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

Refer to the safety manual for general safety requirements.

Materials and Reagents

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

Procedure

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**.

XN 550 will have an alert message of RBC Agglutination?
And asterisks (*) appear next to RBC and its indices.

Step	Action
1	Incubate the CBC sample by putting it in a 37 ° C Dry bath for at least 15 minutes.
2	Mix well and rerun the specimen ***immediately*** after pre-warming.
3	If MCHC is corrected (≤ 37.5g/dL and no asterisk present), 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 a prewarmed CELLPACK DCL and run CBC on XN550 in Manual Analysis mode. Follow steps below: • Rerun sample • Scan the sample accession in the XN IPU. • Mix the sample by gentle inversion at least 10 times. • Run the sample in Manual Analysis mode.
5	If results are valid (see #3 above), • Calculate and apply the dilution factor into the results. • Choose the appropriate run column in Cerner for the rerun group.

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	• Replace **WBC** and **PLT** results from the original run, then validate in Cerner. **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 differential is needed, prewarm several slides in the drybath for 15-30 minutes and make a new peripheral smear from the warmed sample.

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 an 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	Simultaneously mix and warm the sample for at least five (5) minutes, order a rerun and cycle through the analyzer in manual mode.
7	Use the original WBC and PLT results obtained as a guide to verify proper re-dilution of the specimen. WBC/PLT results should be within ±5% from the original run
8	If plasma replacement corrected the symptom, replace the WBC and Platelet with the original values since centrifugation will alter these parameters. Document on Cerner “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.

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C. **Lipemic/Icteric/Hemolyzed Samples:** Lipemia and Icterus falsely elevates HGB and/or MCHC. Result will have a low or normal MCV with MCHC of **≥ 37.5 g/dL**.

XN 550 will have an alert message of Turbidity/HGB Interference? And asterisks (*) appear next to HGB, MCH and MCHC.

Step	Action
1.	Rerun Sample. manually prepare a 1:5 dilution with CellPack DCL and run on XN550 in Manual Analysis mode. If results are valid then validate in Cerner. If dilution does not correct problem, then perform plasma replacement. Proceed to next step.
2.	Take a portion of the specimen and transfer into another tube.
3.	Centrifuge the aliquot at 2000 RPM for 10 minutes to thoroughly separate the cells from the plasma.
4.	Using an MLA pipette, carefully remove the plasma without disturbing the buffy coat.
5.	Change tip on MLA pipette and replace removed plasma with equal amount of CellPack DCL diluent.
6.	Mix the sample, order a rerun and run through the analyzer.
7.	Use the WBC and PLT results obtained in step 1 as a guide to verify proper re-dilution of the specimen.
8.	If the WBC/PLT 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.
9.	Document in report that specimen was grossly lipemic/icteric, and that hemoglobin was corrected for lipemia/icterus.

For Hemolyzed Samples: follow steps below

Step	Action
1.	Take a portion of the specimen and transfer into another tube. Centrifuge the aliquot at 2000 RPM for 10 minutes. Observe plasma for the presence of hemolysis. If hemolyzed, obtain a second sample if possible and check for hemolysis. If the second sample is also hemolyzed, do not perform CBC anymore.
2.	Do not report out any CBC result. Cancel test due to hemolysis.

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D. **Platelet Clumping:** Follow procedure below to process the specimen.

XN 550 will have an alert message of PLT Clumps? or PLT ABN Distribution

And asterisks (*) appear next to PLT result, dashes or asterisk may appear in place of data for the MPV

Please follow the workflow below for any platelet issues

PLT Clumps? – Rerun for PLT ABN Distribution

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REVIEW smear for PLT CLUMPS and **PERFORM** a PLT Estimate

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YES ← **PLT CLUMPS seen** → NO

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1) Delete the platelet count result in Cerner.
2) On the PLT result area, free text as **CLUMPING**. Click **SAVE**,
3) Then double click on the **Comment** section, **ADD** a comment on the **Free Text** box 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.

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COMPARE PLT count result with your PLT Estimate count, if it correlates **THEN** release PLT count result

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Controlled Documents

The following controlled documents support this procedure.

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

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Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Ops Director reviewed/ date	Date change Implemented
New	Procedure for new XN instrument	Yvette Lingat 4/20/2020			