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. Refer to: Policy and Procedures Safety Manual Infection Control and Procedures 11-085-01.

Specimen

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

Quality Control

Symex XN**-**Check (three levels) every 8 hours. For detailed QC procedure refer to Hematology Policy & Procedures, HEM.02-0010 XN QC Procedure.

Procedure

For detailed XN operating procedure refer to Hematology Policy & Procedures, HEM.03-0010 Sysmex XN 9000 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.

XN Retic Limitations, continued

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 by dilution prior to reporting.

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 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.
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 x 106/µL, then: • 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 the results may be affected by the presence of interfering substances.
6	If the flag **is persistent**, rule out that particular XN analyzer for functional errors: • Re-analyze the patient sample in a different XN to ensure there 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:

A. Sysmex XN-9000 Instructions for Use (North American Edition), Sysmex Corporation, Kobe, Japan.
B. Sysmex XN Series Flagging Guide 1166 Rev. 3

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Implemented
New	Procedure for new XN instruments.	Julius Salomon, 7/1/17			