# Resolving Pre-analytical CBC Sample Problems

**Principle**

The presence of cold agglutinins, lipemia, icterus, hemolysis and platelet clumping in the sample can cause erroneous results. These results must be corrected by the CLS 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.

**Materials and Reagents**

37°C Dry bath and timer
CELLPACK DCL
MLA pipette
12 x 75 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 & WAM 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 XN in Manual Analysis mode.<br><br>Follow steps below:<br>• Order a rerun in WAM.<br>• Scan the sample accession in the XN IPU.<br>• Mix the sample by gentle inversion at least 10 times.<br>• Run the sample in Manual Analysis mode. |

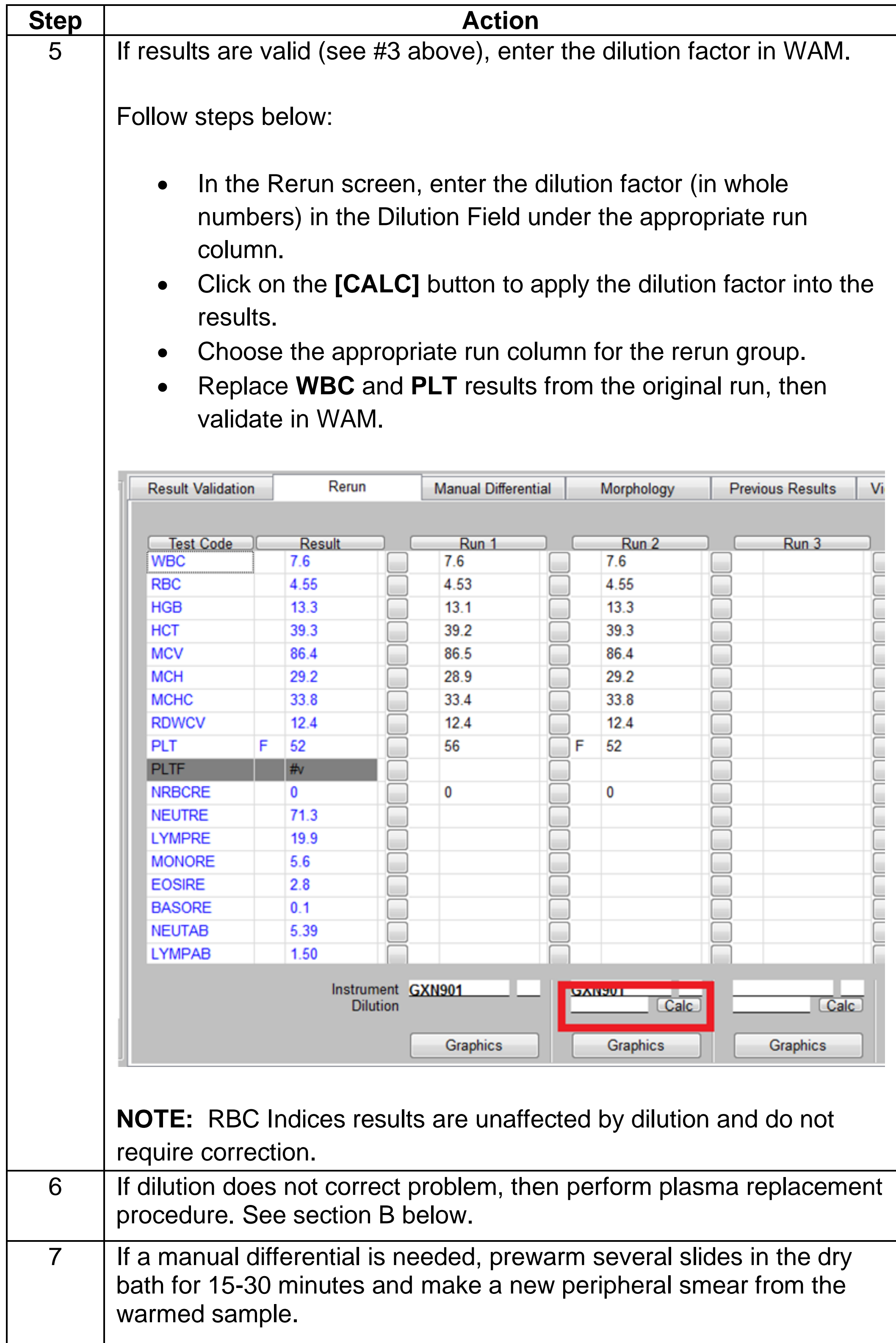
# Resolving Pre-analytical CBC Sample Problems

**Procedure,** continued

| Step | Action |
|---|---|
| 5 | If results are valid (see #3 above), enter the dilution factor in WAM. |

Follow steps below:

- In the Rerun screen, enter the dilution factor (in whole numbers) in the Dilution Field under the appropriate run column.
- Click on the **[CALC]** button to apply the dilution factor into the results.
- Choose the appropriate run column for the rerun group.
- Replace **WBC** and **PLT** results from the original run, then validate in WAM.




**NOTE:** RBC Indices results are unaffected by dilution and do not require correction.

| Step | Action |
|---|---|
| 6 | If dilution does not correct problem, then perform plasma replacement procedure. See section B below. |
| 7 | If a manual differential is needed, prewarm several slides in the dry bath for 15-30 minutes and make a new peripheral smear from the warmed sample. |

# Resolving Pre-analytical CBC Sample Problems

**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 12X75 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 the 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 WAM under comment section *"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/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 & WAM will have an alert message of Turbidity/HGB Interference? And asterisks (*) appear next to HGB, MCH 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 results are valid then validate in WAM. 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. |

# Resolving Pre-analytical CBC Sample Problems

**Procedure**, continued

| | |
|---|---|
| 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 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 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. |

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

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

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

# Resolving Pre-analytical CBC Sample Problems

**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

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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).

- **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.

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

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**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.

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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

YES ↓

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**. Click **SAVE**, do not put anything on 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). Suggest Citrated Plt Count order."**
4) Do platelet count on citrate (if there's an order).

**NOTE:** Presence of small occasional clumping is fine.

NO ↓

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

# Resolving Pre-analytical CBC Sample Problems

**Non-Controlled Documents**

These non-controlled documents support this procedure:

A. Sysmex XN-3100 Instructions for Use (North American Edition), Sysmex Corporation, Kobe, Japan.
B. Sysmex XN Series Flagging Guide 1166-LSS, Rev.4 3/2018.
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.