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| **Department:** | Hematology | **Effective Date:** |  |
| **Author:** | Kim Clark MT(ASCP)  R.J. Bernshausen MT(ASCP) | **Version:** | 1.0 |

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**CLINICAL SIGNIFICANCE:**

Hematology analyzers provide quick and accurate results in most situations. However, spurious results, related either to platelets or to other parameters from the cell blood count (CBC) may be observed in several instances. Spuriously low white blood cell (WBC) counts may be observed because of agglutination in the presence of ethylenediamine tetra-acetic acid (EDTA). Cryoglobulins, lipids, insufficiently lysed red blood cells (RBC), erythroblasts and platelet aggregates are common situations increasing WBC counts. In most of these instances flagging and/or an abnormal WBC differential scattergram will alert the operator.

Several situations lead to abnormal hemoglobin measurement or to abnormal RBC count, including lipids, agglutinins, cryoglobulins and elevated WBC counts. Mean (red) cell volume (MCV) may be also subject to spurious determination, because of agglutinins, excess of glucose or salts and technological considerations. In turn, abnormality related to one measured parameter will lead to abnormal calculated RBC indices: mean cell hemoglobin content (MCHC) is certainly the most important RBC indices to consider, as it is as important as flags generated by the hematology analyzers (HA) in alerting the user to a spurious result. In many circumstances, several of the measured parameters from CBC may be altered, and the discovery of a spurious change on one parameter frequently means that the validity of other parameters should be considered. Sensitive flags now allow the identification of several spurious counts, but only the most sophisticated HA have optimal flagging and more simple HA, especially those without a WBC differential scattergram, do not possess the same sensitivity for detecting anomalous results. Reticulocytes are integrated now into the CBC in many HA, and several situations may lead to abnormal counts.

**PRINCIPLE:**

Several factors affect the results given by the automated hematology analyzers. It is up to the operator to recognize the abnormal flags generated by the analyzers, and correction methods that can be employed. Please see the charts at the end, to help in describing the flagging that occurs with the Siemens’ ADVIA 2120i and ADVIA 120.

**Platelet Aggregation:**

Platelet clumping may give aggregates large enough to be counted as WBC’s which causes a spurious elevation of the total white blood count. These cases are associated with a spurious decrease and the presence of platelet clumps on the peripheral blood smear. The hematology analyzer will flag platelet clumps. Since most of these cases are due to the presence of EDTA dependent platelet agglutinins, there are two correction methods to try:

* Vortexing the specimen vigorously for one minutes, may break up the platelet clumps. This specimen is then repeated on the analyzer. Only the total WBC count and the platelet count may be used from this vortexed specimen.
* A sodium Citrate tube may be collected and ran on the hematology analyzer in place of the EDTA specimen. **Note:** the platelet count must be multiplied by 1.1 if this method is employed. This takes into account the dilution of the sodium citrate in the collection tube.

**Note:** When platelet clumps or platelet satellitism are present, the platelet count from the **analyzer must not be reported**. Please TNP the analyzer platelet count, with a comment that the platelets are clumped. Under the differential, give a platelet estimate. If the platelets appear numerous, but in clumps that give an erroneous decreased result, you can report that the platelets appear adequate, but in clumps.

**Nucleated Red Blood Cells:**

The presence of NRBC’s can give a falsely elevated WBC count. The new hematology analyzers can now perform a differential that will separate the NRBC’s from the total WBC count. A careful observation of the differential scattergram will determine if the NRBC’s are counted in the total WBC count or are omitted. If they are part of the total WBC count, the following formula is used during the manual differential to give a corrected WBC count.

Corrected WBC = Instrument WBC count x 100

100+NRBC’s counted per 100 WBC’s

**Cryoglobin:**

The presence of cryoglobin and/or cryofibrinogen is a sample can affect the WBC count. Examination of a Wright’s stained smear in such cases will show aggregates of amorphous material and occasionally large crystals. A correct WBC count may be obtained by warming the sample to 37°C.

**Abnormal Proteins:**

Monoclonal proteins in high concentrations (myeloma or macroglobulinemia) may precipitate with lysing reagents. This will cause turbidity which may elevate the WBC and HGB. Peripheral blood smears will show prominent rouleaux formation.

Verify the WBC count by comparing with a slide estimate on a Wright’s stained smear. Using the 40x high dry power lens, count at least 10 high power fields. Use the chart below to estimate the WBC count.

**Table: CH040-01**

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| **Average Leukocytes/HPF** | **Estimated Total Leukocyte Count** |
| 2-4 | 4,000-7,000/cumm |
| 4-6 | 7,000-10,000/cumm |
| 6-10 | 10,000-13,000/cumm |
| 10-20 | 13,000-18,000/cumm |

Do not report the WBC count from the analyzer if there is a significant discrepancy between the analyzer and the slide estimate. In this case report the WBC as TNP (do not report auto-differential). Attach a comment to the TNP stating the WBC slide estimate value.

**Cold Agglutinins:**

Cold agglutinins will increase the MCV while decreasing the HCT and RBC. Cold agglutinin interference can usually be corrected by placing the CBC specimen in a 37°C water bath or heat block for 10-20 minutes. The specimen must be tested as quickly as possible after warming. Use the following procedure if warming in a water bath does not correct the problem:

1. Run the specimen normally
2. Centrifuge an aliquot of the specimen
3. Mark the meniscus and remove the plasma from the specimen
4. Replace the plasma with warm (37°C) saline
5. Re-suspend the specimen and run it through the analyzer
6. Verify the diluting results. The WBC and platelet counts should compare between both specimens
7. Use the original WBC, Platelet, and MPV results for the patient report
8. Use the RBC and RBC parameters from the warmed specimen.
9. If this does not correct the agglutination, repeat incubation of the undiluted specimen for up to 1 hour at 37°C.

In some instances, no amount of incubation will reverse the agglutination. If this is the case report only the WBC, HGB and Platelet count. Verify both the WBC and the Platelet count on the peripheral smear. Use canned text to indicate that cold agglutination was not reversible. Use TNP as the result for the RBC, HCT and associated indices.

**Lipemia:**

Lipemia will falsely elevate the HGB and the MCHC. Determine if the specimen is lipemic by inquiring from chemistry department or centrifuging the specimen.

To correct the RBC parameters for lipemia, you can substitute:

* Hemoglobin: Replace with the cellular hemoglobin result
* MCHC: Substitute with the CHCM result
* MCH: Substitute with the CH result

**NOTE:** Please document results if corrected for lipemia.

In rare instances, the degree of lipemia will interfere with the cellular hemoglobin results. In this case, perform a saline replacement procedure using the following steps:

1. Centrifuge an aliquot of the specimen
2. Mark the meniscus and remove the plasma from the specimen
3. Replace the plasma with normal saline
4. Re-suspend the specimen and run it through the analyzer
5. Verify the results by comparing the WBC and platelet counts from the original specimen to the results from the “saline replacement” specimen. These results should agree fairly closely. For example the WBC and Platelet counts should usually agree within 20
6. Use the original WBC, Platelet, and MPV results for the patient report

Use the RBC, HGB and RBC parameters from the specimen where saline replacement was performed.

**Hemolysis:**

Hemolysis will decrease the RBC count. For specimens that are hemolyzed, request a recollect for testing. If it is determined that the hemolysis is occurring in vivo , report the values from the analyzer and attach a message that the specimen was hemolyzed. Hemolysis appears to be in vivo.

**Abnormal Blood Chemistry**

Changes in certain blood chemistries, especially blood urea nitrogen (BUN), sodium and glucose can cause a temporary shrinkage or swelling of erythrocytes when the cells are mixed with an isotonic solution. These changes will result in either falsely decreased or , more commonly, falsely increased MCV (Mean cell Volume ) values when the blood specimen is processed on a hematology analyzer.

These changes are usually detected either due to a large change (delta) in the MCV value from a previous sample or RBC indice values not correlating properly. If it is determined that this is a problem, follow the steps below:

* Add 200 µL of well-mixed whole blood and 200 µL of normal saline to a 12 x 75 mm plastic tube that has been appropriately labeled.
* Cap the plastic tube containing the dilution, invert several times to mix.
* Allow the dilution to incubate for 5 minutes at room temperature.
* Process the dilution in the manual open tube mode.
* Use the HGB as a q.c. check for the dilution process. The correction for the diluted HGB (i.e. 2 times the value) should agree with the original HGB =/- 0.5 g/dL.
* Report the original WBC, RBC, HGB and platelet. Report the HCT and RBC indices from the diluted specimen, **do not correct for the dilution for these values.**

It is the responsibility of the technologist to review the analyzer results for each CBC tested, to ensure there are no spurious results released.

# **References**

1. Siemens HealthCare Diagnostics, ADVIA ®2120*i* / ADVIA® 120 Operator’s Guide, V4.00.00, 2008

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|  | **CH40-02**  **Sample System Flags** | | | | |
| **ADVIA 120 Code** | **ADVIA 2120i  Code** | | **Description of Flag** | | **Action** |
| **BTO** | **BTO** | | BASO Chamber Temp Out | | Troubleshoot problem then Verify  Differential if needed |
| **CC** | **CHCMCE** | | MCHC-CHCM Comparison Error | | Analyze sample for lipemia, cold aggl or high WBC. Correct HGB |
| **TX** | **PXTO** | | PEROX Chamber Temp Out | | Troubleshoot problem then Verify  Differential if needed |
| **RR** | **RBCIFR** | | RBC Irregular Flow Rate | | Rerun sample, Accept if No CC |
| **HR** | **HGBIFR** | | HGB Irregular Flow Rate | | Rerun sample, Accept if No CC |
| **PH** | **HGB-PL** | | HGB Lamp Low | | Accept if No CC |
| **NW** | **PLT-CLM** | | Platelet Clumps | | Verify PLT Count if platelets decreased |
| **NT** | **PLT-NO** | | PLT Noise | | Verify PLT Count if platelets decreased |
| **OT** | **PLTORN** | | PLT Origin Noise | | Verify PLT Count if platelets decreased |
| **VX** | **PX-NV** | | PEROX No Valley | | Verify Differential |
| **CT** | **RTCint** | | Platelet threshold questionable | | Manual Retic |
| **CR** | **RTCIFR** | | Retic Irregular Flow Rate | | Manual Retic |
| **FC** | **RTC-FS** | | Retic Abnormal Distribution | | Manual Retic |
| **NO** | **RTC-NO** | | Noise at retic Origin | | Manual Retic |
| **CL** | **RTC-L** | | Cells analyzed <10,000 | | Manual Retic |
| **RF** | **RTC-FL** | | Retic Irregular Flow Rate | | Manual Retic |
| **CS** | **RTCSAT** | | Cells in saturation >10% | | Manual Retic |
| **SE** | **RTC-SE** | | Abnormal Distriubtion of Retic RBCs | | Manual Retic |
| **PX** | **PX-PL** | | PEROX Light Intensity Low | | Change perox lamp |
| **XS** | **PX-SAT** | | PEROX Saturation | | Rerun sample |
| **PL** | **LAS-PL** | | Laser Light Intensity Low | | Manual Differential |
| **MPO-D** | **MPO** | | Myeloperoxidase deficiency | | Manual Differential |
| **WC** | **WBC-CE** | | WBCB and WBCP do not match | | Rerun sample, Verify Differential |
| **WS** | **WBCSUB** | | WBC Substitution Rule was used. | | Rerun sample, Verify Differential |
| **BC** | **B-SUSP** | | BASO Count Suspect | | Verify Differential |
| **BR** | **B-IRF** | | BASO Irregular Flow Rate | | Rerun sample |
| **NB** | **B-NO** | | BASO Noise | | Rerun sample |
| **VB** | **B-NV** | | BASO No Valley | | Ignore this flag |
| **BS** | **B-SAT** | | BASO Saturation | | Rerun sample |
|  | **NRCELL** | | Suspect Cellular Interference - Unlysed RBCs | | Manual Differential |
| **NR-LPD** | | Suspect Lipid Interference | | Verify Differential | |
| **NRLPLT** | | Suspect Large Plt Interference | | Verify Differential | |
| **NRPXNV** | | No Perox NRBC/Lymph Valley | | Verify Differential | |
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**CH40-03**

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|  |  | **Morphology Flags** |  |
|  | **Code** | **Description of Flag** | **Action** |
|  | **ANISO 2+** | RDW Increased | Scan RBC Morphology |
|  | **MICRO 2+** | MICRO Increased | Scan RBC Morphology |
|  | **MACRO 2+** | MACRO Increased | Scan RBC Morphology |
|  | **HYPO 2+** | HYPO Increased | Scan RBC Morphology |
|  | **HYPER 2+** | HYPER Increased | Scan RBC Morphology |
|  | **RBCF** | RBC Fragments present | Scan RBC Morphology |
|  | **RBCG** | RBC Ghosts present | Scan RBC Morphology |
|  | **ATYP 1+** | LUC Increased | Verify Differential |
|  | **BLASTS 1+** | % Blasts Increased | Manual Differential |
|  | **IG 1+** | Immature Granulocytes | Manual Differential |
|  | **LS 1+** | Bands | Manual Differential |
|  | **MO 1+** | Myeloperoxidase deficiency | Manual Differential |
|  | **LPLT 2+** | Large Platelet | Verify PLT Count if platelets decreased |