Immature Granulocyte Enumeration –
Our Journey from Manual to
Automated Reporting

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Objectives

- Describe the steps necessary to implement the automated IG parameter
- Describe the benefits of the automated IG over the manual differential
- Discuss the methods used to educate the clinical staff on the value of the automated IG count.
- Review the performance of the automated IG count on the new Sysmex XN Automated Hematology Analyzer*

*Pending FDA Clearance. For Investigational Use Only
Not Available for Sale in the United States
Performance Characteristics have not been Established.
University of Iowa Hospitals and Clinics

762 beds including the 190 bed Children's hospital
27 academic departments
Comprehensive tertiary level center with regional burn unit, hemophilia treatment and transplant/trauma center

Clinical Core Laboratory

- 76 Employees (chemistry, heme)
  - 51 are MT/MLTs
  - 25 are Specimen Control/support
- Staffing in Hematology/Coag/UA
  - 12-14 MT/MLTs/day
  - 5 MT/MLTs/evening
  - 3 MT/MLTs/overnight
- Workload
  - 1100 lavender tubes/day (M-F)
  - 175 routine coag tubes/day
  - 140 urines/day
Instrumentation in Hematology

What we currently have –
- HST (2 instruments/1 slidemaker/stainer) and DM96 Cellavision
- Alpha line (1 instrument/1 slidemaker/stainer) and Cellavision
- WAM (Work Area Manager – Middleware)
  Standardization of processing and autovalidation rules

Future Wish List:
- WAM for Body Fluids Module
- Body Fluids on Cellavision
- Combined Line for all instruments

Why use the automated IG count?

Detection of a Left Shift may be clinically important but...
We do not have a good definition for left shift
  ?Band Absolute # or Band %
  ?Presence of Metas, Myelos or Pros
  ?Ratio immature/total granulocyte count (neonatology)
Classification of Immature Granulocytes
*Metamyelocytes, Myelocytes, Promyelocytes*

  - 100 cells vs 32,000
- Consistency in classifying cells
  - Chemical identification vs. visual
- Rumke confidence limits for low-incidence cells
  - At a value of 5%, 95% confidence interval is 1.6-11.3%
**Metamyelocyte vs. Band**

**XE IG Parameter Goals**

- **Efficiency**
  - Reduce smear reviews and manual differentials
- **Improve patient care**
  - Accuracy
  - Precision
- **Reduce TAT**
- **Employee Satisfaction**
Define the IG parameter

- What it includes:
  Metamyelocytes, Myelocytes, Promyelocytes
- How the analyzer generates the results........

DIFF Channel

*STROMATOLYSER 4DL & 4DS, Fluorescent Staining System*
Differential Technology

- **STROMATOLYSER-4DL™ & -4DS™**
  - Maintains integrity of cells
  - Fluorescent stain binds to nucleic acid
  - Enhanced resolution
  - Better separation of cell clusters
  - Complexity of nucleus
  - More sensitivity & specific flagging
**Project Outline – How we approached the project**

- Software Installation - standard for XE-5000
- Correlation Studies (vs. Manual diff)
- Establish Reference Ranges or Review Cutoff
- Reproducibility
- Procedure Changes  
  - Hematology – rebuild CBC and Adif reporting  
  - LIS- include the new parameters of IG and NRBC
- Training
- Communication with Clinical Staff

**Other Considerations**

- Modify reports to included IG parameter  
  (absolute only reported, generated from % cutoff)
- QC material
- Proficiency Testing  
  - CAP Survey FH9
Correlation Study vs. Manual Differentials

• Performed 100 normal, 100 abnormal/200-cell diffs
  – Adhering to lab criteria for classification of cells (bands vs. metas)
  – Selection Criteria
    • Normal vs. Abnormals
      – IG’s are a “Rare Cell Event”
• Statistics generated using EP-evaluator
• Sensitivity Specificity Report Generated

Reference Range

Criteria for what is an “abnormal” differential at UIHC?
  • Bands ≥5%
  • Metas, Myelos or Pro ≥1%
  • Blast ≥1%
  • Abnormal lymphoid cells – various criteria
Setting Review Criteria

• IG Present:
  – If IG% ≤ 2.0% and no other IP Messages
    • Report Automated Differential
  – If IG% ≥2.0%, Review Smear
    • Confidence in IG number not in question, but at ≥2% (user defined), slide reviewed for other abnormalities and manual differential reported
      – Toxic changes associated with leukemoid reactions/sepsis
      – Hematologic abnormalities, such as MPD, CML

Note: All patients whose smears contained promyelocytes also had other flags, and thus slides were reviewed. Eased concern for missing blasts.

Clinical Staff Notification

Power Point on All In House Computer Screens
  ❖ Identifying the IG parameter initial use
  ❖ Identifying the RDW-SD parameter addition
  ❖ Noting the addition of NRBC reports on all CBCs
  ❖ Opportunity to call or email with any questions
Outcome After IG Implementation

- Reduction in Slide Review Rate?
- Overall rate remained 23-28% of diffs to manual review
- Confidence of accurate reporting
- Efficiency of processing samples
- Standardization of process

Looking to the Future

What about 5% for a cutoff value?

What is the clinical significance if this is the only flag?

How might this decrease the number of slides that have manual diffs for review?
Future Design and Performance

Compact Automation Solutions

XN-1000
XN-2000
XN-3000

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Scalable Automation Solutions

Modular Clinical Utility

- **Basic Channels**
  - NRBC Standard With Every XN-CBC
  - IG Standard With Every XN-Diff
  - Body Fluids With Diff

- **Advanced Channels**
  - Thrombopoiesis (IPF)
  - Erythropoiesis (RET-He)
  - Leukopoiesis (WPC) (XN-20 only)
Enhanced Flagging
- Better detection of Platelet Clumps
- Better separation between Monos and Lymphs

Auto-correction of lymphs when NRBC are present

6-part reportable differential including IG% / #

Improved efficiencies
- Rapid TAT
- More reportable automated differential
- Decreased manual interventions

Automated Immature Granulocyte Counts on the new Sysmex XN™ Automated Hematology Analyzer

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Abstract:
The first automated immature granulocyte count (IG) was introduced in 2003 and is available on the Sysmex XT-Series™ and XE-Series™ Automated Hematology Analyzers. The new Sysmex XN™ Automated Hematology Analyzer (XN)™ with WDF channel was designed with improved gating and optimization of leukocyte clusters including IG. The WDF channel uses two reagents: Lysercell WDF and Fluorocell WDF. Surfactants in Lysercell WDF™ hemolyze RBC and platelets and penetrate the cell membranes of WBC. Fluorocell WDF™ stains the nucleic acids and cell organelles. Through cluster analysis of differences in scattered light and fluorescence with the proprietary algorithm, the IG cluster is positioned right above the neutrophil cluster. We compared the performance of the Cellavision® DM96 Automated Digital Cell Morphology System (Cellavision AB, Lund, Sweden) (DM96) WBC differential vs automated IG counts on the XE-5000 and XN hematology analyzers to determine the optimum IG cut-off for autoverification of samples when IGS were reported, but no other sample flags were present.
Methods:
163 samples received in the University of Iowa Hospitals and Clinics hematology laboratory, Iowa City, IA, were analyzed on the Sysmex XE-5000 and XN-1000 analyzer for a complete blood count and differential. Following CLSI guidelines (H20-A2), two slides were prepared on each sample and 200-cell differentials were performed by two technologists utilizing the DM96, and were compared to the XE-5000 and XN automated IG counts. WBC morphology was also noted on each sample. Statistical analysis using the EP Evaluator version 9 was performed on all samples. The data was also evaluated at varying levels of IG to determine the optimum IG cut-off to use for autoverification.

Results:
In 163 samples, IG% ranged from 0.0 - 30.9% and correlation coefficients between the DM96 IG enumeration, XE-5000 and the XN were r= 0.8587, r= 0.8608, and r= 0.9671 respectively. This represents exceptional correlation, considering limitations of the manual differential in enumerating rare cell events. If the level of review for the IG is set at <=2.0 IG%, then 52 and 49 samples of 114 could be auto-verified on the XE-5000 and XN respectively. If the level for review is set a <= 5.0%, then 72 and 83 samples could be auto-verified on the XE-5000 and XN respectively. This represents an increase in auto-verified results of 17% and 30% for the XE-5000 and XN respectively.

Manual vs Automated Immature Granulocyte Correlation
Conclusions

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.

* The XN is for investigational use only in the US pending 510(k) FDA review

Questions??