Analytical Interferences on CBC Specimens

Purpose

This procedure provides instructions for resolving analytical interferences that prompt spurious results on Complete Blood Count (CBC) or its components.

Scope

This procedure is intended for Clinical Laboratory Scientists (CLS), Medical Laboratory Technicians (MLT), and supervisors working in the hematology section.

Policy

The presence of cold agglutinins, lipemia, icterus, hemolysis, platelet clumping, and other intrinsic factors present in the specimen can cause erroneous results. The CLS must address these results on duty 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.

Instrument and Equipment

The following are used for specimen analysis and troubleshooting:

- Instrument: Sysmex XN-10 analyzer or Sysmex XN-550 analyzer (L-series)
- Equipment: 1) 37°C Dry bath, 2) Timer, 3) MLA (or comparable kind) calibrated pipette

---

Materials and Reagents

The following may be needed depending on the specimen situation:

- Materials:
    - Pipette tips,
    - 12 x 75 tubes
- Reagents: CELLPACK DCL

Before you begin

Before troubleshooting any specimen for flags and messages:

- Read and follow the Op Alert triggered thru autoverification rules
- Ensure that the analyzer performs with acceptable quality control (QC) and that maintenance is current.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

Specimen collection and handling assessment

- Compromised samples during the preanalytical process, such as those not properly collected, stored, transported, or containing clots, may cause misleading results. Always use good laboratory practices for inspecting specimens for acceptability and verifying results.
- Clotting and hemolysis during collection and handling can be detected by flags and values generated by the Sysmex XN analyzer during specimen testing.
- Applicator sticks may be used as an initial step to check for micro clots.
- As a confirmatory step, a slide review is recommended.

Specimen rejection

The specimen received for CBC, or its components should be canceled using the appropriate reason citing the preanalytical condition that will cause analytical interference. These include but are not limited to the following:

If	then reject the specimen for testing
the specimen is visibly clotted	by using the Cerner cancellation reason "Clotted Specimen, Test Not Performed."
the specimen was frozen during transport	by using the Cerner cancellation reason "Specimen Temp. Error, Test Not Performed"
the specimen was not collected in EDTA	by using the Cerner cancellation reason "Specimen Container Error, Not Tested."
original specimen volume collected in the correct container and received within stability time is not adequate for testing	by using the Cerner cancellation reason "Insufficient Volume, Test Not Performed."

Refer to SCPMG-LIS-0089 for Cerner Cancel Messages.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

Hemolysis

The different degrees of hemolysis, from mild to moderate and marked, and its nature, affects the clinical reliability of CBC results.

Type	In-vitro	In-vivo
Nature and cause	Most often, the result of preanalytical factors such as blood draws, specimen handling, specimen delivery to the laboratory, or specimen storage.	Mainly due to pathological conditions such as hemolytic disease of the newborn (HDN) and autoimmune hemolytic anemia (AIHA) and/or infections that occur within the body prior to blood being drawn
CBC results integrity	Some or all CBC results or components are invalid and should not be reported.	CBC results or their components are valid
Effects	• Red Blood Cell (RBC) count and Hematocrit (Hct) are falsely decreased • Possibly high MCHC • May falsely increase Platelet (Plt) count	

Refer to the Red Cell Indices succeeding section of this procedure.

Step	Action
1.	Take a portion of the specimen and transfer it into another tube. Centrifuge the aliquot at 2000 RPM for 10 minutes. Observe plasma for the presence of hemolysis.
2.	• Review the results for historical correlation. • Review the slide for RBC morphology that may indicate clinical conditions.
3	• If there are no historical results and the morphology review does not indicate any hematological condition (e.g., sickle cell, etc), do not report any CBC result. Cancel test due to hemolysis. • If the morphology review shows clinical conditions, consider releasing CBC results.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

Lipemia & Icterus

Lipemia and icterus can falsely elevate Hemoglobin (HGB) and/or MCHC. The result will have a low or normal MCV with MCHC of **≥ 37.5 g/dL**.

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

Refer to the Red Cell Indices succeeding section of this procedure.

Intrinsic Morphological Specimen Conditions

Several conditions intrinsic to the specimen and related to its morphology can bring up RDW with an asterisk or as dashes. These can be seen in the following conditions:

• Anisocytosis	• Poikilocytosis
• Dimorphic RBC populations	• Fragmented RBCs
• Rouleaux formation	

Review morphology.

- Report RDW with the asterisk after confirming with slide review; no other abnormality was found.
- Report RDW as NM (Not Measured) if there’s no value.

Measurand	Hemolysis	Lipemia & Icterus	**Cold agglutinins**	**Severe cold agg**	**Dimorphic Pop & Rouleaux**
RBC	**Falsely decreased**		Possible asterisk(*)	Possible asterisk(*)	Possible asterisk(*)
Hgb		**Falsely elevated**	Possible asterisk(*)	Possible asterisk(*)	Possible asterisk(*)
Hct	**Falsely decreased**	Low or normal	Possible asterisk(*)	Possible asterisk(*)	Possible asterisk(*)
MCV	Low or normal	Low or normal	Falsely elevated/ Possible asterisk(*)	Falsely elevated/ Possible asterisk(*)	High; Possible asterisk(*)
MCH			Falsely elevated/ Possible asterisk(*)	Falsely elevated/ Possible asterisk(*)	Possible asterisk(*)
MCHC	Possibly high	**Falsely elevated**	>37.5	>37.5; Possible asterisk(*)	Possible asterisk(*)
RDW			Possible asterisk(*)	Possible asterisk(*)	Dashes, possible asterisk
Morphology		**Normal**	Red cell aggregates (grape-like clusters) resolved at 37C	Red cell aggregates (grape-like clusters) resolved at 37C	Anisocytosis, Rouleaux (stack of coins)

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

Troubleshooting Red Cell Indices

Consider the MCHC and the MCV together when evaluating results and the reasons for the interference. Differentiate the possible interference reasons based on MCV results.

Pattern of Results	Encountered in
Low or Normal MCV High MCHC (>37.5 g/dL)	• Hemolysis • Plasma electrolyte abnormalities (i.e., low sodium) affecting hematocrit results • Severe lipemia • Icterus • Severe leukocytosis affecting hemoglobin measurement • Abnormal plasma protein precipitation affecting hemoglobin measurement *Refer to the Troubleshooting Chart below*
High MCV High MCHC (>37.5 g/dL)	• RBC Agglutination • Rouleaux

I. Perform the following steps to identify and resolve interference manifesting **high MCV with high MCHC**:

Step	Action
1	Incubate the CBC sample 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 the resulting corrected values.
4	In 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 XN in Manual Analysis mode.
5	Coordinate the run on XN with WAM: a. Order a rerun in WAM. b. Scan the sample accession in the XN IPU. c. Mix the sample by gentle inversion at least 10 times. d. Run the sample in Manual Analysis mode.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

Troubleshooting Red Cell Indices, continuation:

6	A. If MCHC is corrected (≤ 37.5g/dL and no asterisk present), then the other indices have been corrected for cold agglutinin. • In the Rerun screen, enter the dilution factor (in whole numbers) in the Dilution Field under the appropriate run column. • Click the [CALC] button to apply the dilution factor to the results. • RBC, Hgb, and Hct will be recalculated based on the dilution. **NOTE:** RBC Indices results are unaffected by dilution and do not require correction. • Choose the appropriate run column for the rerun group. • Proceed to the result of the corrected values. Replace **WBC** and **PLT** results from the original run, then validate in WAM.

Result Validation | Rerun | Manual Differential | Morphology | Previous Results | Vi

Test Code		Result	Run 1		Run 2	Run 3
WBC		7.6	7.6		7.6	
RBC		4.55	4.53		4.55	
HGB		13.3	13.1		13.3	
HCT		39.3	39.2		39.3	
MCV		86.4	86.5		86.4	
MCH		29.2	28.9		29.2	
MCHC		33.8	33.4		33.8	
RDWCV		12.4	12.4		12.4	
PLT	F	52	56	F	52	
PLTF		#v				
NRBCRE		0	0		0	
NEUTRE		71.3				
LYMPRE		19.9				
MONORE		5.6				
EOSIRE		2.8				
BASORE		0.1				
NEUTAB		5.39				
LYMPAB		1.50				

Instrument GXN901 | GXN901
Dilution | Calc | Calc
Graphics | Graphics | Graphics

B. If MCHC is not corrected, proceed to the next steps for plasma replacement.

Continued on next page

Analytical Interferences on CBC Specimens
, Continued

Troubleshooting Red Cell Indices, continuation:

In cases with high cold agglutinin titers, a plasma replacement using warm CELLPACK DCL may be necessary to reduce the interference from the antibody.	
7	Pre-Warm a CELLPACK DCL at 37°C dry bath for at least 15 minutes.
8	Aliquot 1 mL of a well-mixed prewarmed specimen sample in a 12X75 mm tube.
9	Centrifuge the 1 mL aliquot for 2000 RPM for 10 minutes to separate plasma from red cells.
10	Using an MLA pipette, carefully remove the plasma without disturbing the buffy coat.
11	Change tip on MLA pipette and add back the same amount of removed plasma with Prewarmed CELLPACK DCL diluent.
12	Simultaneously mix and warm the sample for at least five (5) minutes, order a rerun and cycle through the analyzer in manual mode.
13	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.
14	If plasma replacement corrected the symptom, replace the WBC and Platelet with the original values since centrifugation will alter these parameters. Document on WAM under internal comment section *"Severe cold agglutinin, RBC and indices corrected by plasma replacement."*
15	If plasma replacement does not correct the symptom, request for a redraw where sample is kept at 37°C at all times.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

II. Perform the following steps to identify and resolve interference manifesting **low or normal MCV with high Hgb and MCHC**:

Step	Action
1.	Order a rerun in WAM. Then manually prepare a 1:5 dilution with Cellpack DCL and run on XN in Manual Analysis mode. If the results are valid, then validate in WAM. If dilution does not correct the problem, then perform plasma replacement. Proceed to the next step.
2.	Take a portion of the specimen and transfer it 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 the tip on the MLA pipette and replace the removed plasma with an equal amount of Cellpack DCL diluent.
6.	Mix the sample, order a rerun, and run through the analyzer.
7.	Use the WBC and RBC results obtained in step 1 as a guide to verify proper re-dilution of the specimen.
8.	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 reported from the results from step 1.
9.	Document in the report that "*Specimen was grossly lipemic/icteric, and that hemoglobin was corrected for lipemia/icterus.*"

Platelet Clumping

Follow the procedure below to process the specimens where:

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

And asterisks (*) appear next to PLT, MPV, and IPF results.

Continued on next page

Analytical Interferences on CBC Specimens

, Continued

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 Alerts 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 a PLT IP message,** 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 a PLT IP message,** 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** ⟶ IF NO, proceed to step 4

↓

1) Delete the platelet count in WAM.
2) Then double-click on the **PLT Result** area, and a window will pop up; choose **DNR;** this will be reported as **CLUMPING SEEN**. Click **SAVE**, do not put anything in the **Free Text** box.
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). Thrombocytopenia Panel [257863], which includes Immature Plt Fraction (IPF), which is an indicator of marrow recovery for platelets), can be ordered."
4) Validate the result. Note occasional platelet clumps in morphology as Few.

Continued on next page

Analytical Interferences on CBC Specimens

Controlled Documents

The following controlled documents support this procedure. Locally approved versions will have a different document number.

Document Number	Title
SCPMG-LIS-0089	Job Aid_Cerner Cancel Messages

Non-Controlled Documents

The following non-controlled documents support this procedure.

- Sysmex America, Inc. Lincolnshire, IL. *XN-Series Automated Hematology Systems Flagging Interpretation Guide*. Document Number: 1166-LSS, Rev. 6, March 2021.
- Sysmex America, Inc. Lincolnshire, IL. *XN-L Series Flagging Guide.* Document Number: 1399-LSS, Rev. 3, February 2021.

Author(s)

SCPMG Hematology Working Group
Eleanor E Callasan
Julius Salomon