# Sysmex® XN-Series Automated Hematology Systems

## Flagging Interpretation Guide

**Document Number: 1166-LSS, Rev. 3**

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This manual was created by Sysmex America, Inc. Questions or comments regarding the content of this manual can be directed to:

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## Introduction

The XN-Series Flagging Interpretation Guide is designed to serve many objectives including:

- Providing users with an explanation of criteria used for the XN-Series Interpretive Program (IP) Messages.
- Suggesting actions to be taken when samples generate IP Messages.
- Suggesting actions to resolve sample related problems.

The following sections introduce the IP Messages. Definitions and examples of each message are presented with suggested actions to be taken by qualified personnel to verify the presence of specific cell types and obtain a correct result when interference occurs. These action steps are merely suggested guidelines and not requirements. Always follow your local laboratory procedures for repeat testing or confirmation of results.

The XN-Series analyzers are designed to aid in the separation of specimens into POSITIVE and NEGATIVE categories according to preset criteria. The system bases its judgments on comprehensive surveys of numerical data, particle size distributions, and scattergrams and provides easy-to-understand flags and messages indicating the analyzer's findings. These flags and messages are referred to as IP Messages. The IP Messages may be classified as either Suspect IP Messages or Abnormal IP Messages. The IP Messages generated by the analyzer determine if the sample is judged as POSITIVE or NEGATIVE.

Suspect IP Messages are generated by analyzer software algorithms. Abnormal IP Messages are based on numerical user defined settings.

A specimen is judged NEGATIVE when there are no IP messages generated. NEGATIVE does not necessarily indicate a normal sample; however, the results are generally reported without review.

The XN-Series analyzers will generate a POSITIVE when an IP Message is present. ERROR will be generated when there is an analysis error. These judgments indicate the possibility of sample abnormality. These results should be reviewed carefully and may require further examination in accordance with your local laboratory protocol.

All analyzer flags, error messages and results must be interpreted together and in consideration of the patient's clinical condition prior to results being reported from the laboratory. Any asterisk (*) next to a parameter indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting. Protocols for comparison of current results to previous results (delta checking) as well as critical value alerts are also useful for identifying potentially erroneous results prior to reporting to the clinician.

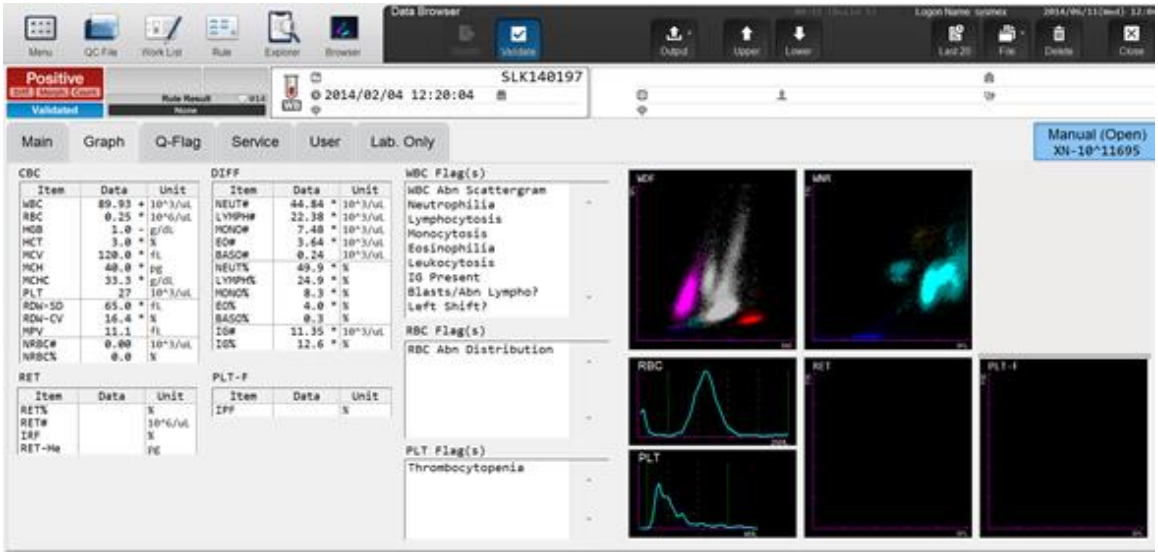
NOTE: System configuration, analysis mode and discrete testing mode used may determine availability of certain IP messages. Refer to the Instructions for Use for detailed list of IP Messages.

## Abnormal, WBC Abn Scattergram

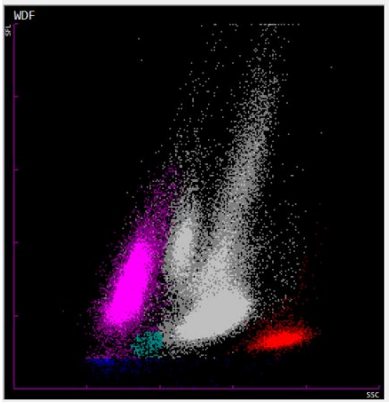
The WBC Abn Scattergram IP message is generated whenever clustering in the WNR or WDF scattergrams is abnormal. Dashes may appear in place of data that was not calculated.

In the example below, clustering failed between the Monocytes, Immature Granulocytes and the Neutrophils on the WDF scattergram.

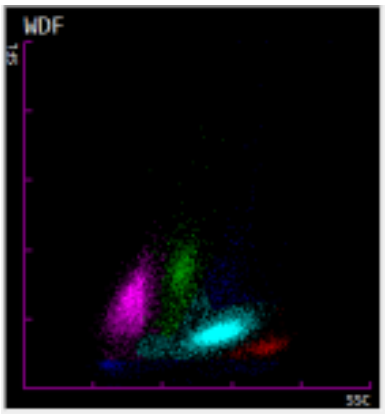
XN-Series Results




Abnormal WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)




NOTE: If the analyzer has reported the WBC from the WDF channel, the WBC result will have the “&D” indicator adjacent to it.

**Abnormal, WBC Abn Scattergram**
**(continued)**

Suggested Action Steps:

1. Dashes (— —) in place of numeric data:
    - Verify WBC, NRBC and differential results according to your laboratory's policy. Possible actions may include:
        - repeating the sample
        - performing a manual differential
2. Asterisk (*) next to results:
    - Verify WBC, NRBC and differential results according to your laboratory's policy. Possible actions may include:
        - scanning the slide for abnormal cells or platelet clumping and to estimate the WBC and NRBC counts
        - performing a manual differential if abnormal cells are observed
    - If no abnormalities are found when reviewing the smear and the WBC and NRBC estimates match the analyzer reported WBC and NRBC, the results with asterisks (*) may be reported.

## NRBC Present Message

The threshold for the NRBC Present message is user defined and programmable. NRBC Present only appears when the NRBC result (NRBC/100 WBC) exceeds the programmed limit.

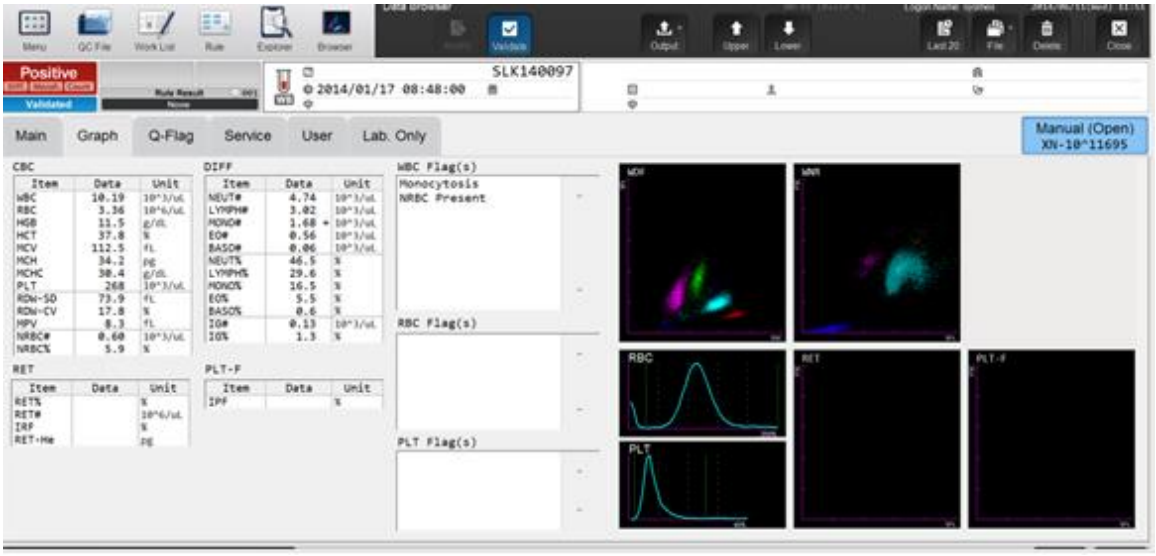
The thresholds for this message must be set regardless of whether the lab is using Sysmex WAM™ or other middleware products. Good laboratory practice is to set all analyzer user defined flag limits to the same limits used in Sysmex WAM or other middleware products in use by the laboratory.

The NRBC Present message alerts the operator to the presence of cells accurately quantitated by the analyzer. When this message is present, it is recommended to review a smear to detect other clinically relevant findings and report the analyzer NRBC results. If indicated based on smear review, comment on abnormal morphology or other clinically relevant findings as described in your laboratory protocol.

The XN-Series analyzers identify and count NRBCs simultaneously while counting WBCs. No further correction of the WBC count is required.

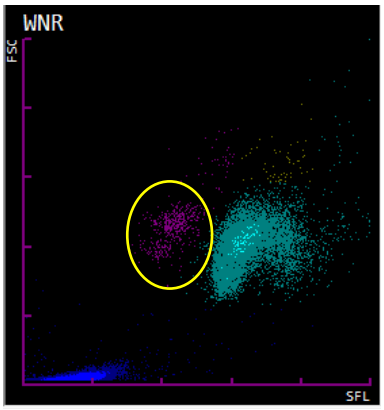
If NRBCs are greater than 0.01/100 WBC, the lymphocyte counts are corrected.

XN-Series Results

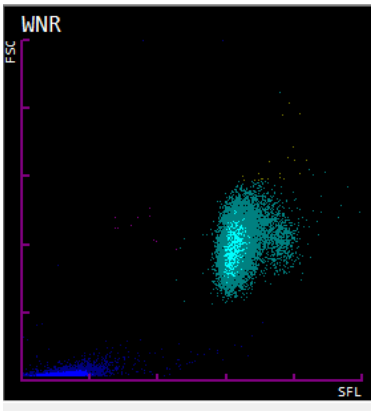


## NRBC Present Message (continued)

Abnormal WNR Scatter (Close-Up)

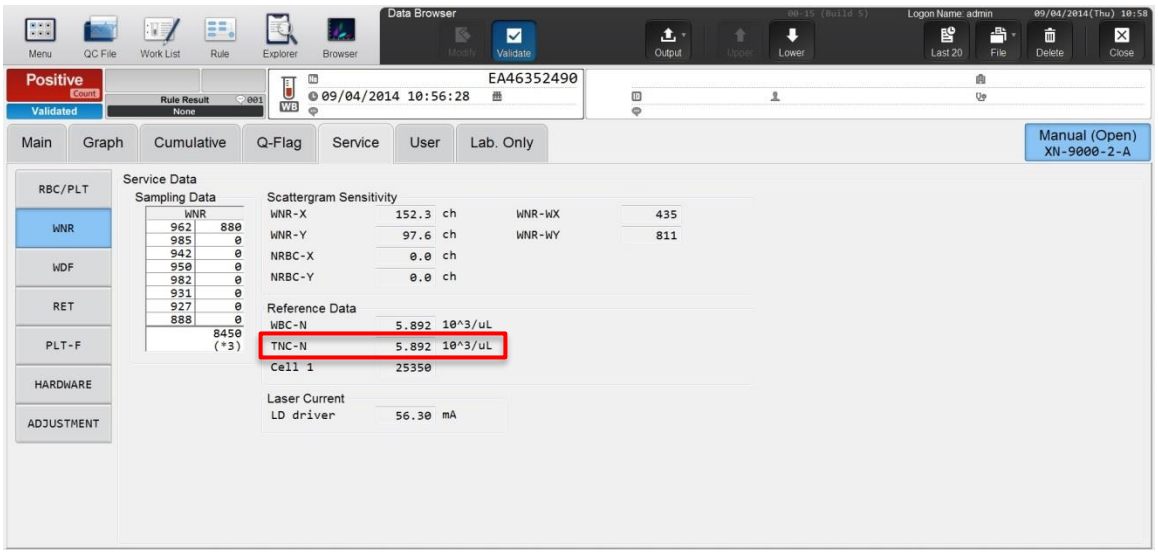



Normal WNR Scatter (Reference)




(NRBC population circled)

An uncorrected WBC count can be found in the Service/WNR tab of the Browser Screen if needed. Use the TNC-N as the uncorrected WBC count.




NOTE: Results from the Service Tab are not directly reportable by the laboratory and must be confirmed first.

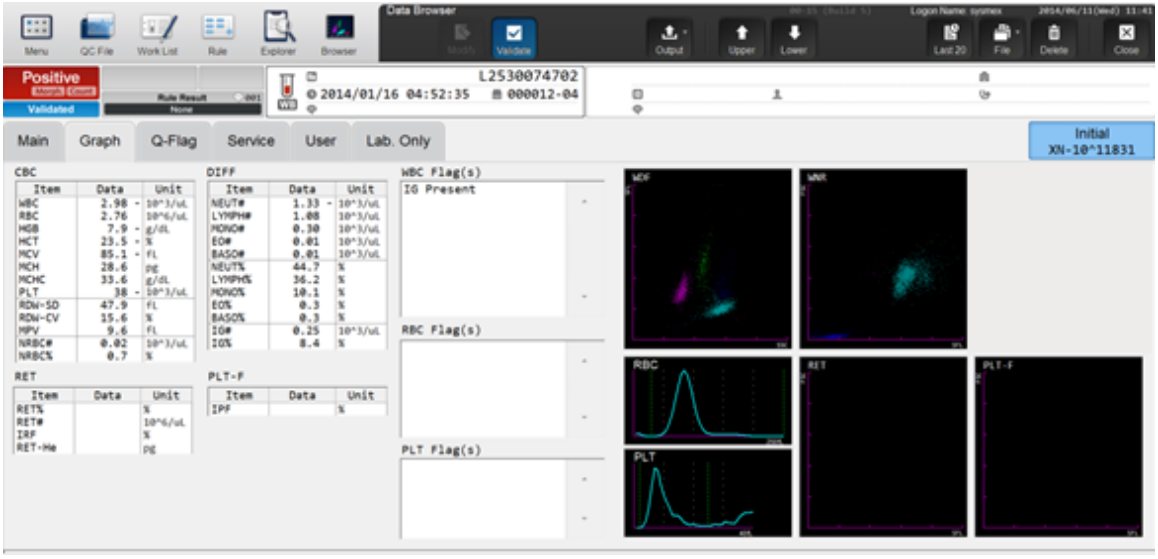
## IG Present Message

XN-Series analyzers report a 6-part differential that is comprised of Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Immature Granulocytes. The Immature Granulocyte % / # results include metamyelocytes, myelocytes and promyelocytes.

The threshold for the IG Present message is user defined and programmable. It only appears when the IG% or IG# exceeds the programmed limit. It is suggested that the IG Present message threshold be set at 5% or 0.5 x10^3/uL.

The IG Present message alerts the operator to the presence of cells accurately quantitated by the analyzer. When this message is present, it is suggested to review a smear to detect other clinically relevant findings and report the analyzer differential results. If indicated based on smear review, perform a manual differential or comment on clinically relevant findings as described in your laboratory protocol.

XN-Series Results



## IG Present Message (continued)

Suggested Action Steps:
When the IG Present message is displayed:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
    - immature granulocytes – promyelocytes, myelocytes and metamyelocytes
    - band cells in increased numbers
    - toxic granulation or vacuolation of neutrophils
    - other abnormal cells

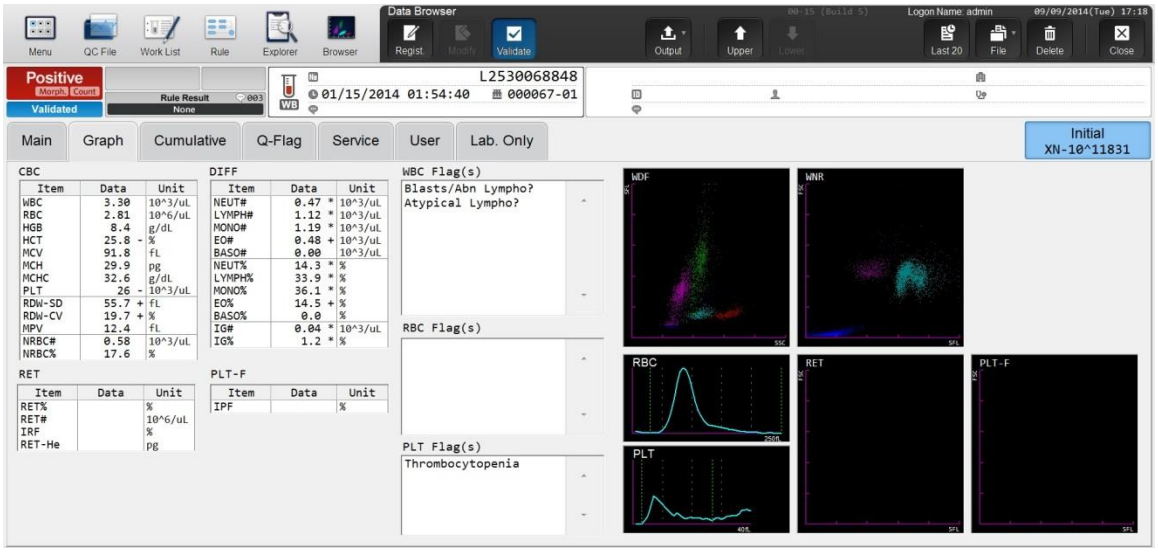
    Report any abnormal cells according to your laboratory protocol.
2. If the IG% or IG# has an asterisk (*), verify differential results according to your laboratory's policy. Possible actions may include:
    - scanning the slide for abnormal cells
    - performing a manual differential if abnormal cells are observed
3. If no abnormalities are found when reviewing the smear, the results with asterisks (*) may be reported.

## Suspect, Blast / Abn Lympho?

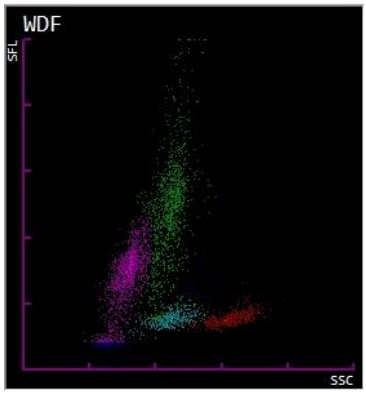
The Blast / Abn Lympho? IP message indicates that the analyzer has detected abnormal clustering in the region for blasts and abnormal lymphocytes in the WDF scattergram.

An asterisk (*) appears next to the Neutrophil, Lymphocyte, Immature Granulocyte and Monocyte % and #. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

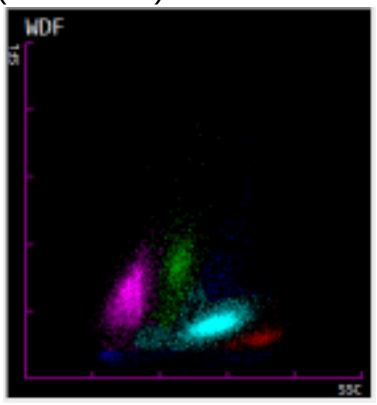
XN-Series Results




WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)



**Suspect, Blast / Abn Lympho?**
**(continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
    - blasts – lymphoblasts, myeloblasts, and myelomonoblasts
    - immature granulocytes – promyelocytes, myelocytes, metamyelocytes
    - atypical or immature lymphocytes
    - other abnormal cells

   Report any abnormal cells according to your laboratory protocol.

   NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as blasts and other large cells may migrate to this area during smear preparation.

2. If no abnormalities are found, the results with the asterisk (*) may be reported.
3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
    - repeating the sample
    - performing a manual differential

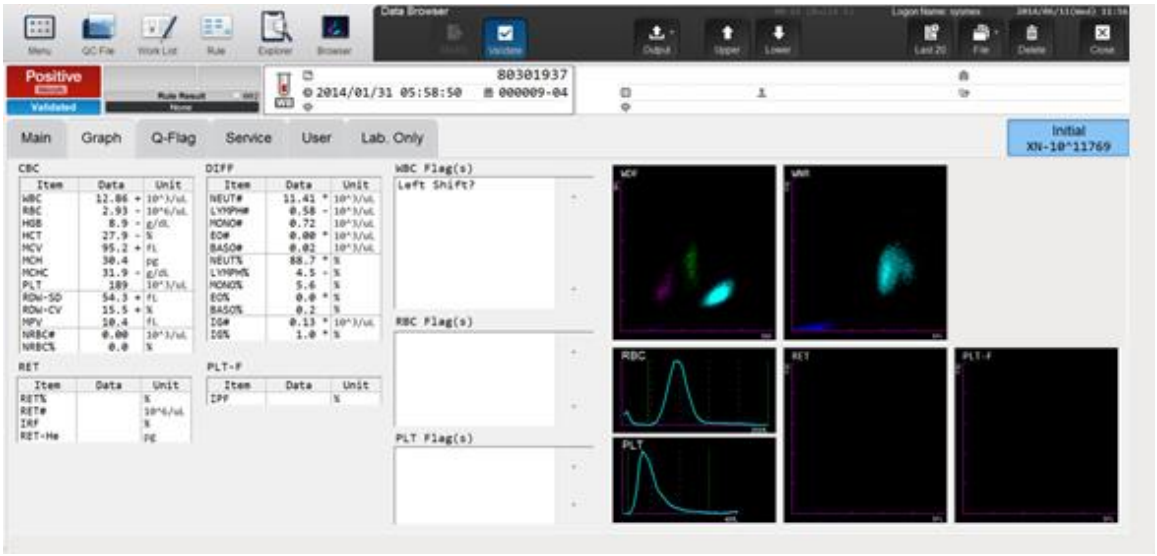
**Suspect, Left Shift?**

The Left Shift? IP message indicates that the analyzer has detected abnormal clustering in the region for left shift (bands) in the WDF scattergram. When bands are present, they are included in the neutrophil population.

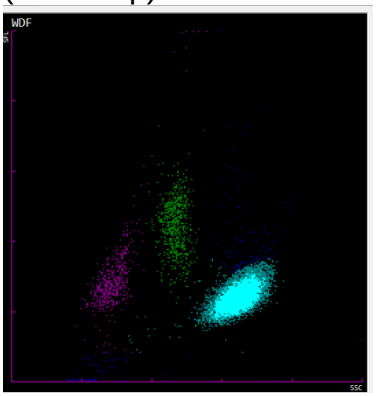
If the WBC is <0.50 x $10^3$/µL in the Whole Blood (WB) mode or <0.20 x $10^3$/µL in the Low WBC (LW) mode the, Left Shift? IP flag will not be generated.

An asterisk (*) appears next to the Neutrophil and Eosinophil % and #. The IG% and IG# may also have an asterisk. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

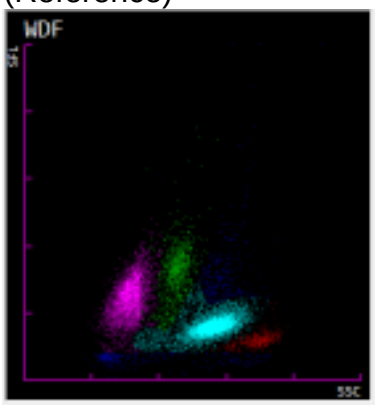
XN-Series Results




WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)



**Suspect, Left Shift?**
**(continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
    - band cells in increased numbers
    - toxic granulation or vacuolation of neutrophils
    - other abnormal cells

   Report any abnormal cells according to your laboratory protocol.

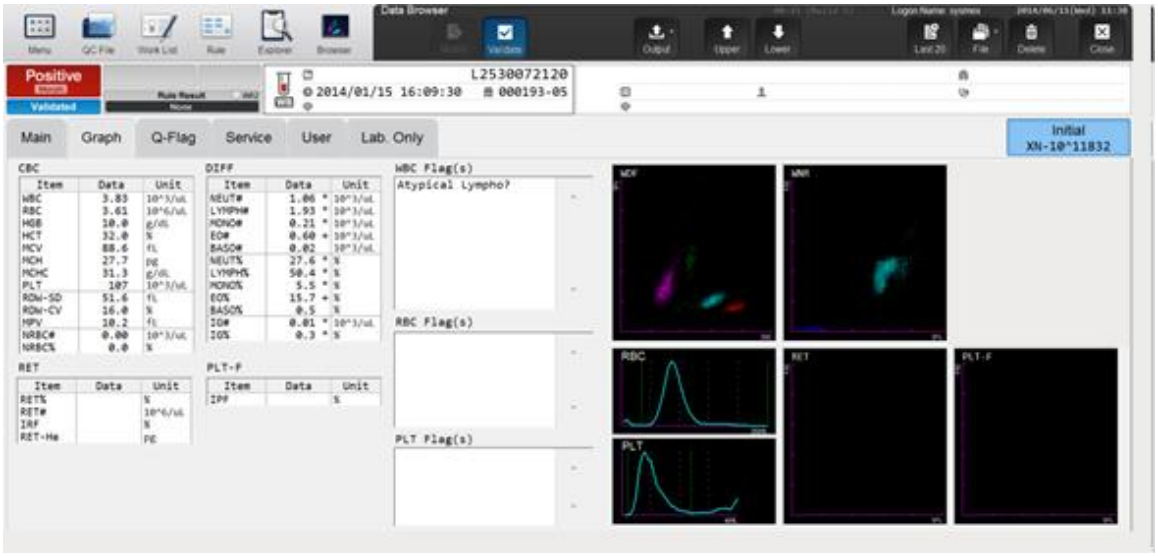
2. If no abnormalities are found, the results with the asterisk (*) may be reported.
3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
    - repeating the sample
    - performing a manual differential

## Suspect, Atypical Lympho?

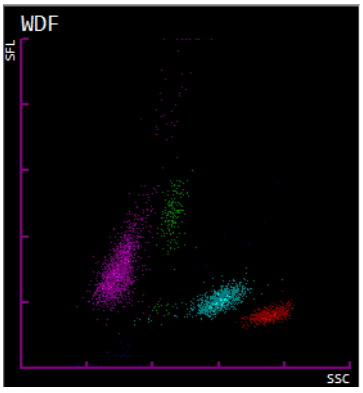
The Atypical Lympho? IP message indicates that the analyzer has detected significant clustering in the region for atypical lymphocytes that is located in the upper left lymphocyte region on the WDF scattergram.

An asterisk (*) appears next to the Neutrophil, Lymphocyte, Monocyte, Eosinophil and Immature Granulocyte % and #. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

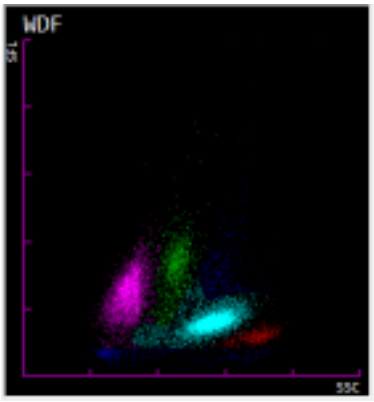
XN-Series Results




WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)



**Suspect, Atypical Lympho?**
**(continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
    - atypical or variant lymphocytes
    - abnormal or atypical monocytes
    - immature lymphocytes, such as seen in ALL or CLL
    - immature monocytes
    - smudge cells
    - other abnormal cells

    Report any abnormal cells according to your laboratory protocol.

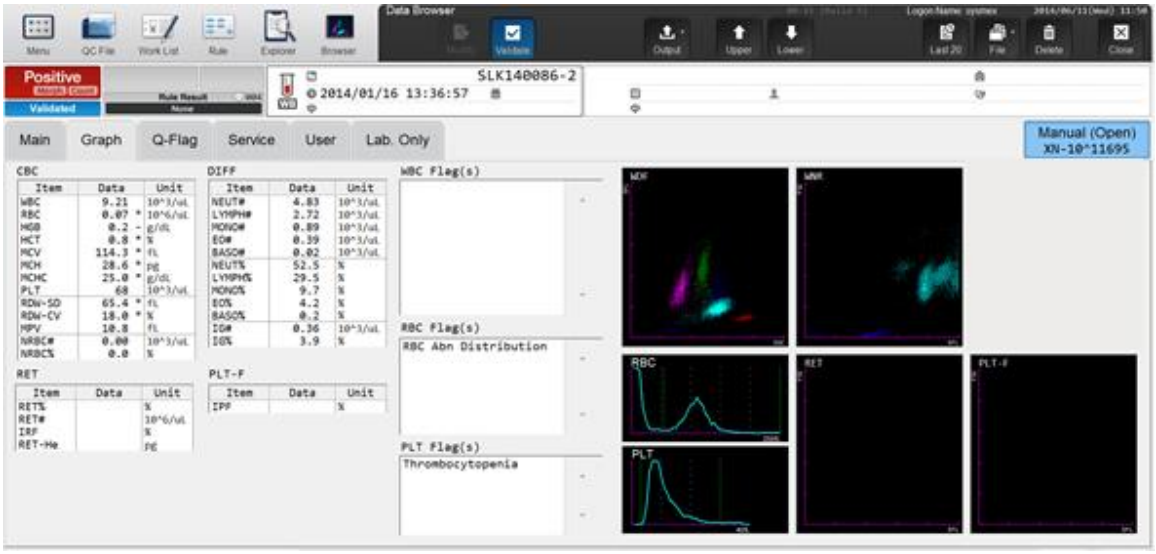
2. If no abnormalities are found, the results with the asterisk (*) may be reported.
3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
    - repeating the sample
    - performing a manual differential

## Abnormal, RBC Abn Distribution

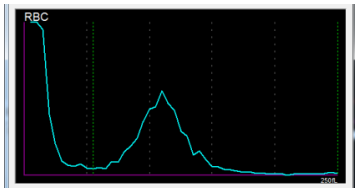
The RBC Abn Distribution IP Message is generated when the histogram pattern from the RBC channel is abnormal or when RBC < 0.50 x $10^6$/µL. Judgment for RBC flags other than RBC Abn Distribution is not performed when this flag is generated.

Dashes appear in place of affected results. For example, if there are multiple peaks present on the RBC histogram, there would be dashes in place of results for the RDW-SD and RDW-CV. Sometimes this IP Message can cause the RDW-SD and RDW-CV to be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

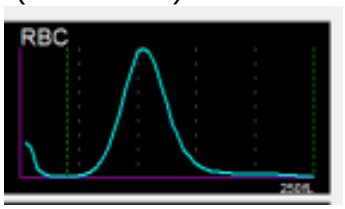
XN-Series Results




RBC Histogram (Close-Up)




Normal RBC Histogram (Reference)



**Abnormal, RBC Abn Distribution (continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of abnormal RBC morphology such as:
    1. increased anisocytosis
    2. multiple RBC populations
    3. fragmented RBCs
    4. poikilocytosis
    5. rouleaux or RBC agglutination (refer to suggested action for “RBC Agglutination?” if present)

    Report any abnormal RBC morphology according to your laboratory protocol.

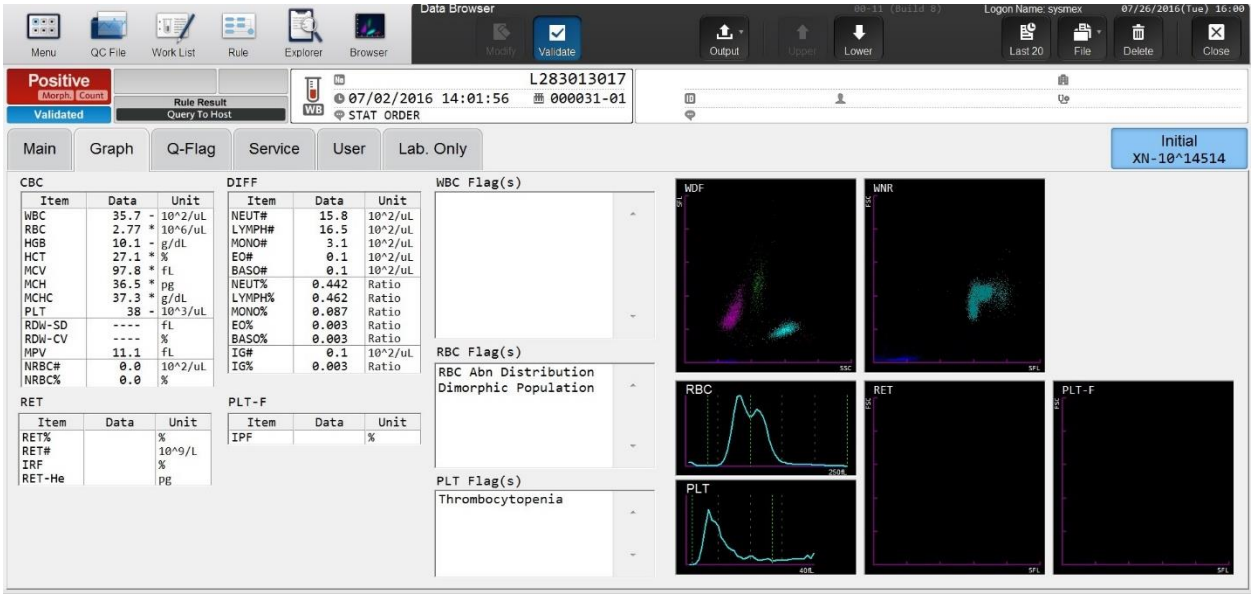
2. If no abnormalities are found, the results with the asterisk (*) may be reported.
3. If dashes (— —) are in place of numeric data, verify results according to your laboratory’s policy. Possible actions may include repeating the sample or reporting RBC morphology from smear review.
    a. Follow your laboratory protocol for handling suppressed or unreportable results in your Laboratory Information System (LIS), if dashes (— —) are displayed in place of numeric data. This is addressed in some LIS by using a code of “HIDE” or “Not Measured” in place of the results that are suppressed.
4. If the RBC morphology is normal and the MCHC is abnormal (<30 or >37.5 g/dL) an interfering substance or condition may be present. Refer to the suggested guidelines for the HGB/Turbidity Interference? IP Message.

## Abnormal, Dimorphic Population

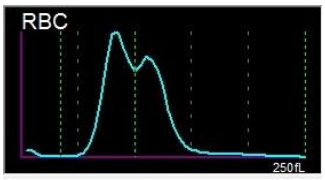
The Dimorphic Population IP Message is generated when there are multiple peaks in the RBC histogram pattern. This message may occur with the RBC Abn Distribution IP Message.

Dashes appear in place of results for the RDW-SD and RDW-CV. This message may cause certain RBC parameters to be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

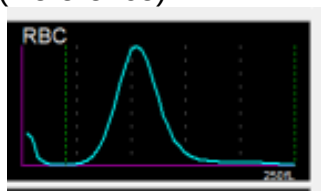
XN-Series Results




RBC Histogram (Close-Up)

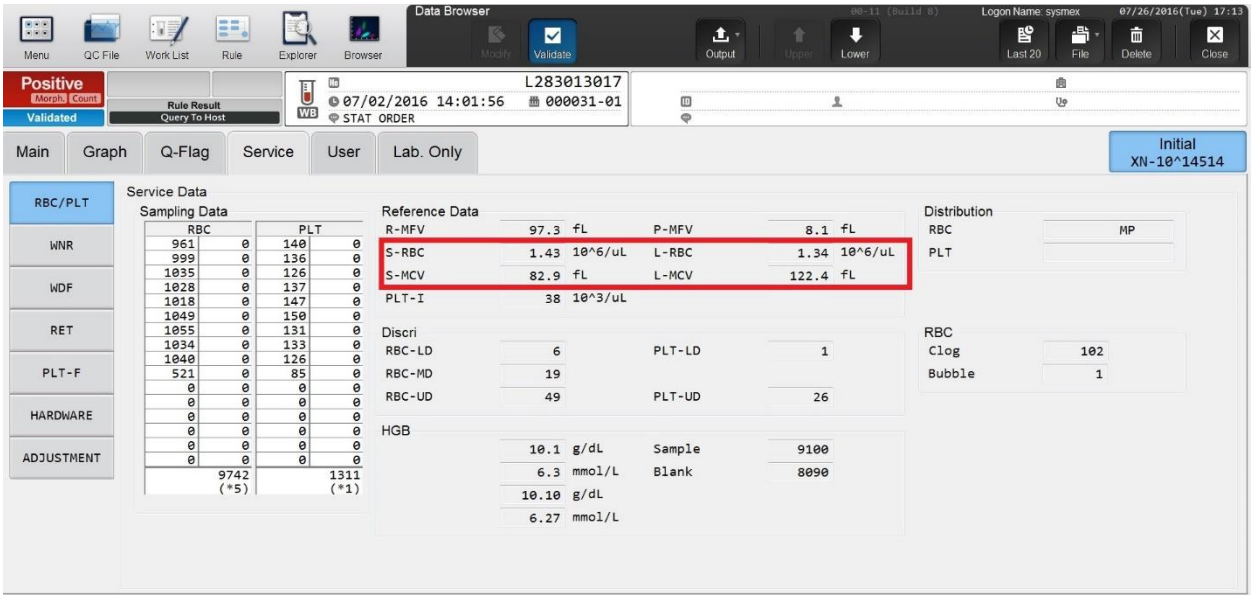



Normal RBC Histogram (Reference)



## Abnormal, Dimorphic Population (continued)

The RBC count and MCV for the two populations shown on the RBC histogram can be found in the Service/RBC/PLT tab of the Browser Screen if needed.




NOTE: Results from the Service Tab are not directly reportable by the laboratory and must be confirmed first.

**Abnormal, Dimorphic Population**
**(continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of abnormal RBC morphology such as:
    - increased anisocytosis
    - multiple RBC populations
    - fragmented RBCs
    - poikilocytosis
    - rouleaux or RBC agglutination (refer to suggested action for “RBC Agglutination?” if present)

    Report any abnormal RBC morphology according to your laboratory protocol.

2. If no abnormalities are found, the results with the asterisk (*) may be reported.
3. If dashes (— —) are in place of numeric data, verify results according to your laboratory’s policy. Possible actions may include repeating the sample or reporting RBC morphology from smear review.
    a. Follow your laboratory protocol for handling suppressed or unreportable results in your Laboratory Information System (LIS), if dashes (— —) are displayed in place of numeric data. This is addressed in some LIS by using a code of “HIDE” or “Not Measured” in place of the results that are suppressed.
4. If the RBC morphology is normal and the MCHC is abnormal (<30 or >37.5 g/dL) an interfering substance or condition may be present. Refer to the suggested guidelines for the HGB/Turbidity Interference? IP Message.

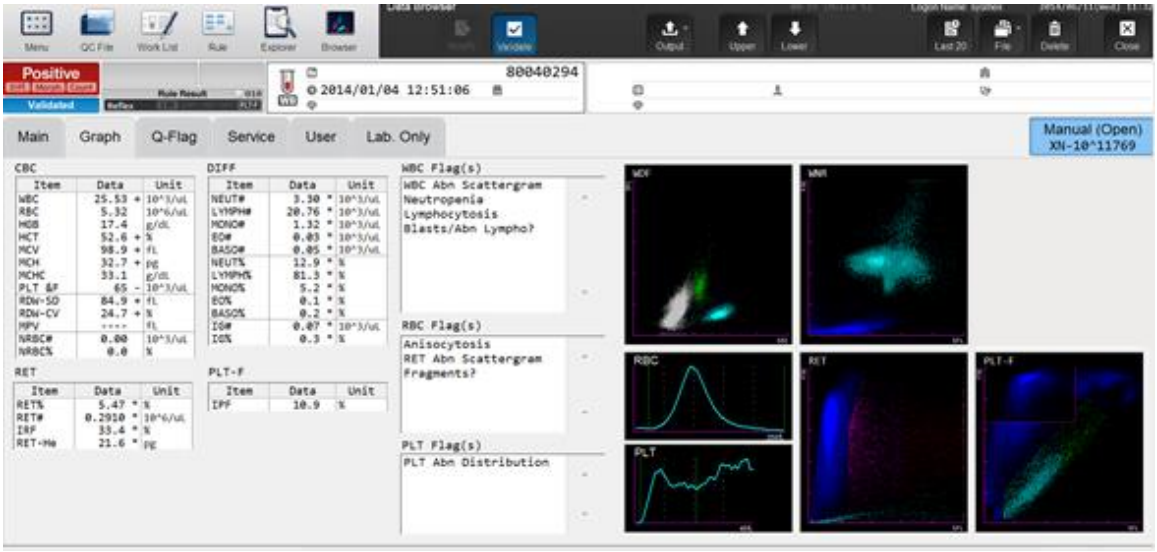
## Abnormal, RET Abn Scattergram

The RET Abn Scattergram IP Message can only be generated on the XN-Series if the reticulocyte parameter is ordered. This IP Message indicates that the analyzer has detected increased activity in the RET-THR (threshold) area of the RET scattergram or increased activity in the RET-UPP (Upper Particle Plateau) area on the RET-EXT scattergram.

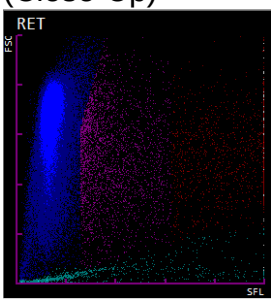
RET-EXT Scattergram: The RET-UPP area (green area past reticulocytes) is abnormal due to NRBCs, Howell-Jolly Bodies, parasites or stress reticulocytes. These are not included in the reticulocyte count.

Asterisks (*) appear next to the RET%, RET#, IRF and RET-H*e* parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

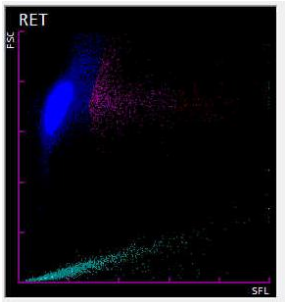
XN-Series Results




RET Scatter (Close-Up)




Normal RET Scatter (Reference)



**Abnormal, RET Abn Scattergram (continued)**

Suggested Action Steps:

1. Prepare a 1:5 dilution with CELLPACK® DCL diluent to minimize interference. Run the 1:5 dilution in the manual mode (NOT the pre-dilute mode). Dilutions greater than 1:5 should not be used.

   NOTE: Do not use undiluted CELLPACK® DST for any dilutions.

2. Check that the RBC (x5) on the diluted sample matches the original RBC count to ensure that dilution errors have not occurred. Also, check that the diluted RBC count is not less than 0.50 x $10^6$/µL. In flow cytometry adequate particles must be present for accurate gating to occur. If the diluted sample's RBC count is <0.50 x $10^6$/µL, make a lower dilution (i.e. 1:2 or 1:3) in order to increase the RBC count.

3. If the RET Abn Scattergram? flag is eliminated, multiply the absolute reticulocyte count by 5 and report all results according to your laboratory protocol. The reticulocyte % and IRF do not need to be multiplied by the dilution factor since these percentages/ratios should remain the same regardless of the dilution factor. RET-H*e* also does not need to be multiplied by the dilution factor since it is measured at the cellular level and is unaffected by dilution.

4. If the flag is not eliminated, or the RBC count is <0.50 x $10^6$/µL, follow your local laboratory protocol. Possible actions include:
   - reviewing the peripheral smear for the presence of polychromasia, parasites, NRBCs, Howell-Jolly Bodies or basophilic stippling. If present, report the results with a comment saying that the results may be affected by the presence of interfering substances.
   - performing the reticulocyte by an alternate method.

5. Decisions to report with a comment, perform a dilution or perform an alternate method should be based on your local laboratory protocol.

## Suspect, RBC Agglutination?

The RBC Agglutination? IP Message is determined by calculation and size comparison of certain RBC items (MCHC, MCH, RBC, Upper RBC histogram discriminator [RU%] *).

*The RU% is not a reportable parameter, but it is used in the RBC Agglutination algorithm.

Asterisks (*) appear next to the RBC, HGB, HCT, MCV, MCH, MCHC and RET # parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

XN-Series Results (initial run)


XN-Series Results (after warming at 37ºC)

**Suspect, RBC Agglutination?**
**(continued)**

Suggested Action Steps:

1. Follow your laboratory protocol and scan the peripheral smear for the presence of agglutinated RBCs or visually check the sample tube for agglutination.

2. If agglutinated RBCs are present, warm the sample at 37ºC for 15-30 minutes according to your laboratory policy. Reanalyze the warmed sample in the manual mode after mixing by manual inversion 10 times. Make a new peripheral smear from the warmed sample if agglutination is severe and WBCs and PLTs cannot be accurately assessed.

NOTE: Sometimes agglutination can be so severe that warming the sample does not enable accurate analysis.

3. In cases with high cold agglutinin titers, a plasma replacement using warm CELLPACK® DCL may be necessary to reduce the interference from the antibody. Further warming post-plasma replacement may also be necessary.

    a. To perform a plasma replacement
        i. Centrifuge an aliquot of blood from the primary tube to separate the cells from the plasma.
        ii. Using a pipette, remove a measured amount of plasma removing as much plasma as possible without disturbing the buffy coat.
        iii. Add back the same amount of CELLPACK DCL as the volume of plasma removed in step ii. (Example: If 0.5 mL of plasma is removed then add back 0.5 mL of CELLPACK DCL.)
        iv. Cap the tube and mix the sample by manual inversion until the cells are fully re-suspended in the CELLPACK DCL.
        v. Reanalyze the sample in the manual mode.

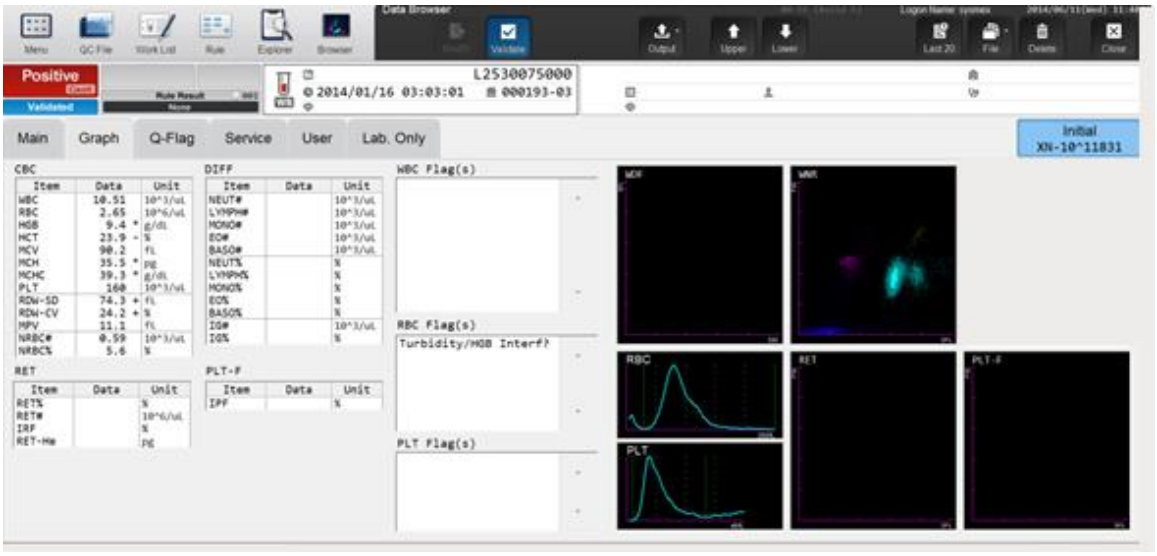
4. In cases where a warm-reacting antibody has caused agglutination, a plasma replacement may reduce the interference from the antibody. Room temperature CELLPACK DCL may be used to replace the plasma.

**Suspect, Turbidity/HGB Interference?**

The Turbidity/HGB Interference? IP Message occurs when the MCHC is >37.5 g/dL and indicates that turbidity may be present in the diluted and lysed sample. This turbidity could interfere with the HGB detection light path and falsely increase the HGB value. Other interfering substances or conditions may impact the hematocrit and also cause an MCHC >37.5 g/dL.

Asterisks (*) appear next to the HGB, MCH and MCHC parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

XN-Series Results




NOTE: An MCHC up to 37.5 g/dL may indicate a normal specimen on the high end of normal range in which case no action is needed. This may occur more often in samples with higher hemoglobin and hematocrit results.

Consider the MCHC and the MCV together when evaluating results and the reasons for the interference. Refer to the following table for possible interferences and corrective actions.

**Suspect, Turbidity/HGB Interference? (continued)**

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

Troubleshooting Chart

*Always follow your local laboratory procedure for repeat testing or rejection of samples*

| Low Sodium Affecting Hematocrit? | RBC Agglutination? | Severe Lipemia, Icterus, Abnormal Protein or Leukocytosis Affecting Hemoglobin Measurement or Hemolysis? |
|---|---|---|
| 1. Perform a 1:5 dilution of sample with CELLPACK DCL<br>2. Allow the dilution to equilibrate for ten to fifteen minutes<br>3. Rerun after equilibration<br>4. Correct results for dilution factor prior to reporting.<br>NOTE: MCV, MCH, MCHC, RDW-SD, RDW-CV, IPF%, MPV, Ret-He, IRF and differential percent results are unaffected by dilution and do not require correction. | 1. Prewarm at 37°C for fifteen to thirty minutes then rerun<br>2. Severe cold agglutinins or rouleaux may require dilution or plasma replacement with CELLPACK DCL.<br>3. For severe cold agglutinins, additional incubation at 37°C may be necessary following dilution or plasma replacement. | 1. Perform a 1:5 dilution of sample with CELLPACK DCL<br>2. Repeat diluted sample<br>3. Correct results for dilution factor prior to reporting.<br><br>Lipemia or Icterus Only<br>Perform a plasma replacement procedure<br><br>Hemolysis:<br>Recollect a new sample. |

### Suspect, Iron Deficiency?

The Iron Deficiency? IP Message is determined by calculation and size comparison of certain RBC items (MCV, RDW-CV).

NOTE: This flag is not used in the North American market.

### Suspect, HGB Defect?

The HGB Defect? IP Message is determined by calculation and size comparison of certain RBC items (MCV and RDW-CV).

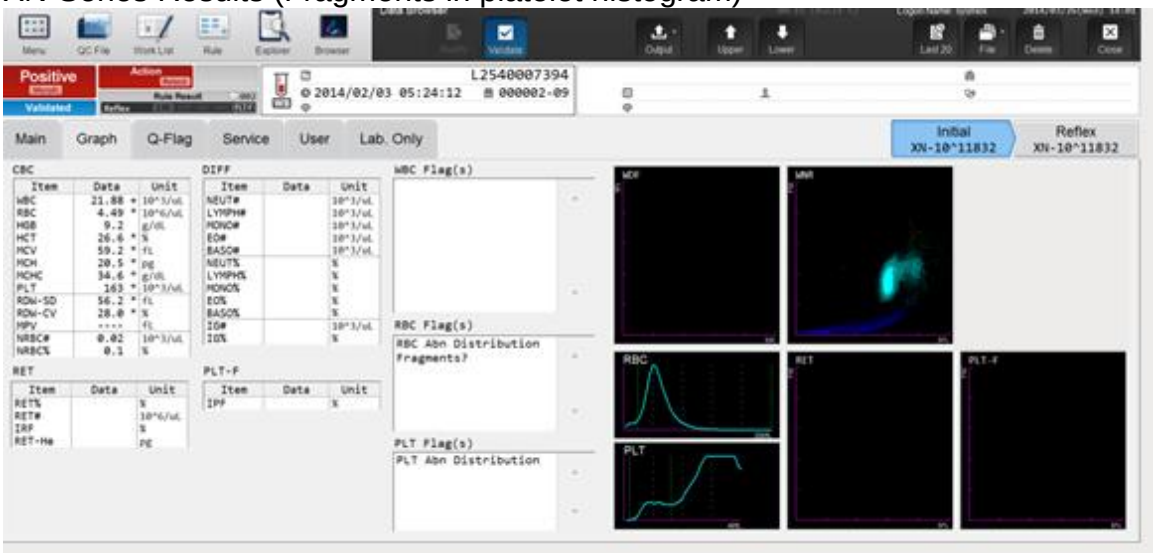
NOTE: This flag is not used in the North American market.

**Suspect, Fragments?**

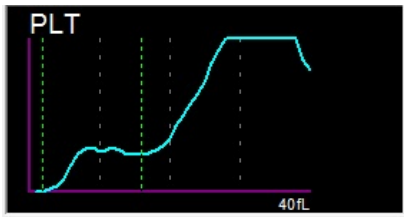
The Fragments? IP Message is determined from RET Scattergram and/or by calculation and size comparison of certain RBC and PLT items (MCV, RDW-SD, MCHC, RBC Lower Discriminator [RL]*, PLT Upper Discriminator [PU]*, PLT Upper Discriminator % [PU%]*).

* RBC lower discriminator, PLT upper discriminator, % of the PLT upper discriminator. These parameters are not reportable, and are used only in the algorithm for the Suspect, Fragments flag.

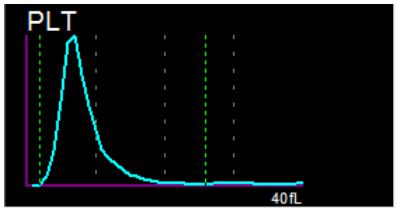
XN-Series Results (Fragments in platelet histogram)




Platelet Histogram (Close-Up)



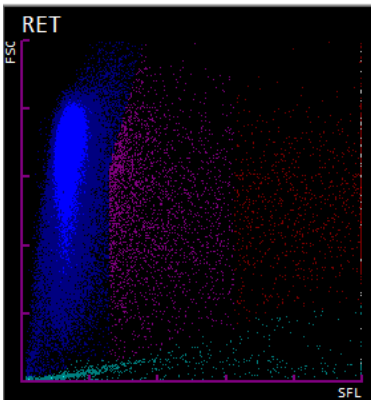

Normal Platelet Histogram (Reference)



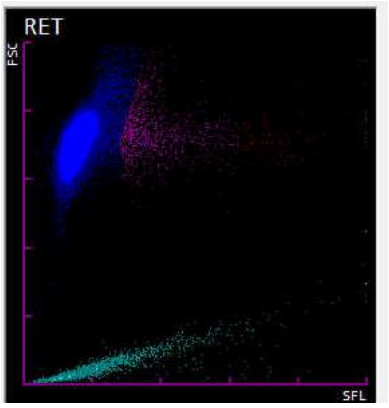
**Suspect, Fragments?**
**(continued)**

XN-Series Results (Fragments in reticulocyte scatterplot)


Reticulocyte Scatterplot (Close-Up)




Normal Reticulocyte Scatterplot (Reference)




Suggested Action Steps:

1. Scan the peripheral smear for the presence of fragmented RBCs and other poikilocytosis according to your local laboratory protocol.

2. Report the presence of any clinically significant RBC morphology according to your local laboratory protocol.

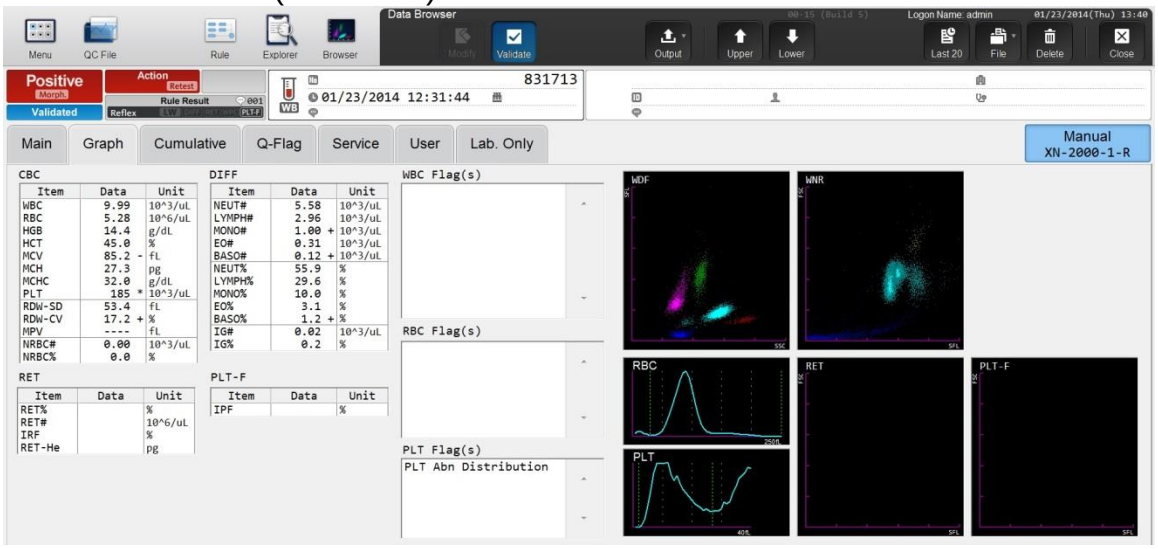
## Abnormal, PLT Abn Distribution

The PLT Abn Distribution IP Message is generated by calculation and size comparison of certain PLT items (PDW*, % of PLT lower discriminator [PL%] *, % of upper discriminator [PU%] *, platelet mean-frequent volume [PMFV]*, platelet large cell ratio*, MPV, platelet upper discriminator [PU]*).

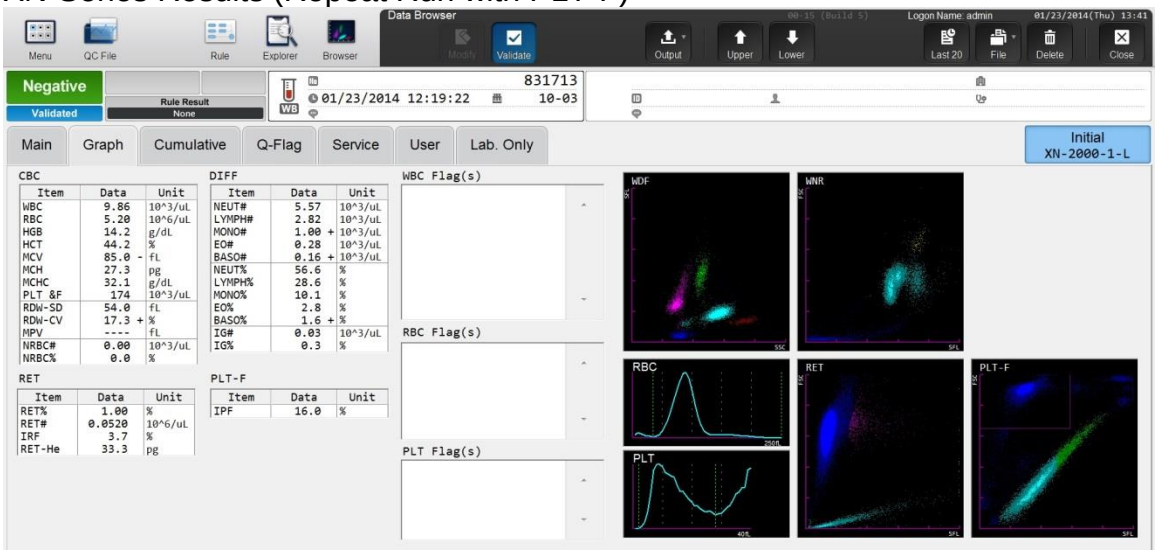
*These are all non-reportable parameters that are used as part of the flagging algorithm.

Dashes may appear in place of data for the MPV or the MPV may be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your local laboratory protocol prior to reporting.

XN-Series Results (Initial Run)




XN-Series Results (Repeat Run with PLT-F)



**Abnormal, PLT Abn Distribution**
**(continued)**

Suggested Action Steps:

1. Review results according to your local laboratory protocol. Possible actions include:
   a. For analyzers with the PLT-F capability, re-analyze the sample with CBC and PLT-F. By ordering the CBC and PLT-F the platelet measurement is done using a nucleic acid stain specific for platelet organelles and flow cytometry. The PLT-F result will have "&F" to the left of the result indicating the result was obtained in the PLT-F channel. In the absence of other IP Messages, the PLT-F may be reported with no further action. If on rerun an asterisk (*) is present on the PLT result OR if the analyzer does not have PLT-F capability, proceed to Step 1b.

   b. Scan the peripheral smear to estimate the platelet count and review for the presence of abnormal RBC or PLT morphology such as:
      - large or giant platelets
      - small platelets
      - platelet clumps
      - fragmented RBCs
      - microcytic RBCs
      - parasites

      If abnormal RBC, PLT or other morphology is noted, report according to your local laboratory protocol.

   NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

2. If platelet estimate confirms accuracy of analyzer count, it may be reported.

3. If platelet estimate does not confirm accuracy of analyzer count, confirm with an alternate method such as a manual platelet count according to your local laboratory protocol. Report any clinically significant RBC and/or PLT morphology according to your local laboratory protocol.

4. If platelet clumps have interfered, perform one of the alternate procedures recommended in the section Suggested Actions for PLT Clumps? IP Message.

5. If dashes (— —) are displayed in place of the MPV result, follow your laboratory protocol for handling suppressed or unreportable results in your Laboratory Information System (LIS). This is addressed in some LIS by using a code of "HIDE" or "Not Measured" in place of the results that are suppressed.

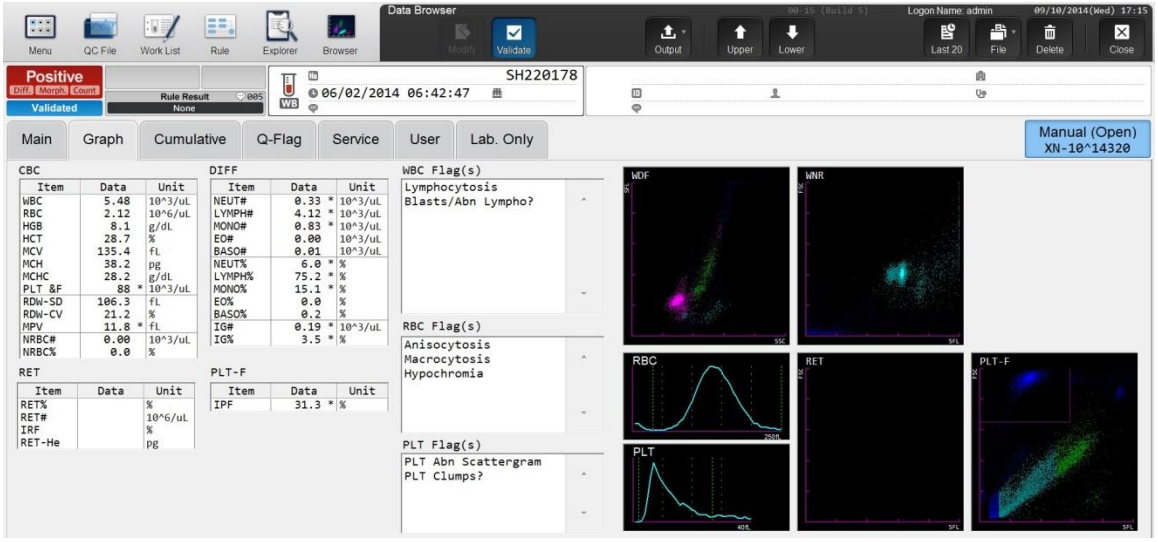
## Abnormal, PLT Abn Scattergram

NOTE:  This flag does not apply to analyzers without PLT-F capability.

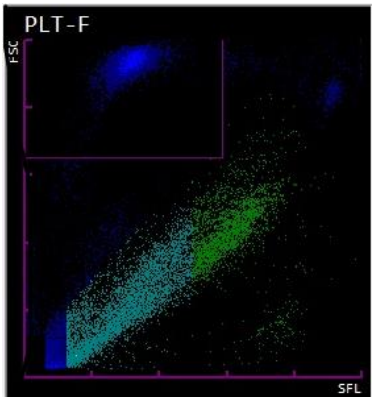
The PLT Abn Scattergram IP Message can only be generated when a PLT-F count is performed. This IP Message occurs when clustering in the platelet and IPF area on the PLT-F Scattergram is abnormal.

The PLT-F, IPF% and IPF# are reported with an asterisk (*). Dashes may appear in place of data for the MPV or the MPV may be reported with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

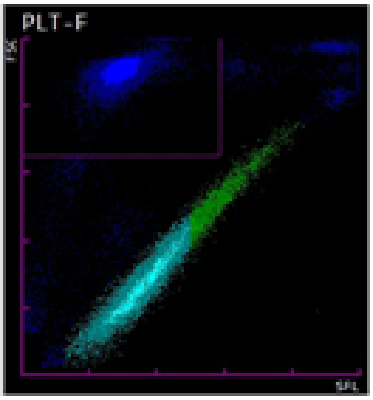
XN-Series Results




PLT-F Scatter
(Close-Up)




Normal PLT-F Scatter
(Reference)



**Abnormal, PLT Abn Scattergram**
**(continued)**

Suggested Action Steps:

1. Review results according to your laboratory protocol. This may include scanning the peripheral smear to estimate the platelet count and reviewing for the presence of abnormal RBC or PLT morphology such as:
    - large or giant platelets
    - platelet clumps
    - fragmented RBCs
    - microcytic RBCs
    - parasites

    If abnormal RBC, PLT or other morphology is noted, report according to your local laboratory protocol.

    NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

2. If platelet estimate confirms accuracy of analyzer count, the PLT and IPF results may be reported.

3. If platelet estimate does not confirm accuracy of analyzer count, confirm with an alternate method such as a manual platelet count according to your local laboratory protocol. Report any clinically significant RBC and/or PLT morphology according to your local laboratory protocol.

4. If platelet clumps have interfered, perform one of the alternate procedures recommended in the section Suggested Actions for PLT Clumps? IP Message.

5. If dashes (— —) are displayed in place of the MPV or IPF results, follow your laboratory protocol for handling suppressed or unreportable results in your Laboratory Information System (LIS). This is addressed in some LIS by using a code of “HIDE” or “Not Measured” in place of the results that are suppressed.

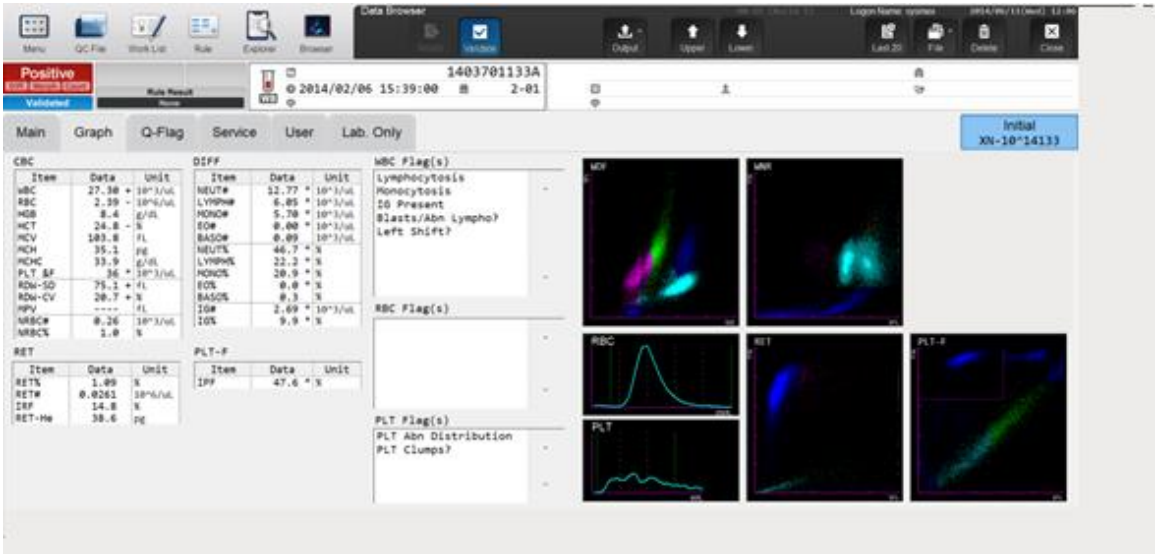
**Suspect, PLT Clumps?**

The PLT Clumps? IP Message is determined by abnormal clustering in the WNR, WDF or PLT-F scattergrams. In the WDF and PLT-F scattergrams the FSC-W measurement is also used to identify platelet clumps.

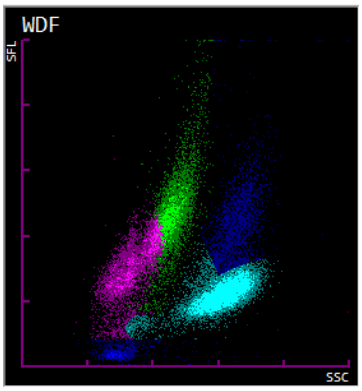
NOTE: PLT-F is not available on all XN-series analyzer configurations.

Asterisks (*) will appear next to the PLT, MPV and IPF results. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

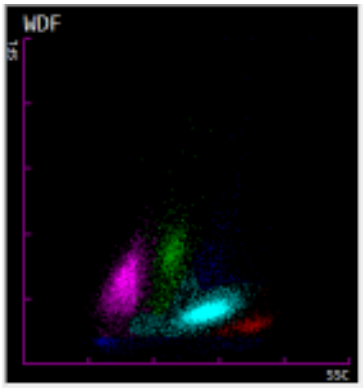
XN-Series Results




WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)



**Suspect, PLT Clumps?**
**(continued)**

Suggested Action Steps:

1. Follow your local laboratory protocol. Possible actions may include:
    a. Checking the sample for the presence of clots
    b. Reflexing to PLT-F. If the PLT-F run has no asterisk on the platelet result, IP Messages or Action Messages, the platelet result may be reported without further review according to laboratory procedures.

    NOTE: For analyzers without PLT-F capability, follow your laboratory procedure when the "PLT Clumps?" flag is present.

    c. Scanning the peripheral smear, especially the feathered edge, for the presence of abnormal morphology including:
        - fibrin strands
        - platelet clumps
            i. If any of the above are present, verify the WBC and PLT by a manual slide estimate.
            ii. If the WBC and PLT estimates match the analyzer counts, report the results according to your local laboratory protocol.
            iii. If the estimates do not match the analyzer counts, refer to the next step to obtain an accurate count.

    NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

2. If platelet clumps or fibrin strands have interfered, perform one of the following alternate procedures to obtain an accurate count:
    a. Re-draw specimen in EDTA and sodium citrate tubes if possible. Analyze re-drawn EDTA tube. If the repeat run has no PLT Clumps? IP Message, report these results.
    b. If there is still a PLT Clumps? IP Message and platelet clumps are present on smear review it could be an in vitro reaction with EDTA. Analyze the sodium citrate tube. Obtain only the WBC and PLT counts from the sodium citrate tube as sodium citrate alters RBC morphology and indices.
    c. Multiply the WBC and PLT results from the sodium citrate tube by the factor used by your laboratory.
    d. If recollection is not possible or if platelet clumps persist when using sodium citrate, estimate the platelet count and report as decreased, adequate or increased and comment on the platelet clumps according to your local laboratory protocol.

**Suspect, PLT Clumps?**
**(continued)**

NOTES:

1. There are different methods for handling samples with platelet clumps. These methods include vortexing of the original sample, recollection and analysis of a new sample in sodium citrate or adding amikacin to the original sample and reanalyzing. Individual facilities should define and validate the method, anticoagulant type and any dilution factors used.

2. Some samples with severe platelet clumping may not be resolved (or only be partially resolved) using any method. In such samples, the only option is to not report the numeric platelet result and instead report a platelet estimate from a review of a stained smear.

3. The incidence of completely unflagged instances of pseudothrombocyopenia is very low. However, to identify samples with pseudothrombocytopenia due to platelet clumping, multiple approaches must be employed together. Approaches for detecting platelet clumping may include:
    a. Smear review based on analyzer generated or user defined flags such as “Thrombocytopenia” or “Platelet Abnormal Distribution”, etc.
    b. Reflexing to a second platelet method, such as PLT-F based on the platelet result and / or flagging
    c. Use of delta checks comparing the current result against previous results
    d. Review of smears based on both PLT and Mean Platelet Volume (MPV) results. (Low PLT with high MPV may indicate the presence of platelet clumps.)
    e. Use of review criteria for results that fall between the “thrombocytopenia” threshold and a critical low value that might warrant transfusion support.

## Body Fluid Analysis IP Message

## Abnormal, WBC Abn Scattergram

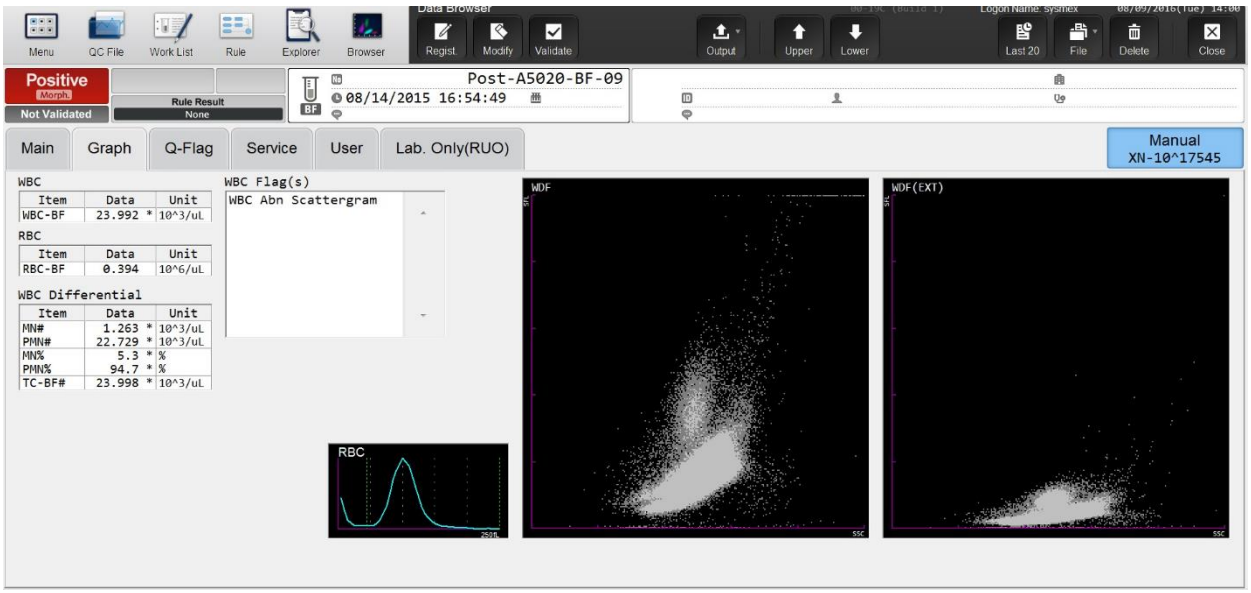
The WBC Abn Scattergram IP message is generated during body fluid analysis whenever clustering in the WDF scattergram is abnormal. This flag can also be generated when the HF-BF#* or HF-BF%* result exceeds the HF-BF user defined limits in the WBC Abnormal Flag (Body Fluid Analysis) setting.

*These parameters are not reportable, and are used only in the algorithm for the WBC Abn Scattergram flag during body fluid analysis.

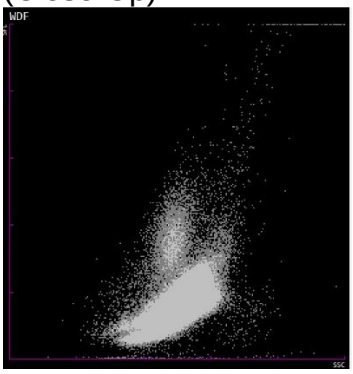
Dashes may appear in place of data that was not calculated.

NOTE: Body fluid analysis is not available on all XN-series analyzer configurations.

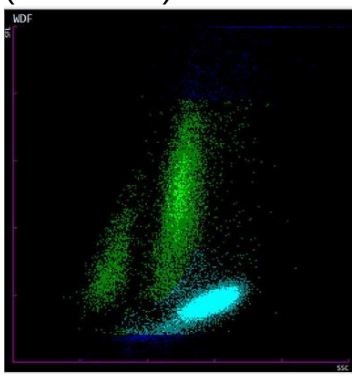
XN-Series Results




WDF Scatter (Close-Up)




Normal WDF Scatter (Reference)



**Abnormal, WBC Abn Scattergram (continued)**

Suggested Action Steps:

1. Dashes (— —) in place of numeric data:
    - Verify WBC-BF, TNC-BF and differential results according to your laboratory's policy. Possible actions may include:
        - repeating the sample
        - diluting the sample with CELLPACK DCL diluent to minimize interference.
        - performing a manual cell count
        - performing a manual differential
2. Asterisk (*) next to results:
    - Verify WBC-BF, TNC-BF and differential results according to your laboratory's policy. Possible actions may include:
        - performing a manual cell count
        - performing a manual differential
    - If the WBC ABN Scattergram IP Message is present due to HF-BF results exceeding the HF-BF user defined limits– scan the slide for the presence of mesothelial and/or abnormal cells. Report results according to your laboratory protocol.
    - If no abnormalities are found when reviewing the smear and the WBC and TNC estimate matches the analyzer reported WBC-BF and TNC-BF, the results with asterisks (*) may be reported.

NOTE: Decisions to report with a comment, perform a dilution or perform an alternate method should be based on your local laboratory protocol.

## Action and Error Messages

### Difference between WNR and WDF. Check the results.

This message is generated based on the ratio of the Total Nucleated Count in the WDF channel (TNC-D) to the Total Nucleated Count in the WNR Channel (TNC-N). The ratio is calculated as: (TNC-D / TNC-N). The message is generated when the ratio is > 1.3 or < 0.77.

Suggested Action Steps:

1. Rerun the sample
2. If the message is not eliminated, verify WBC and differential results according to your laboratory's protocol. Possible actions may include:
    a. Scanning the slide for abnormal cells and to estimate the WBC count
    b. Performing a manual differential if abnormal cells are observed
    If no abnormalities are found when reviewing the smear and the WBC estimate matches the analyzer reported WBC, the results may be reported according to your laboratory's protocol.

NOTE: If the analyzer has reported the WBC from the WDF channel, the WBC result will have the "&D" indicator adjacent to it.

### Difference between RBC and RET. Check the results.

This message is generated based on the ratio of the RBC result from the RET channel (RBC-O) and the RBC result from the impedance channel. The ratio is calculated as: (RBC-O / RBC). The message is generated when the ratio is > 1.2 or < 0.8.

Suggested Action Steps:

1. Rerun the sample
2. If the message is not eliminated, follow your laboratory protocol. Possible actions may include:
    a. Scanning the peripheral smear for the presence of abnormal RBC morphology such as rouleaux or RBC agglutination (refer to suggested action for "RBC Agglutination?" if present), polychromasia, parasites, NRBCs, Howell-Jolly Bodies. Report any abnormal RBC morphology according to your laboratory protocol.
    b. Verifying the reticulocyte using an alternate method.

## Action and Error Messages (continued)

**Difference between PLT and PLT-F. Check the results.**

This message is generated based on the ratio of the PLT-F result to PLT result from the impedance channel. The ratio is calculated as: (PLT-F / PLT). The message is generated when the ratio is > 2.0.

NOTE: This message does not apply to analyzers without PLT-F capability.

Suggested Action Steps:

1. Rerun the sample.
2. If the message is not eliminated, follow your laboratory protocol. Possible actions may include:
   a. Scanning the peripheral smear to estimate the platelet count and reviewing for the presence of abnormal RBC or PLT morphology such as:
      - large or giant platelets
      - platelet clumps
      - fragmented RBCs
      - microcytic RBCs
      - parasites

   If abnormal RBC, PLT or other morphology is noted, report according to your laboratory protocol.

   NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

3. If platelet estimate confirms accuracy of either platelet count (PLT-F or PLT-I) from the analyzer, it may be reported according to your laboratory protocol.

4. If platelet estimate does not confirm accuracy of analyzer count, confirm with an alternate method such as a manual platelet count according to your local laboratory protocol. Report any clinically significant RBC and/or PLT morphology according to your laboratory protocol.

## Action and Error Messages (continued)

### Suspect sample, check the sample

This message is generated based on an algorithm using RBC results and particle counts from the WNR scatter.

Suggested Action Steps:

1. Remix and rerun the sample.
2. If the message is not eliminated, follow your laboratory protocol.
    a. If the results from the initial and repeat runs are consistent, and consistent with patient history report according to your laboratory protocol.
    b. If the results from the initial and repeat runs are consistent, there is no previous patient history and the results are abnormal, confirm as required by your laboratory protocol using smear review or an alternate method.
    c. If the results from the initial and repeat runs are NOT consistent, consider insufficient or non-mixing in manual mode, an overfilled tube (e.g., no air space in tube to enhance hand or automated mixing) or a clotted or fibrinous sample. Reject or recollect the sample based on your local laboratory protocol.
    d. If there are any flags or asterisked results on the repeat run, follow your laboratory protocol for that flag.

## Action and Error Messages (continued)

### Insufficient blood volume (short sample)

This error message is generated by the sample aspiration sensor based on the absorbance of the diluted sample. Results are suppressed when this error message is generated.

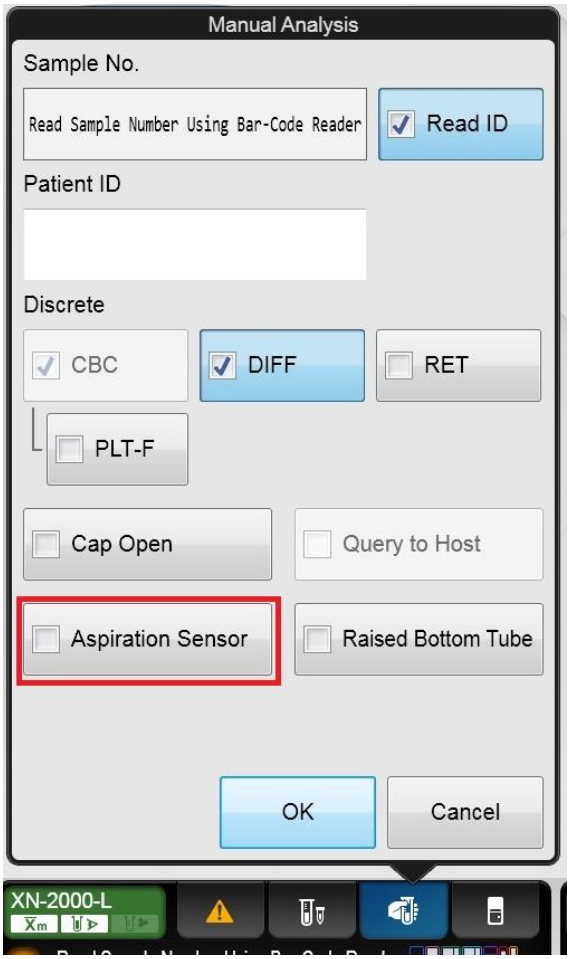
Suggested Action Steps:

1. Check the sample for clots and that the minimum volume requirements have been met; remix and rerun the sample.
2. If the message is not eliminated, follow your laboratory protocol.
    a. If a sample is suspected of having low hemoglobin, turn off the aspiration sensor in the Manual Analysis dialog box, remix and rerun the sample in the manual mode.

       NOTE: Enable the aspiration sensor prior to testing subsequent samples.

    b. If this error message is occurring on multiple samples, refer to the analyzer Instructions for Use for troubleshooting information.

Manual Analysis Dialog Box, Aspiration Sensor Disabled



## Interfering Substances

Some abnormal samples may interfere with automated cell counting methods. The following is a list from the Sysmex XN-Series Instructions for Use of possible substances that may interfere with these parameters.

NOTE: Compromised samples, 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.

WBC: Leukocyte aggregation, possibility of PLT clumps, cryoprotein, cryoglobulin, fibrin, giant platelets (Platelets > 1,000,000/µL)

RBC: Erythrocyte aggregation (Cold agglutinin), microerythrocytes, possibility of fragmented RBCs, leukocytosis (> 100,000/µL), giant platelets (Platelets > 1,000,000/µL)

HGB: Leukocytosis (> 100,000/µL), lipemia, abnormal protein. The effect of abnormal proteins and lipemia may be removed by plasma replacement or plasma blank procedures.

HCT: Erythrocyte aggregation (Cold agglutinin), microerythrocytes, possibility of fragmented RBCs, leukocytosis (> 100,000/µL), severe diabetes (hyperglycemia), uremia, spherocytosis

PLT: Possibility of PLT clumps, pseudothrombocytopenia, giant platelets, microerythrocytes, possibility of fragmented RBCs, fragmented leukocytes, cryoprotein, cryoglobulin

RET: Erythrocyte aggregation (Cold agglutinin), giant platelets, possibility of PLT clumps, fragmented leukocytes, Malaria, Howell-Jolly bodies

NOTE: The Sysmex XN-Series Analyzer is designed to flag abnormal samples that may contain interfering substances. These results should be reviewed carefully and may require further examination in accordance with your local laboratory protocol.

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