#### Evaluating Flagged Patient Results from the XN-3100 Hematology Analzyer

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| Background | This procedure describes how to respond to the Abnormal and Suspect Flags on the Sysmex XN-3100 Hematology Analyzer |

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| Policy | * Evaluation of results will be performed only by Clinical Laboratory Scientists or Medical Laboratory Technicians
* Hold result for any abnormal or suspect flag until further investigation resolves issue
* CBCND orders on patients with NO previous history which display BLAST flag will have a smear review performed to verify the presence of blast cells
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| Analyzer flag categories |

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| Flag category  | Description |   |
| Negative | Sample has no abnormal flags. Results are generally reported without review. Other conditions requiring follow-up are flagged in LIS (i.e. critical result, delta failure, etc.) |
| Positive | Sample has one or more parameter indicator flags or Abnormal/Suspect IP flags requiring follow-up action |
| Error | Sample analysis error occurred (i.e. sample aspiration error, etc.) These results should be reviewed carefully and may require further examination |
| Action | Operator action is required. Perform test indicated on printout (i.e. Perform PLT-F, perform smear review, etc.) |

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| Parameter indicator flags |

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| Indicator flag | Description |
| ( \* ) Asterisk | Reliability of the data is suspect. Corrective action is required |
| ( @ ) At sign | Data exceeds the linearity limit |
| ( ! ) Exclamationmark | Data exceeds the upper acceptable background check value limit. Repeat background count |
| + or - | Data exceeds the upper or lower reference range limits |
| (----) vote out  | Data cannot be displayed due to analysis error or a parsing error |
| ++++ | Data exceeds the display limit |
| [ ] | Indicates that the analysis order does not exist |
| &  | Corrected results (may be seen with WBC, Lymph, PLT-F) |

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| WBC Abnormal & Suspect IP messages |

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| Message | Explanation | Action |
| WBCAbnormal Scattergram | Clustering in the WNR or WDF scattergrams is abnormal.NOTE: If the analyzer has reported the WBC from the WDF channel, the WBC result will have the “&D” indicator adjacent to it. | * If WBC count is <0.5K/µL and the sample was run in closed mode, the analyzer automatically performs an extended WBC count time. If WBC count is <0.5K/µL and the sample was run in manual mode, re-run the sample in the Low WBC mode.
* If dashes (- - -) are present in place of data, perform and report manual differential.
* If asterisk (\*) next to data, perform scan to verify WBC, NRBC, and auto DIFF. Assess for presence of abnormal cells or platelet clumping.
* If WBC, NRBC, and DIFF confirmed and no abnormal WBCs or platelet clumps are seen, report instrument results.
* If WBC are not confirmed by smear estimate, instrument WBC is not reportable. Result WBC field with free-text comment: “Unable to accurately determine WBC count”. Result Absolute differential parameters with ETC comment NCAL (not calculated); percent differential parameters may still be reported.
* If auto DIFF or NRBC counts not confirmed or abnormal WBC or PLT clumps are seen, perform manual diff
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| WBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| NRBC Present. | This message appears when the NRBC result exceeds 5 nrbc/100 WBCNote: NRBC’s are counted simultaneously while counting WBCs. No further correction of the WBC count is required. If NRBCs are >0.01/ 100WBC, the lymph counts are corrected. | * Perform smear review to detect the presence of malignant cells and confirm NRBC’s
* If malignant cells seen, perform manual diff.
* If malignant cells not seen, and this is the only WBC flag, accept instrument results.
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| IG present | * Numerical based flag that occurs when >5% IGs are detected
* Immature granulocyte %/# results include metamyelocytes, myelocytes and promyelocytes
 | * Perform and result manual diff and assess for presence of: Immature granulocytes, Toxic granulation or vacuolization of neutrophils or other abnormal cells
* When >5% IGs are detected by the analyzer, the automated differential will not add up to 100 cells in the LIS. Therefore, regardless of the presence of IGs upon review, a manual differential must be performed and reported in order for the cell count to equal 100.
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| IG with asterisk (\*) | An asterisk (\*) appears next to the IG %/# indicates these results may be unreliable. | * Perform smear review to verify DIFF and to assess for presence of Immature granulocytes, Toxic granulation, vacuolization of neutrophils, other abnormal cells
* If immature granulocytes or other abnormal WBCs are seen, perform and report manual differential
* If no immature granulocytes or other abnormal WBCs are seen, and no other flags present, report the Auto Diff.
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| WBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| Left Shift? | * The instrument 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.
* An asterisk (\*) appears next to Neutrophil & Eosinophil % and #. The IG % / # may also have an asterisk
* If the WBC is <0.50 x 103/µL in the Whole Blood mode or <0.20x103/µL in the Low WBC mode, the Left Shift IP flag will not be generated
 | * If dashes (---) are present in place of data, perform manual differential
* Otherwise, if this is the only WBC flag, append ETC comment LSHFT (Left Shift) to Auto diff Neutrophil % result and accept instrument results. No action is required.
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| WBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| Blast?/Abn Lympho? | * The instrument 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.
 | * Perform smear review to verify DIFF and assess for presence of Blasts, Immature granulocytes, Atypical, immature lymphocytes or other abnormal cells
* Review feathered edge and sides of smear as blasts and other large cells may migrate to this area during smear preparation.
* If abnormal WBCs are noted or there are dashes (---) in place of data, perform and report manual differential.
* If auto DIFF is not confirmed, perform and report manual differential.
* If no abnormal WBCs are found and the auto DIFF is confirmed, report instrument results.
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| Atypical Lympho? | * The instrument detected significant clustering in the region for atypical lymphocytes in the upper left lymphocyte region on the WDF scattergram
* An asterisk (\*) appears next to the neutrophil, lymph, mono, eos, and IG% and # and these results may be unreliable
 | * If dashes (---) are present in place of data, perform manual differential
* Perform smear review to verify DIFF and assess for presence of Atypical/variant lymphs, Blasts, abnormal or atypical monocytes, immature lymphocytes, smudge cells or other abnormal cells
* If abnormal WBC’s are seen, perform and report manual diff
* If auto diff not confirmed, perform and report manual diff
* Otherwise, report auto diff
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| RBC Abnormal & Suspect IP messages |

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| Message | Explanation | Action |
| RBC Abn distribution | * The RBC <0.5 x 106/µL or the RBC histogram pattern from the RBC channel is abnormal
* 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 RDW-SD and CV. The RDW-SD and CV results may also be marked with an asterisk (\*).
 | * Review blood count results. If MCHC is abnormal (< 30.0 or >37.5) an interfering substance may be present. Refer to RBC agglutination or HGB interference flags
* Review blood smear for the presence of abnormal RBC morphology such as rouleaux or RBC agglutination, multiple RBC populations, fragmented RBC, and report RBC morphology
* If dashes (---) or asterisks (\*) appear in place of data for the RDW-CV, result as \* with ETC comment UTD (Unable to determine
* If this is the only flag, accept instrument results. No action is required.
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| Dimorphic population | * Multiple peaks in RBC histogram pattern.
* Dashes (---) appear in place of affected results for RDW-SD and CV.
 | * If dashes (---) appear in place of data for the RDW-CV, result as \* with ETC comment UTD (Unable to determine)
* If this is the only flag, accept instrument results. No action is required
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| RBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| RET Abn Scattergram | * Retic abnormal scattergram (occurs only if RETIC is ordered)
* Increased activity in the RET-THR (threshold) area of the RET scattergram or increased activity in the RET-UPP (Upper Particle Plateau) area of the RET-EXT scattergram
* RET-EXT Scattergram: The RET-UPP area(green area past reticulocytes) is abnormal due NRBCs, Howell-Jolly bodies, parasites, or stress reticulocytes. These are not included in the reticulocyte count
* Asterisks (\*) appear next to the RET% / #, IRF and RET-H*e*. The (\*) indicates these results may be unreliable.
 | * Prepare and run sample dilution - see Sample Dilution Procedure section. Do NOT use dilutions greater than 1:5.
* Check that the RBC (x5) on the diluted sample m original RBC count within 10% to ensure dilution errors have not occurred. Also, verify that the diluted RBC count is not < 0.50
* If <0.50, make a lower dilution (i.e., 1:2 or 1:3) to increase the RBC count and ensure that adequate particles are present for accurate gating to occur
* If the RET Abn Scattergram flag resolves, multiply the absolute Retic count by the dilution factor and report result. The Retic % and IRF do not need to be multiplied by the dilution factor since these percentages / ratios should remain the same upon dilution
* If flag persists, send out for alternate Reticulocyte test
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| RBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| RBC agglutination | * Occurs when MCHC is >37.5 g/dl
* Determined by calculation and size comparison of certain RBC parameters (MCHC, MCH, RBC, RU%\*)
* Asterisk (\*) appear next to the RBC, HGB, HCT, MCV, MCH MCHC and RET# parameters, indicating that these results may be unreliable
* The RU% is the upper RBC histogram discriminator. This is not a reportable parameter but it is used in the RBC agglutination algorithm
* Consider MCHC and MCV together when evaluating results and the reasons for interferences:
* If MCHC ↑ and MCV ↑, likely RBC agglutination or rouleaux
 | * If neonate ( < 28 days) and MCHC is <40, no action needed, result may be released
* All other ages and MCHC is <37.5, no action needed, result may be released
* Warm sample at 37oC for 15-30 minutes. Reanalyze warmed sample in the manual mode after mixing by manual inversion 10 times.
* If flag resolves, report warmed results with appended ETC comment R37 (37C result, possible cold agglutinin) to the MCHC.
* If flag persists, perform plasma replacement using warm Cellpack DCL. Refer to the plasma replacement procedure
* In cases where warm reacting antibody has caused agglutination, a plasma replacement with room temp Cellpack DCL may be used to replace the plasma
* If flag persists after plasma replacement and MCHC is

< 40, release results with appended ETC R37 |

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| RBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| Turbidity/HGB interference | * Occurs when the MCHC is >37.5 g/dL. Indicates interference with HGB and/or HCT analysis
* Asterisks (\*) appear next to HGB, MCH and MCHC indicating these results may be unreliable
* Consider MCHC and MCV together when evaluating results and the reasons for interferences
* If MCHC ↑ and MCV ↑, likely RBC agglutination or rouleaux
* If MCHC ↑ and MCV ↓ or normal, likely due to: hemolysis, electrolyte abnormality (i.e. low Na), severe lipemia, icterus, severe leukocytosis, and/or abnormal protein precipitation
 | * If RBC Agglutination suspected, follow actions for "RBC Agglutination?" message
* Otherwise, proceed to prepare and run sample dilution - see Sample Dilution Procedure section
* If flag resolves, then correct HGB result for dilution factor and report. If HGB result changes >10% from original analysis, then also recalculate MCH and MCHC, otherwise original MCH and MCHC can be reported
* If flag persists, proceed to examine the plasma for gross lipemia, hemolysis, or icterus
* If sample is grossly lipemic or icteric, perform plasma blank procedure orperform plasma replacement using CELLPACK DCL. Refer to the appropriate corrective action procedure
* If sample is grossly hemolyzed, request recollection. If recollection is not possible, report analyzer results EXCEPT RBC, HCT, MCV, MCH and MCHC - result as ETC comment UGH (Unable to measure due to gross hemolysis. Re-draw is recommended).
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| RBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| Hypochromia | Occurs when MCHC <25 g/dL | * Review all RBC parameters for hypochromic anemia correlation (i.e. typically ↓ HGB, ↓ HCT, ↓ MCV)
* Review available chemistry results for possible interfering condition (i.e. electrolyte abnormality or low electrolyte, hyperglycemia, etc.).
* Perform scan to verify hypochromic RBC morphology
* If hypochromic RBC morphology confirmed and/or interfering condition excluded, then report results. Perform smear review after the results are released, and enter comments
* If hypochromic RBC morphology NOT confirmed or unable to exclude possible interference, proceed to prepare and run sample dilution - see Sample Dilution Procedure section
* If results from dilution significantly differ from original results, correct RBC, HGB, HCT for dilution factor. MCV, MCH, MCHC do not require dilution factor correction. Report WBC, PLT and DIFF from original (undiluted) sample
* If results from dilution NOT significantly different form original results, then report original results.
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| RBC Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| Fragments? | * Possibility of fragmented RBCs (schistocytes)
* RBC lower discriminator, PLT upper discriminator, % of the PLT upper discriminator. These parameters are not reportable, and are used only in the algorithm for this flag.
 | * If the sample was run in manual mode, re-run the sample to include PLT-F
* If the sample was run in closed mode, the analyzer automatically performs a reflex PLT-F count.
* The PLT-F will have “&F” to the left of the result indicating that result was obtained in the PLT-F channel.
* Report instrument PLT-F result
* Refer to the appropriate PLT abnormal IP message for corrective action if PLT is flagged.
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| PLT Abnormal & Suspect IP messages |

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| Message | Explanation | Action |
| PLT Abnormal Scattergram | * Generated only when a PLT-F is performed
* Occurs when clustering in the platelet and IPF area on the PLT-F Scattergram is abnormal
* PLF-F, IPF% and IPF # are reported with an asterisk (\*). Dashes (---) may appear in place of data for MPV or MPV may be reported with an asterisk (\*). The asterisk (\*) indicates these results may be unreliable
 | * Follow the corrective action required for "PLT Abn Distribution" flag
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| PLT Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| PLT Abnormal Distribution | * Generated by calculation and size comparison of PDW, % PL, % PU, PMFV, platelet large cell ratio, PLCR\*, MPV, and [PU]\*
* These are non-reportable parameters used as part of the flagging algorithm
* Dashes (---) in place of data for MPV or MPV data with an asterisk (\*) indicates these results may be unreliable.
 | * If sample was run in the manual mode, re run the sample to include PLT-F after checking for clots
* If sample was run in the closed mode, the analyzer automatically performs a reflex PLT-F count
* The PLT-F will have “&F” to the left of the result indicating that result was obtained in the PLT-F channel.
* If flag resolves and no other flags are present, report the PLT-F result.
* If other flags or an asterisk (\*) is present on the PLT-F result, proceed to the step below:
* Check sample for clots/fibrin strands if not previously done
* If clot/fibrin strands present, request recollection.
* If patient cannot be recollected (i.e. premature neonate, hard stick, etc), clinical correlation of specimen results and decision to recollect should be obtained with consultation of patient caregiver
* If clot/fibrin strands not present, Vortex sample (1-2 minutes) and re-run with PLT-F (Note: Must program sample with "/" in manual mode to prevent auto- filing.)
* Ensure a slide has been made and all other non-PLT flags have been resolved prior to vortexing the sample as vortexing may cause WBC distortion/destruction. Only the PLT-F may be reported from a vortexed sample.
* If flag/asterisks resolves, report instrument results
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| PLT Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| PLT Abnormal Distribution, cont |  | * If flag persists, proceed to perform smear review to estimate PLT count and assess for presence of abnormal RBC and/or PLT morphology (i.e. large or giant platelets, small platelets, platelet clumps, fragmented RBCs, microcytic RBCs, parasites)
* Review feathered edge and sides of smear as platelet clumps and fibrin strands may migrate to this area during slide prep
* If PLT-F is confirmed by smear estimate, report PLT-F. Perform smear review after results are released, and enter morphology comments from smear review as needed.
* If PLT-F not confirmed by smear estimate **and** significant platelet clumping observed on smear, instrument PLT result is not reportable. Result PLT field with one of the appropriate ETCs:
* PLTCN: "Platelet clumps noted on smear but count appears normal." (i.e. within normal range)
* PLTCI: "Platelet clumps noted on smear but count appears increased." (i.e. above normal range)
* PDF: "Platelet count may be falsely decreased due to platelet clumping. Suggest redraw in citrate tube." (i.e. below normal range)
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| PLT Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| PLT Clumps? | * Determined by abnormal clustering in the WNR, WDF and PLT-F scattergrams
* In the WDF and PLT F scattergrams, FSC-W measurement is also used to identify platelet clumps.
* Asterisks (\*) will appear next to the PLT, MPV and IPF indicating that results may be unreliable and action is required prior to reporting results
 | * If sample was run in the manual mode, re run the sample to include PLT-F after checking for clots
* If sample was run in the closed mode, the analyzer automatically performs a reflex PLT-F count
* The PLT-F will have “&F” to the left of the result indicating that result was obtained in the PLT-F channel.
* If flag resolves and no other flags are present, report the PLT-F result.
* If other flags or an asterisk (\*) is present on the PLT-F result, proceed to check sample for clots/fibrin strands if not previously done.
* If clot/fibrin strands present, request recollection
* If patient cannot be recollected (i.e. premature neonate, hard stick, etc), clinical correlation of specimen results and decision to recollect should be obtained with consultation of patient caregiver
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| PLT Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| PLT Clumps?cont |  | * If clot/fibrin strands not present, proceed to vortex sample (1-2 minutes) and re-run with PLT-F (Note: Must program sample with "/" in manual mode to prevent auto- filing.)
* Ensure a slide has been made and all other non-PLT flags have been resolved prior to vortexing the sample as vortexing may cause WBC distortion/destruction. Only the PLT-F may be reported from a vortexed sample
* If flag resolves, report instrument results
* If flag or asterisks persists, proceed to perform a smear review to assess for presence of platelet clumps and fibrin strands (check feathered edge).
* If fibrin strands or platelet clumps are NOT seen, report instrument results.
* If fibrin strands or platelet clumps are seen, proceed to perform smear review to estimate WBC and PLT counts and to assess abnormal morphology
* If PLT-F and WBC are confirmed by smear estimate, report instrument results. Perform smear review after results are released, and enter comments from smear review as needed
* If PLT-F is not confirmed by smear estimate, instrument PLT result is not reportable. Result PLT field with one of the appropriate ETCs:
* PLTCN: "Platelet clumps noted on smear but count appears normal." (i.e. within normal range)
* PLTCI: "Platelet clumps noted on smear but count appears increased." (i.e. above normal range)
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| PLT Abnormal & Suspect IP messages, cont |

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| Message | Explanation | Action |
| PLT Clumps?cont |  | * PDF: "Platelet count may be falsely decreased due to platelet clumping. Suggest redraw in citrate tube." (i.e. below normal range)
* If WBC are not confirmed by smear estimate, instrument WBC is not reportable
* Result WBC field with free-text comment: “Unable to accurately determine WBC count” AND request re-draw in citrate. Result Absolute differential parameters with ETC comment NCAL (not calculated); percent differential parameters may still be reported
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| Thrombocytopenia | PLT count <50 K/uL  | * If patient has history of Thrombocytopenia, no further action is necessary and report PLT count
* If Patient’s initial PLT testing or PLT has dropped from Normal PLT count to ≤ 50 K/uL (critical result) then check sample for clots and review blood smear for PLT clumps or satellitism
* If specimen is not clotted and platelet satellitism or platelet clumps are Not seen, report instrument results
* If specimen is not clotted and platelet satellitism or platelet clumps are present in smear review
* If inpatient, recollect in citrate tube
* If outpatient, do not report the platelet count append the ETC “NOPLT” to the PLT test code PDF
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| Calculations | * MCV = (HCT/RBC) x10
* MCH = (HGB/RBC)X10
* MCHC = (HGB/HCT)X100
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| Plasma Replacement procedure | Follow the steps below to perform a Plasma Replacement as indicated

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| Step | Action |
| 1 | Place an aliquot of whole blood into a secondary tube. |
| 2 | Centrifuge tube to separate cells from plasma. |
| 3 | Using an MLA pipette, remove a measured amount of plasma from the tube. Remove as much plasma as possible without disturbing the buffy coat |
| 4 | Replace the volume of plasma removed with an equal volume of CELLPACK DCL. Example: If 200 µL plasma removed, add 200 µL CELLPACK DCL back into tube. |
| 5 | Cap the tube and mix by inversion until RBCs are fully re-suspendedNote: steps 2 through 5 may need to be repeated for strong cold agglutinins |
| 6 | Analyze the plasma replaced aliquot in manual mode |

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| Specimen Dilution procedure | Follow the steps below to perform a Sample Dilution as indicated

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| Step | Action |
| 1 | Prepare a 1:5 dilution of the sample using CELLPACK DCL into a secondary tube |
| 2 | Allow the dilution to equilibrate for 10-15 minutes prior to running |
| 3 | Mix tube by inversion 10 times prior to analysis. Refer to *Running Whole Blood Samples on the XN-3100 Hematology Analyzer* procedure, Manual Analysis (Open) Mode section.Note: Do **NOT** use the analyzer's Pre-Dilution mode |

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| Specimen Dilution procedure, cont |

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| Step | Action |
| 4 | * Correct indicated parameter by multiplying by the dilution factor prior to reporting result
* To report results obtained from a sample dilution, the following parameters must be corrected by multiplying the dilution factor: WBC, RBC, HGB, HCT, PLT (PLT-F), Absolute DIFF, Absolute RET.
* Example: If RBC result from 1:5 dilution is 0.725, calculate correct RBC result by multiplying dilution result by 5: 0.725 x 5 = 3.625 (x106/µL)
* Parameters that generally do not need correcting for the dilution are MCV, MCH, MCHC, DIFF %, RET %, and RDW.
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| References | * Sysmex XN 3100 Operator’s manual
* Sysmex XN series Automated hematology Systems, Flagging interpretation guide, March 2018
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 *End*