DCL diluent.
7.	Mix the sample, order a rerun and cycle through the analyzer.
8.	Use the WBC and RBC results obtained in step 1 as a guide to verify proper re-dilution of the specimen.

9.	If the RBC results are within ±5% of results in step 1, report the Hgb, MCH and MCHC from the re-diluted sample. The other results are resulted from the results from step 1.
10.	Document in report that specimen was grossly lipemic and that hemoglobin was corrected for lipemia.

D. **Icteric Samples:** Severely icteric samples may falsely elevate the HGB value and related indices.

Step	Action
1.	Process blood through the XN analyzer. Order rerun if indicated.
2.	Manually prepare a 1:5 dilution with CELLPACK DCL and run on XN in Manual Analysis mode.
3.	If results are valid, correct results for dilution factor for WBC, RBC, HGB, HCT and PLT, then validate in WAM. **NOTE:** RBC Indices, RDW, IPF%, MPV, Ret-He, IRF and differential percent result are unaffected by dilution and do not require correction.
4.	Document in report that specimen was grossly Icteric, and that hemoglobin was corrected for Icteric.

D. **Platelet Clumping:** Platelet results will be flagged as “Platelet clumps” operator alert in WAM. Follow procedure below to process specimen

Please follow the workflow below for any platelet issues

OP Alert (1st run): PLT Clumps? – Rerun for PLTF or PLT ABN Distribution
XN will automatically **RERUN** the PLTF

↓

Depending on your sample you may or may not get any OP Alert on the PLTF (this is your 2nd run).

- **IF** the PLTF result is **good**, i.e., there's no asterisk (*) and no PLT IP message, **then you can release this result**.
- **IF** the PLTF result has an **asterisk (*) and/or there's a PLT IP message**, then proceed to the vortex procedure below.

↓

VORTEX specimen for a minimum of **1 minute** and **PREPARE** a smear for review

↓

RERUN sample manually as PLTF (this is your 3rd run)

- **IF** the PLTF result is **good**, i.e., there's no asterisk (*) and no PLT IP message, then you can **release** this result from the **3rd run**.
- **IF** the PLTF result has an **asterisk (*) and/or there's a PLT IP message**, then proceed to the smear review and PLT Estimate procedure below.

↓

REVIEW smear manually (not Cellavision) for PLT CLUMPS and **PERFORM** a PLT Estimate

↓

YES ← **PLT CLUMPS seen** → NO

↓

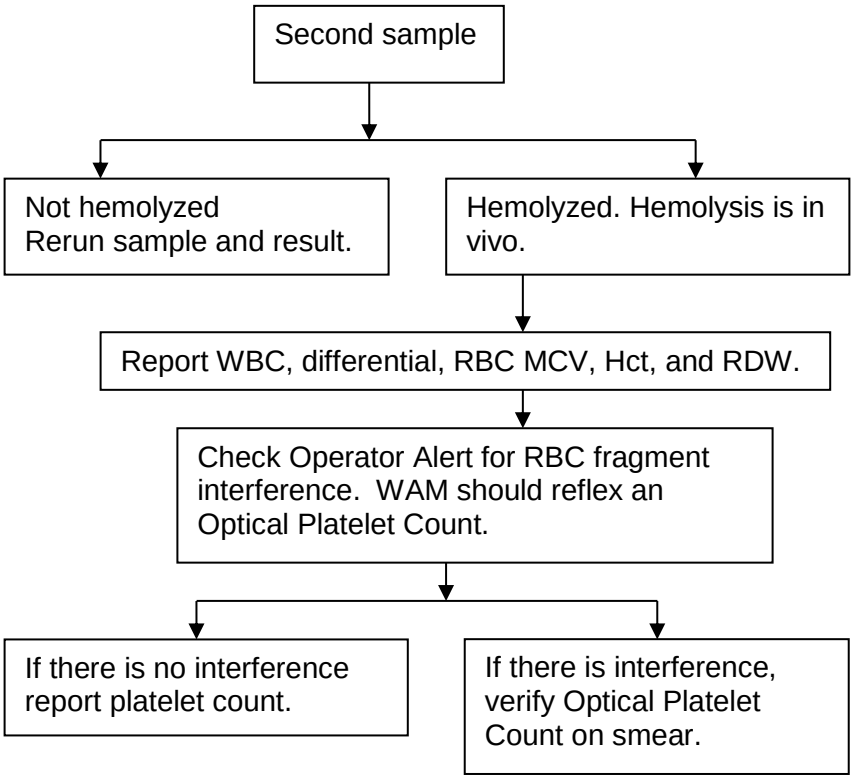
1) Delete the platelet count in WAM.
2) Then double click on the PLT result area, a POP screen will appear, choose **DNR** this will be reported as **CLUMPING SEEN**.
3) Then on the comment section, **ADD** a comment of: **"PLT estimate appears decreased/adequate/increased (whatever applies). Suggest Citrated Plt Count order."**
4) Do platelet count on citrate (if there's an order).

NOTE: Presence of small occasional clumping is fine.

COMPARE PLT count result from 3rd run with your PLT Estimate count, if it correlates **THEN** release PLT count result from the **3rd run**.

Procedure, continued

E. **Hemolyzed Samples:** Follow the steps below to process a hemolyzed sample. MCH and MCHC will be high.

Step	Action
1	Take a portion of the specimen and transfer into another tube. Centrifuge the aliquot at 2000 RPM for 10 minutes. Observe sample plasma for the presence of hemolysis.
2	Obtain a second sample, if possible and process. If the second sample is also hemolyzed, the hemolysis may be in vivo. If you are unable to obtain a second sample proceed to step "3". 
3	Report the WBC, differential and Hgb from the hemolyzed specimen.
4	Do not report the RBC, HCT, MCH, & MCHC from the hemolyzed specimen.
5	Check the operator alerts for possible interference from RBC fragments. • Report Optical Platelet Count. • Do not report the impedance platelet count.

References:

A. Sysmex XN-9000 Instructions for Use (North American Edition), Sysmex Corporation, Kobe, Japan.
B. Sysmex XN-Series Automated Hematology Systems. Flagging Interpretation Guide, Sysmex Corporation, Kobe, Japan.
C. Koepke, John. Practical Laboratory Hematology. Churchill Livingstone Inc. 1991. p. 24-25, 36-39.
D. Cornbleet J., *Spurious results from automated hematology cell counters. Lab Medicine.* 1983;8:509-514.
E. Stewart, Charles and Koepke, John. *Basic Quality Assurance Practices for Clinical Laboratories*, Van Nostrand Reinhold, 1989, p 189.
F. Gulati GL, Asselta A, Chen C. *Using vortex to disaggregate platelet clumps*, Laboratory Medicine, 28:665, 1997.

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Implemented
New	Procedure for new XN instruments	Julius Salomon, 7/1/17			
Major	1. Revised cold agglutinin procedure under sample issues section. Removed the manual hematocrit procedure and added 1:5 dilution. 2. Added a severe cold agglutinin procedure using plasma replacement 3. Expanded the Icteric samples 1:5 dilution procedure.	Marlon Esguerra, 10/8/18			