

Reticulocyte Count (XN Series)

Principle

Reticulocytes are immature, non-nucleated erythrocytes retaining a small network of basophilic organelles, comprised of RNA and Protoporphyrin. The enumeration of reticulocytes provides a simple effective means to determine red cell production and regeneration.

By flow cytometry method using a semiconductor laser, a two-dimensional scattergram is plotted, with the X-axis representing the intensity of the side fluorescent light (SFL), and the Y-axis representing the intensity of the forward scattered light (FSC). This scattergram displays groups of reticulocytes, mature red blood cells and platelets. The scattergram is divided into three RET zones based on the intensity of the fluorescent light, and the ratio of the reticulocytes in each zone to the total number of reticulocytes is calculated.

The blood cell signals from the optical detector block (which analyzes RET channel) can be obtained by sending signals from the forward scattered light, side scattered light, and side fluorescent light to the applicable waveform processing circuits of the analog unit, where noise is eliminated and the required blood cell signals are picked up. The control unit converts the analog-to-digital-converted cell signals into scattergram data and sends the data to the IPU.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing scheduled maintenance.

Specimen

Whole blood should be collected in EDTA-2K or EDTA-3K anticoagulant.

Quality Control

Sysmex XN-Check (three levels). For detailed QC procedure refer to XN QC Procedure.

Procedure

For detailed XN operating procedure refer to Sysmex XN 3100 Procedure.

XN Retic Limitations

- XN RET% is linear from 0.00 to 30.00%. Any result greater than 30% should be diluted and rerun.
- If any of the following is present, the XN may erroneously report a high reticulocyte count: Erythrocyte aggregation, Giant platelets, PLT clumps, Fragmented leukocytes, Malaria, Howell-Jolly bodies. The **RET Abn Scattergram** IP Message and **asterisks (*)** appear next to the RET%, RET#, IRF and RET-He parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed by dilution prior to reporting.

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Procedure Follow steps below:

| Step | Action |
|------|--|
| 1 | Prepare a 1:5 dilution with CELLPACK® DCL diluent to minimize interference. Dilutions greater than 1:5 should not be used. NOTE: Do not use CELLPACK® DST for any dilutions. |
| 2 | Run the 1:5 dilution in the manual mode (NOT the pre-dilute mode). Program sample with MRN (NOT Accession #), test for CBC and RET ONLY . |
| 3 | 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 $0.50 \times 10^6/\mu\text{L}$. In flow cytometry adequate particles must be present for accurate gating to occur. If the diluted sample's RBC count is $<0.50 \times 10^6/\mu\text{L}$, make a lower dilution (i.e. 1:2 or 1:3) to increase the RBC count. |
| 4 | IF the RET Abn Scattergram? flag is eliminated, multiply the absolute reticulocyte count by 5 and report all results. 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. |
| 5 | IF the flag is NOT eliminated, or the RBC count is $<0.50 \times 10^6/\mu\text{L}$, then: <ul style="list-style-type: none">Review 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 <i>"The results may be affected by the presence of interfering substances"</i>. |
| 6 | IF the flag is persistent , rule out that particular XN analyzer for functional errors: <ul style="list-style-type: none">Re-analyze the patient sample on a different XN to ensure that it is not a functional error.Begin troubleshooting by running a known normal patient sample to see if RET is abnormal.IF Scattergram occurs on all patients or only the one in question. Call Sysmex TAC or IR for service if analyzer is the issue. |

- References:**
- System XN-3100 Instructions for Use (North American Edition), Sysmex Corporation, Kobe, Japan.
 - System XN Series Flagging Guide 1166 Rev. 4