Comparison of Automated Immature Granulocyte (IG) Count on Sysmex XE-2100 Analyser with Manual Microscopy

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The enumeration of immature granulocytes (IG) is routinely done by morphologists counting 100 cells on a stained blood film by visual microscopy. This is highly labour intensive, time consuming, imprecise and also subject to personal bias. With the introduction of the Sysmex XE-2100 analyser and the XE pro and XE IG Master software packages (Sysmex Corp., Kobe, Japan), it is now possible to enumerate immature granulocytes automatically using fluorescence flow cytometry technique.
The present study evaluated the efficiency and accuracy of the automated IG counts (Sysmex XE-2100) against manual visual microscopy (NCCLS H20-A protocol). 244 routine samples with automated IG counts > 2.0% were included in the study. Statistical analysis showed good agreement between the two methods. For IG counts without the asterisk flag (low reliability flag), the correlation is r=0.81; for IG counts with the asterisk flag, the correlation is r=0.67. Overall (combining the two groups with and without the asterisk flags), the correlation is r=0.72.
In our study, we have shown that the extended differential counts including the IG counts from the Sysmex XE-2100 can significantly reduce the number of manual differentials performed in a haematology laboratory. However there is a need for education of the clinicians for the meaning of automated immature granulocyte counts (combined counts of metamyelocytes, myelocytes and promyelocytes) before automated IG counts can become acceptable and reportable in routine doctors' reports. In addition, reference intervals for IG counts should be established.

(Sysmex J Int 16 : 12-16, 2006)

Key Words Automated Immature Granulocyte Counts, IG Counts, Sysmex XE-2100, Manual Differential Count, Extended Differential Count

Received 26 Aug, 2005; Accepted 20 Sep, 2005

INTRODUCTION

Immature granulocytes (IG) are increased in numbers in infections, diseases of bone marrow and inflammatory diseases. Traditionally, the counting of immature granulocytes has always been done manually by morphologists on a stained blood film using visual microscopy. Normally 100 white cells are counted and immature granulocytes are classified and counted as metamyelocytes, myelocytes and promyelocytes according to the stages of maturation. Manual differential count is very labour intensive, time consuming, imprecise (because of the small number of cells counted) and subject to personal bias. Previously, automated IG counts were not available. The presence of immature granulocytes were normally only flagged (as suspect flag) by all the haematology analysers available. With the introduction of the Sysmex XE-2100 analyser and the XE pro and XE IG Master software packages (Sysmex Corp., Kobe, Japan), it is now possible to enumerate and report IG counts automatically using fluorescence flow cytometry technique. The Sysmex XE-2100 does not count metamyelocytes, myelocytes and promyelocytes separately; instead they are counted together as immature granulocytes. Compared to the manual differential counts, the automated IG counts are much more precise and more accurate because of the large number of cells being counted. In this study, our objectives were to evaluate the agreement between the manual and automated methods. We also examined the stability, reproducibility and linearity of the automated method.

MATERIALS AND METHODS

Collection of Samples

Blood samples were collected into Greiner Vacuette K_3EDTA tubes (1.8 mg K_3EDTA per ml of blood as anticoagulant). They were processed by the Sysmex XE-2100 within one to two hours of arrival in the laboratory.

Selection of samples

Over a period of 5 weeks in July / August 2004, routine samples with automated IG count of > 2.0% were selected for the study. 244 samples were included in the study (3 samples were excluded from the study because of poor white cell morphology on the blood films). There were 208 (85%) adult samples, 35 (14.5%) paediatric samples and 1 (0.5%) neonatal sample. The automated IG counts ranged from 2.1 to 17.3 % (0.03 - 2.29 x 10^9/L). 72 % of the samples have IG counts between 2 - 5%. There were 13 samples with IG count of >10%. See ***Fig. 1***.

Manual IG count

For this study, we followed the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) H20-A protocol - Reference Leucocyte Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard [1)]. Three manual blood films were prepared within 2 hours of sample arrival in the laboratory. They were fixed with methanol and stained using Wright's Stain. Two experienced (>10 years morphology experience) qualified morphologists were chosen for the study. Each performed a 200-cell differential count on 2 separate blood films. Metamyelocytes, myelocytes and promyelocytes were counted as immature granulocytes. Band forms and blasts were counted separately. The average IG% counts from the two morphologists were used for the comparison. The manual IG absolute counts were calculated by multiplying the average IG% counts with the WBC counts.

Automated (Sysmex XE-2100) IG Counts

For this study, XE pro and XE IG Master (Version 00-03, Sysmex Corp., Kobe, Japan) were installed on the laboratory's Sysmex XE-2100 analyser by Sysmex engineer. Throughout the study period, quality controls (including the automated IG counts) were closely monitored using the low, normal and high levels of e-CHECK controls supplied by Sysmex Corporation.

IG counts are enumerated in the DIFF Channel of the Sysmex XE-2100 which employs the fluorescence flow cytometry technique by using semi-conductor laser. The DIFF Channel differentiates the leucocyte populations by using information obtained from side scatter light and side fluorescence light intensity. Side scatter light intensity indicates the internal complexity of the white cells whereas side fluorescence light intensity indicates the RNA and DNA contents of the white cells. Immature granulocytes contain more RNA and DNA compared to neutrophils; therefore they provide stronger side fluorescence light intensity which results in the separation of the immature granulocytes from the neutrophils. If the IG counts were judged to be less reliable because of poor separation from neutrophils, an asterisk will appear next to the IG results. For the purpose of comparison to the manual counts, we attempted to separate the automated IG counts into two groups, those with and those without asterisks.

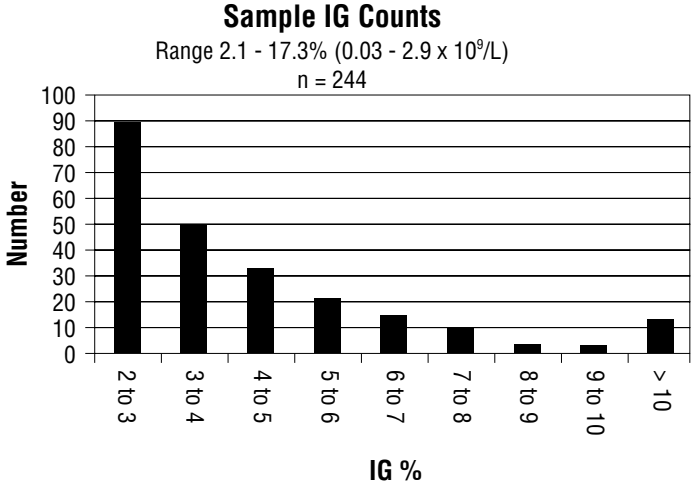


Fig. 1 *Distribution of sample IG counts (automated)*

RESULTS

Reproducibility

Reproducibility studies were carried out on three samples by 10 consecutive repeat analysis on the Sysmex XE-2100 analyser (9 times only on one sample because of insufficient blood). The IG counts of the three samples were 3.0% (0.28 x 10^9/ L), 8.6% (0.84 x 10^9/ L) and 18.6% (2.57 x 10^9/ L) respectively. See ***Table 1***.

Stability

Stability studies were carried out on two samples stored at 4 °C. The IG counts were analysed by the Sysmex XE-2100 analyser at 0 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 24 hours, 30 hours and 48 hours after storage. See ***Fig. 2***.

Linearity

Linearity study was performed on one sample with automated IG count of 18.6% (2.57 x 10^9/ L). The sample was diluted 1 in 2, 1 in 4, 1 in 6, 1 in 8 and 1 in 10 with Cellpack (whole blood diluent supplied by Sysmex Corp. for use in the Sysmex XE-2100) and the automated IG counts obtained. Regression analysis was performed on the expected IG values against the IG values obtained from the Sysmex XE-2100. The regression equation is y=1.01x - 0.0323 and the correlation is r=1.00. See ***Fig. 3***.

Comparison of manual and automated IG counts

For the comparison, we separated the samples into two groups - those with and those without the asterisk low reliability flag. There were 173 samples in the group without the asterisk flag and 71 samples in the group with the asterisk flag. ***Fig. 4*** shows the regression of the manual IG absolute counts and the automated Sysmex XE-2100 absolute IG counts without the asterisk flag. The regression equation is y=0.7902x + 0.1371 (n=173) and the correlation is r=0.81.

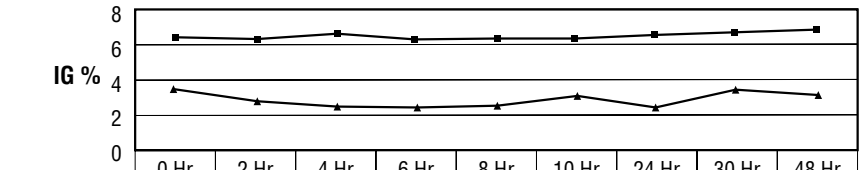
Fig. 5 shows the regression of the manual IG absolute counts and the automated Sysmex XE-2100 absolute IG counts with the asterisk flag. The regression equation is y=0.8855x + 0.2002 (n=71) and the correlation is r=0.67.

Fig. 6 shows the regression of the manual IG absolute counts and the combined automated Sysmex XE-2100 absolute IG counts with and without the asterisk flag. The regression equation is y=0.8718x + 0.1392 (n=244) and the correlation is r=0.72.

The regression studies showed good agreement between the manual IG counts and the automated Sysmex XE-2100 IG counts. We have observed a slight reduction in correlation (r=0.81 vs r=0.67) of the IG counts in the presence of the asterisk flag. This indicated that the XE IG Master's algorithm is quite efficient in determining the reliability and accuracy of the IG counts.

	IG%					IG# X 10^9/L				
	Runs	Range	Mean	CV	SD	Runs	Range	Mean	CV	SD
Sample 1	10	2.6 - 3.3	3.0	7.0	0.21	10	0.24 - 0.31	0.28	7.1	0.02
Sample 2	10	8.2 - 8.8	8.6	2.7	0.23	10	0.80 - 0.86	0.84	3.3	0.03
Sample 3	9	17.7 - 19.3	18.6	2.5	0.46	9	2.48 - 2.66	2.57	3.2	0.08
Average			10.1	4.1	0.30			1.23	4.5	0.04

Table 1 *Reproducibility of XE-2100 IG% and IG# counts*



	0 Hr	2 Hr	4 Hr	6 Hr	8 Hr	10 Hr	24 Hr	30 Hr	48 Hr
Sample 1	3.5	2.9	2.6	2.6	2.7	3.1	2.5	3.5	3.3
Sample 2	6.3	6.2	6.6	6.3	6.3	6.2	6.5	6.7	6.8

Fig. 2 *Stability of XE-2100 IG% counts*

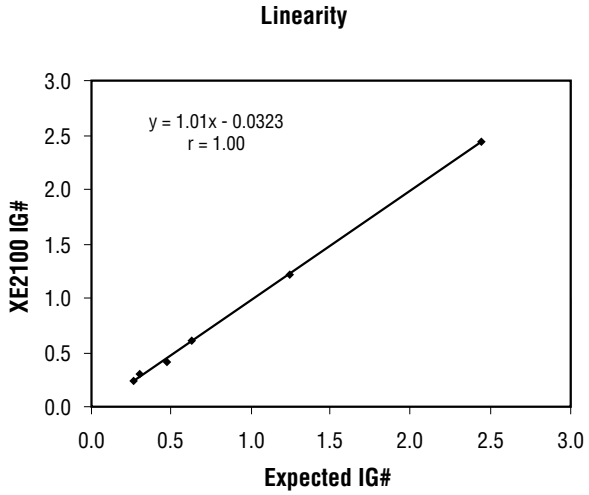


Fig. 3 *Linearity of IG# count*

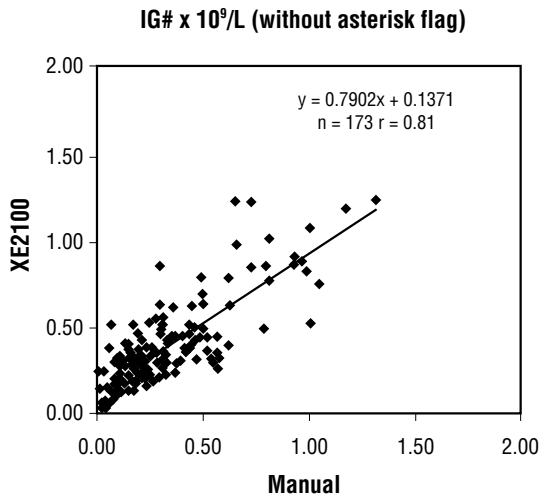


Fig. 4 *Correlation between manual and XE-2100 IG# counts (without asterisk)*

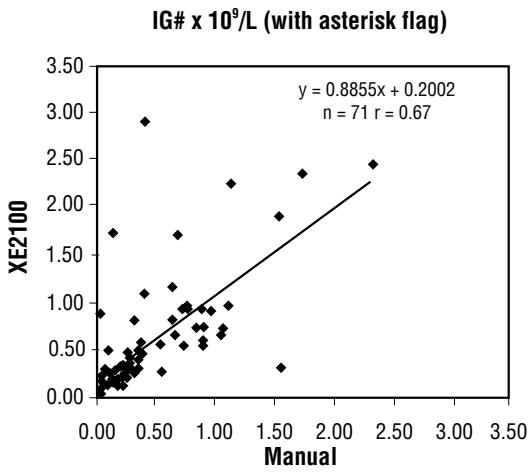


Fig. 5 *Correlation between manual and XE-2100 IG# counts (with asterisk)*

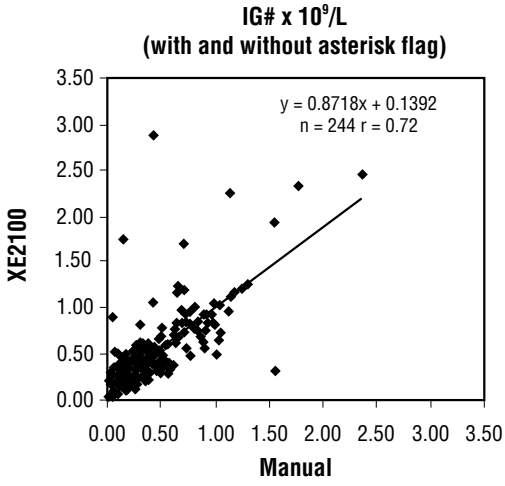


Fig. 6 *Correlation between manual and XE-2100 IG# counts (with and without asterisk)*

DISCUSSION

In this study, 244 routine samples with automated IG counts >2.0% were included. We have observed good agreement between the manual IG counts and the automated IG counts by the Sysmex XE-2100. The XE IG Master software uses a special algorithm to determine the reliability of the IG counts. In case of low reliability, the IG results will be displayed with an asterisk flag. The correlation of IG counts without asterisk is r=0.81. The correlation of IG counts with asterisk is r=0.67. The correlation of the combined IG counts with and without asterisk is r=0.72. This showed that the XE IG Master is quite efficient in providing accurate and reliable IG counts that are reportable (those counts without the asterisk). It is also capable of alerting the morphologists to confirm on the blood films those IG counts with asterisk present. In this study, we have found that a significant number of those IG counts with asterisk were in fact accurate and reportable. However, we did observe a few outliers in this group of samples. The majority of the outliers have higher automated IG counts compared to the manual counts. With further refinement of the asterisk algorithm, it may be possible in future to improve on its sensitivity and specificity. The presence of toxic granulation in the neutrophils seemed to have the tendency of triggering the asterisk flag. In our study, we have observed the presence of toxic granulation in 8% (6 out of 71) of samples with the asterisk flag whereas toxic granulation was present in only 3% (2 out of 173) of samples without the asterisk flag.

The automated IG counts by the Sysmex XE-2100 showed excellent reproducibility. The mean CV for the percentage and absolute IG counts were 4.1% and 4.5% respectively. The automated IG counts also showed excellent stability. The automated IG counts by the Sysmex XE-2100 remained stable for up to 48 hours on

samples stored at 4°C. We also demonstrated that the automated IG count by the Sysmex XE-2100 has good linearity.

We believe the automated IG counts from the Sysmex XE-2100 can significantly reduced the number of manual differentials performed in the haematology laboratory. In our laboratory, we currently examine blood films and manual differentials would be performed and reported if immature granulocytes are present. A significant number of these manual differentials would become unnecessary simply by reporting the Sysmex XE-2100 differential count with the IG count (extended differential count). Since the automated IG count by the Sysmex XE-2100 has already been approved by the FDA (Food and Drug Administration, U.S.A.) as a reportable parameter, haematology laboratory using the Sysmex XE-2100 analyser now has the option of reporting the automated extended differential counts and thereby significantly reduce the number of manual differential counts. From our study, we have observed the automated IG counts are highly reliable (r=0.81) when the asterisk flags are absent. Provided the IG asterisk and other significant machine flags are absent, the extended differential counts including the IG counts can be safely released to the clinicians without manual checking. In the presence of the asterisk flags, the automated IG counts are relatively less reliable and they should be confirmed on blood films before reporting. There is need for education of the clinicians for the meaning of automated immature granulocyte counts (combined counts of metamyelocytes, myelocytes and promyelocytes) before automated IG counts can become acceptable and reportable in routine doctors' reports. In addition, reference interval for IG counts should be established. Recently, Bruegel M et al. 2004 [2] did a study on healthy blood donors to establish the reference interval for immature granulocytes. Based on the data from that study and using the 95th percentile figures, the suggested reference interval for IG# is 0 - 0.03 x 10^9 /L for men and women. For IG%, the suggested reference interval is 0 - 0.5% for men and 0 - 0.4% for women. Further studies may be required to establish whether there is a significant difference of IG% count between normal men and women. In our laboratory, we only provide reference intervals for the absolute differential counts. We would endeavour to establish our own reference interval for IG count in future.

ACKNOWLEDGEMENT

We would like to express our thanks to the staff of Haematology SEALS Randwick for their efforts towards this study especially the two morphologists performing the manual differentials. We would also like to thank Roche Diagnostics Australia for their support and the supply of the XE pro and XE IG Master software.

References

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2) *Bruegel M et al.: Reference values for immature granulocytes in healthy blood donors generated on the Sysmex XE-2100 automated haematology analyser. Sysmex J. Int., 14, 5-7, 2004.*