Objectives

- Describe the advantages of the new technologies available on the XN-Series
- Utilize case studies to demonstrate how fluorescent flow cytometry technology used on the XN can improve efficiency and productivity.
- Understand and be able to explain the advantages of utilizing non-traditional Hematology parameters to aid the clinician in the diagnosis and treatment of anemia and thrombocytopenia)
XN-Series

It all begins here....

XN-Series: Addresses Needs Of All Segments

Single Analytical Module

Multiple Options
Compact Automation Solutions

Scalable Automation Solutions
XN-Series Technology

Sysmex XE vs. XN Technology
Fluorescent Flow Cytometry

- **Side Fluorescent Light**: DNA/RNA information
- **Side Scattered Light**: Cell inside structure information
- **Forward Scattered Light**: Cell size information
- **Dichroic Mirror**
- **Laser Beam** wavelength=633nm
- **WNR Channel**
WNR Channel

Reagent Reaction

- BASO
- LYMPH
- MONO
- Granulocyte

- NRBC
- RBC

WNR Channel Scattergram - Normal Pattern

- NRBC
- BASO
- WBC
- Debris
- SFL
- FSC
- SFL
- Debris
Acute Erythroid Leukemia (AML-M6)

Information from XN-Series

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>2.36 x 10^9/L</td>
</tr>
<tr>
<td>RBC</td>
<td>2.49 x 10^12/L</td>
</tr>
<tr>
<td>HGB</td>
<td>90 g/L</td>
</tr>
<tr>
<td>HCT</td>
<td>0.281/L</td>
</tr>
<tr>
<td>MCV</td>
<td>112.9 fL</td>
</tr>
<tr>
<td>MCH</td>
<td>36.1 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>320 g/L</td>
</tr>
<tr>
<td>PLT</td>
<td>118 x 10^9/L</td>
</tr>
<tr>
<td>PLT%</td>
<td>81 x 10^9/L</td>
</tr>
<tr>
<td>RDW-SD</td>
<td>70.9 fL</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>17.5 %</td>
</tr>
<tr>
<td>MPV</td>
<td>11.9 fL</td>
</tr>
<tr>
<td>NRBC</td>
<td>1.01 x 10^9/L</td>
</tr>
</tbody>
</table>

Flags

- WBC Flag(s):
  - Neutropenia
  - Leukocytopenia
  - NRBC Present
  - Blast?

- RBC Flag(s):
  - Anisocytosis
  - Macrocytosis
  - Anemia
  - Fragments?

- PLT Flag(s):

Blood smear (May-Grum staining)

- PB: Myeloblast 10.0, Promyelto 0.0, Myeloblast 1.0, Meta 0.0, Band 0.0, Segmented N. 27.8, Lymphocytes 60.0, Monocytes 2.0, Basophils 0.0, Eosinophils 0.0, Agranular Lymph 0.0, NRBC 75.0 x 10^9 WBC, Megakaryo 0.0 x 10^9 WBC
Advantage of Fluorocell WNR

WNR Channel

- Fluorescent Flow Cytometry Technology
- Maximized Efficiency
- NRBC the first time – all the time
  - No additional steps
  - Accurate WBC Counts in the presence of NRBCs
  - No additional reagent needed
- Virtually eliminates interference from:
  - Lyse resistant RBCs
  - Lipids
WDF Channel  Scattergram - Normal Pattern

LYMPH

MONO

NEUT+BASO

EO

Immature Granulocytes
IG – XN DIFF Abstract

Automated Immature Granulocyte Counts on the new Sysmex XN Automated Hematology Analyzer
Nancy Rosenthal¹, Barbara Connell³, Bonnie Brown¹, Julie Kruger¹, Mary Capper¹, Kimberly Blaine⁷
¹University of Iowa Hospitals and Clinics, Iowa City, IA, United States ²Sysmex America, Inc., Mundelein, IL, United States

Conclusion: The WDF Channel provides accurate automated IG counts as confirmed by a respectable correlation between the DM96, XE-5000 and the XN. These results were excellent considering the low levels of IGs observed and the well-known limitations of manual differentials and rare cell events. Reporting the automated IG count using a cut-off of <=5% would increase the number of auto-verified results by 30% on the XN analyzer and would improve productivity and efficiency in the laboratory.

So what’s different about the WDF?

Enhanced Flagging

XN flagging system improves work flow by reducing manual reviews
Enhanced Flagging Algorithms

SAFLAS method
(Sysmex Adaptive Flagging Algorithm based on Shape-recognition)

Detects abnormal cells - (with high sensitivity)

LDA (Linear Discriminant Analysis)

WDF SAFLAS Method

Normal | Abnormal (Blasts?) | Abnormal (Abn Lympho?)

[Images of cell analysis graphs]
**WDF Channel**

- Enhanced Flagging
- Better separation between lymphs and monos (SAFLAS)
- Better identification of platelet clumps (multiple channel detection)
- Auto-correction of lymphs when NRBCs are present
- 6-part differential (including IG)

**Low WBC Analysis Mode**

<table>
<thead>
<tr>
<th>WB (Whole Blood) mode</th>
<th>LW (Low WBC) mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Rate: x 61.1</td>
<td>Dilution Rate: x 61.1</td>
</tr>
<tr>
<td>Analysis Volume: 68.3µL</td>
<td>Analysis Volume: 174.6µL</td>
</tr>
<tr>
<td>Whole Counts: 9,530</td>
<td>Whole Counts: 28,600</td>
</tr>
</tbody>
</table>

Analysis Time extended to 3 times the Whole Blood WBC count time
Low WBC Analysis Mode

- Analysis time extended to 3 times WB WBC count time
- Better accuracy and precision on counts < $0.50 \times 10^3$
- No Vote Outs – Differential results on all counts
- Increased reportable differentials

PLT-F Channel
PLT-F Channel

PLT performance

- Impedance platelet analysis (size) has limitations in the identification and discrimination of platelets from interfering particles with the same size.

- Possible interferences
  - RBC fragments counted as platelets: falsely high
  - Microcytic RBCs counted as platelets: falsely high
  - Large platelets counted as RBC: falsely low

Reagent Reaction

- RBC
- PLT
- IPF
PLT-F Channel

Reportable Parameters
- PLT-F
- IPF

Fluorocell PLT Staining

- Dye binds some structures more strongly.
- Dye binds diffusely spread throughout.

WBCs were stained by both dyes.
Binding sites of Fluorocell PLT

Fluorocell PLT stains nucleic acid rich organelle
- Rough-surfaced endoplasmic reticulum (ribosomal RNA)
- Mitochondria (MtDNA)

PLT-F separates platelets from RBC fragment by the differences in staining

- CD41/CD61 positive = Platelets
- Staining by PLT-F dedicated reagent
  - Platelets - Strong, especially within the cell
  - RBC fragments - Weak, only cell membrane
Interference with Routine Impedance Count

β-Thalassemia Major with numerous fragmented red cells

XE: PLT-I

Microcytic RBC

XE: PLT-O

Microcytic RBC

XE: PLT-F

Microcytic RBC

Improved Performance of PLT-F

Acute Prolymphocytic Leukemia / chemo: white blood cell fragments

XE: PLT-O

WBC cytoplasm fragments

XE

PLT-I = 477*10^9/L
PLT-O = 111*10^9/L
PLT-CD61 = 152.2*10^9/L
IPF% = 13.9%

XE

PLT-I = 514*10^9/L
PLT-O = 140.8*10^9/L
PLT-CD61 = 152.2*10^9/L
IPF% = 12.9%

XE

PLT-I = 28*10^9/L
PLT-O = 24.2*10^9/L
PLT-CD61 = 24.2*10^9/L
IPF% = 1.1%

XE

PLT-I = 41*10^9/L
PLT-O = 74*10^9/L
IPF% = 41.2%

XE

PLT-I = 34*10^9/L
PLT-O = 63*10^9/L
IPF% = 12.9%

XE

PLT-I = 20*10^9/L
PLT-O = 24.2*10^9/L
PLT-CD61 = 24.2*10^9/L
IPF% = 1.1%

XE

PLT-I = 0.3*10^9/L
PLT-O = 74.6*10^9/L
IPF% = 41.2%
PLT-F Channel

- Second method of platelet
- Fluorescent Dye specific for platelet organelle
- Extended count time (6 times) for accurate platelet enumeration, especially in low platelet counts
- Good comparison with CD41/CD61
- Minimizes interference from RBC fragments, microcytic RBC’s and WBC fragments
- Automated Action message and reflex with on board rules
Reticulocyte Channel

RET Channel

Reagent Reaction

WBC  RET  RBC  PLT
Reticulocyte Parameters

- Reticulocytes
  - # and % of immature RBC's
- Immature Reticulocyte Fraction
  - Newly released from the marrow, a direct cellular measurement of erythropoiesis
- Reticulocyte Hemoglobin
  - Direct cellular measure of iron availability
Reticulocyte Hemoglobin (Ret-He/CHr)

- Measured at cellular level
- Monitor acute changes in hemoglobin incorporation into the erythron
  - Real-time estimate of iron availability in bone marrow
- Shown as a more sensitive tool for early detection of iron deficiency
  - Changes rapidly, more sensitive screen than Hb
  - Less variation than acute phase reactants
- Provides additional information for managing iron requirements for ESA therapy
  - Limitation in specificity addressed by interpreting Ret-He results in conjunction with other tests and clinical picture

K-DOQI Guidelines Evaluation of Anemia

Initial anemia evaluation
- Cellular Assessment
  - CBC
    - Hgb < 12 g/dL
    - RBC indices
    - Absolute Retic
    - WBC & Diff
    - Platelet
- Iron Assessment
  - Serum ferritin
  - Serum TSAT or Hb content of reticulocytes

Iron Assessment
- HD-CKD Target
  - Hemoglobin > 11 g/dl
  - Tsat > 20%
  - Ferritin > 200 ng/ml or Reticulocyte Hgb > 29 pg/cell

NKFK-DOQI May 2006
Reticulocyte Channel

- Operational Efficiency
  - Reduced Interference From:
    - WBCs
    - Howell Jolley Bodies
    - Parasites
    - Sickle cells
  - Quick and automatic
- Monitor RBC development at the cellular level
  - IRF for Retic production
  - RET-He for iron incorporation in hemoglobin of the erythron
- Clinically relevant information for the management of anemia in conjunction with other available clinical information.
Body Fluid Analysis

Body fluid mode (target species)

Cerebrospinal fluid (CSF)
Pleural fluid
Peritoneal fluid
Synovial fluid

WDF Scattergram
Body Fluid Analysis

- No pre-analysis preparation
- No additional reagents
- Automated background counts
- Rapid analysis

XN Technology Summary

- WNR Channel
  - Maximized efficiency
  - NRBC the first time – all the time
  - Accurate WBC Counts in the presence of NRBCs
- WDF, Low WBC
  - Enhanced Flagging
  - 6-part differential (including IG)
  - Improved Sensitivity and Specificity
**XN Technology Summary**

- **Low WBC**
  - Better accuracy and precision on counts < $0.50 \times 10^3$
  - No Vote Outs – Differential results on all counts
  - Increased Reportable differentials

- **PLT-F**
  - Fluorescent Dye specific for platelet organelle
  - Extended count time (6 times) for accurate low platelet enumeration
  - Good comparison with CD41/CD61
  - Automated Action message and reflex with on board rules

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**XN Technology**

Hematology Technology of the Future Today