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 Drybath and timer
Heparinized 75mm Hematocrit tubes
Cellpack
MLA pipette, 500 uL/300 uL
Sysmex Analyzer
Micro-hematocrit centrifuge
Critoseal
Statspin Centrifuge
MLA tips

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 Drybath 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, perform a SPUN HCT.
5	If a manual diff is needed, prewarm several slides in the drybath for 15-30 minutes before making the smear.
6	Report only the **WBC, HGB, HCT and PLT** count. Enter **NOT MEASURED** for RBC, MCV, MCH, MCHC and RDW. Then **ADD** the comment: • "**Unable to report RBC and RBC Indices due to strong cold agglutinins.**" on **ALL** parameters reported as NOT MEASURED. • **"Hematocrit is a SPUN HCT."** for HCT.

Procedure, continued

B. **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 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 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.

C. **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 and run on XN in Manual Analysis mode, then validate in WAM.

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
PLT ABN Distribution
XN will automatically **RERUN** for PLTF

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Depending on your sample you may or may not get any OP Alert on the PLTF (this is your 2nd run).
Regardless, we will proceed to do a **RERUN** after vortexing the sample.

CLS to **ADD RERUN** in WAM

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

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RERUN sample (this is your 3rd run)

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

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YES ← PLT CLUMPS seen → NO

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IF **LARGE/NUMEROUS PLT Clumps** seen, then:

1) If platelet appears normal, report as "**Clumping Seen**". Report platelet smear estimate as "**Adequate**".
2) If platelet count appears decreased, report as "**Clumping Seen**". Report platelet smear estimate as "**Decreased**".

NOTE: Presence of small occasional clumping is fine

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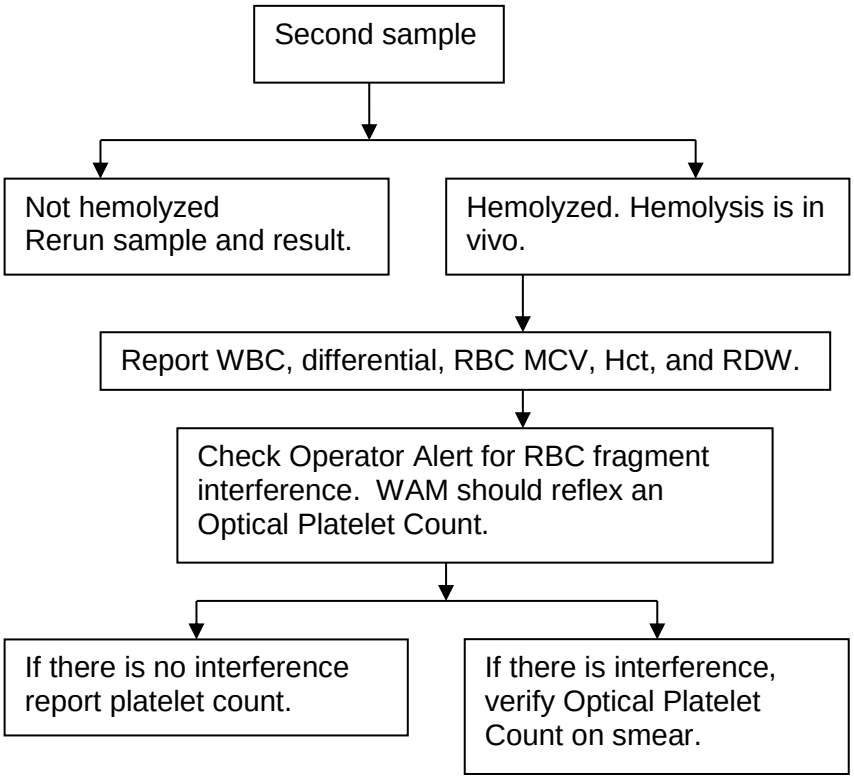
COMPARE PLT count result from 3rd run with your PLT Estimate count, if it correlates **THEN** release PLT count result.

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. Koepke, John. Practical Laboratory Hematology. Churchill Livingstone Inc. 1991. p. 24-25, 36-39.
C. Cornbleet J., *Spurious results from automated hematology cell counters. Lab Medicine.* 1983;8:509-514.
D. Stewart, Charles and Koepke, John. *Basic Quality Assurance Practices for Clinical Laboratories*, Van Nostrand Reinhold, 1989, p 189.
E. 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			