The Future of Hematology – XN-Series Technology

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 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

- FSC
- NRBC
- Debris
- SFL
- BASO
- WBC
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 \times 10^{10}/L</td>
</tr>
<tr>
<td>RBC</td>
<td>2.49 \times 10^{11}/L</td>
</tr>
<tr>
<td>HGB</td>
<td>90 g/L</td>
</tr>
<tr>
<td>HCT</td>
<td>0.281 L/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 \times 10^{9}/L</td>
</tr>
<tr>
<td>PLTf</td>
<td>91 \times 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 \times 10^{9}/L</td>
</tr>
</tbody>
</table>

WBC | 0.74 \times 10^{10}/L 31.4 %
LYMPH | 1.42 \times 10^{10}/L 60.2 %
MONO | 0.17 \times 10^{10}/L 7.2 %
EO | 0.01 \times 10^{10}/L 0.4 %
BASO | 0.02 \times 10^{10}/L 0.8 %
IG | 0.06 \times 10^{10}/L 25 %
RET | 1.66 \times 10^{9}/L 1.87 %
IRF | 27.3 %
RET-He | 29.3 pg |
IPF | 3.2 \times 10^{10}/L 3.5 %

Flags

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

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

- PLT Flag(s)

**Blood smear (May-Giemsa staining)**

- PB
  - Myeloblast: 10.0
  - Promyelocyte: 0.0
  - Myelocyte: 1.0
  - Metamyelocyte: 0.0
  - Band: 0.0
  - Segmented N.: 27.8
  - Lymphocyte: 60.0
  - Monocyte: 2.0
  - Basophils: 0.0
  - Eosinophils: 0.0
  - Atypical Lymph: 0.0
  - NRBC: 75.0\% WBC
  - Megakaryocyte: 1.0\% WBC

Acute Erythroid Leukemia (AML-M6)
Advantage of Fluorocell WNR

- 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

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

- LYMPH
- MONO
- NEUT
- EO
- Atypical LYMPH
- Immature WBC
IG – XN DIFF Abstract

Automated Immature Granulocyte Counts on the new Sysmex XN Automated Hematology Analyzer
Nancy Rosenthal¹, Barbara Connell², Bonnie Brown¹, Julie Kruger¹, Mary Capper¹, Kimberly Blaine³

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 dot plots showing classification into groups]
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 : 58.3µL</td>
<td>Analysis Volume : 174.6µL</td>
</tr>
<tr>
<td>Whole Counts 1 : 9,530</td>
<td>Whole Counts 2 : 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 x 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

PLT-F Channel

Reagent Reaction

- RBC
- PLT
- IPF
PLT-F Channel

Reportable Parameters
- PLT-F
- IPF

Fluorocell PLT Staining

- **PLT**
  - Dye binds some structures more strongly.

- **RBC**
  - Low RBC background (because of using only one staining dye, oxazine)

- **WBC**
  - WBCs were stained by both dyes.

- **Low RBC background**
- **High RBC background**
- **WBCs were stained by both**
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

ß-Thalassemia Major with numerous fragmented red cells

- Microcytic RBC

Improved Performance of PLT-F

Acute Promyelocytic Leukemia / chemo: white blood cell fragments

- WBC cytoplasm fragments
Burn injury

PLT measurement over time in the severe burn injury patient.

The feature: Huge micro-spherical FRCs appeared because of the heat shock.
### Burn injury

PLT measurement over time in the severe burn injury patient.

#### Day 3

<table>
<thead>
<tr>
<th></th>
<th>PLT-I ($x10^3/uL$)</th>
<th>PLT-O ($x10^3/uL$)</th>
<th>PLT-F ($x10^3/uL$)</th>
<th>CD61</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT-I</td>
<td>632</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT-O</td>
<td>211</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT-F</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD61</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Day 4

<table>
<thead>
<tr>
<th></th>
<th>PLT-I ($x10^3/uL$)</th>
<th>PLT-O ($x10^3/uL$)</th>
<th>PLT-F ($x10^3/uL$)</th>
<th>CD61</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT-I</td>
<td>201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT-O</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT-F</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD61</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### PLT-F Channel

Scattergram - Normal Pattern

![PLT-F Channel Scattergram](image)
Low PLT + Low IPF
Consistent with disorder of production

Low PLT+ High IPF
Destruction consistent with autoimmune or other destruction mechanism (ITP, TTP, DIC)

Immature Platelet Fraction to Assess Bone Marrow Recovery

How well can IPF predict platelet recovery following peripheral blood HPC transplantation?

Zucker, M. Laboratory Hematology, 2006 12:125 - 130
Potential IPF Applications

- HIT
- Drugs
- Corticosteroids
- Estrogens
- Thrombopoietic growth factors
- Other chemokines (eg. IL-11)
- Platelet substitutes
- Factor VIIa

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
Serum Iron Studies Are Important, but…..

<table>
<thead>
<tr>
<th>Iron Deficiency Anemia</th>
<th>Anemia of Inflammatory Chronic Disease</th>
<th>Functional Iron Deficiency Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron: (Will be High with Iron therapy)</td>
<td>Low or Normal or High</td>
<td></td>
</tr>
<tr>
<td>TIBC:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin Saturation: (Changes with dietary protein)</td>
<td>Normal Low in 10% of cases</td>
<td></td>
</tr>
<tr>
<td>Ferritin: (Acute phase reactant)</td>
<td>Normal or High</td>
<td>Normal or High 100-400 ng/ml (5-200μg/l) with persistent anemia</td>
</tr>
<tr>
<td>sTfR:</td>
<td>Normal</td>
<td>SI High but may be unreliable in ESRD</td>
</tr>
<tr>
<td>Recommendation</td>
<td>More useful to use sTfR / log ferritin</td>
<td></td>
</tr>
</tbody>
</table>

Iron Studies Don’t Give a Clear Picture

- Wide Biological Variation
- Acute Phase Reactants
- Slow Response to Therapy
Iron Metabolism

If we want to know about iron metabolism at the cellular level... Shouldn’t we directly measure changes to developing RBC?

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

NKF/K-DOQI May 2006
Recommendations for Screening

“A low CHr concentration has been shown to be the strongest predictor of ID in children.”

“For infants with Hb <11.0 mg/dL or with significant risk of ID or IDA, SF and CRP or CHr levels should also be measured to increase the sensitivity and specificity of the diagnosis.”

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 x 10³
  - 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

---

XN Technology

Hematology Technology of the Future Today