

**THE AUTOMATED
COUNT ON THE SYSMEX
HAEMATOLGY**



**NUCLEATED RED CELL
XE-2100™
ANALYSER**

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INTRODUCTION

Figure 1 - The XE-2100



- **The XE 2100™ (Sysmex) (Figure 1) is a top of the range, recently introduced fully automated blood cell counter which uses fluorescence flow cytometry to quantitate the standard five cell differential, nucleated red blood cells, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), reticulocyte count and immature reticulocyte fraction. The analyser has the ability to measure platelets in two ways, by conventional impedance as well as an “optical” platelet count using a fluorescent dye.**
- **The analyser has a maximum throughput of 150 samples per hour and uses 200 ml of blood in closed mode and 130 ml in open mode. The workstation has the capacity to store data on 10,000 samples, including graphics.**
- **A new diagnostic feature of the instrument is when the analyser is run in NRBC mode it quantitates the nucleated red cell count (NRBC) and automatically corrects the total white cell count (WBC) and lymphocyte count.**

- In the NRBC channel a surfactant causes contraction of red blood cells and platelets, while staining nucleic acids of WBCs and nucleated red cells. The XE-2100 counts the nucleated red blood cells using side fluorescence and forward light scatter information obtained by illuminating the specimen with a semiconductor laser. The surfactant in the reagent, STROMATOLYSER-NR lyses red blood cell membranes, leaving only the nuclei of the NRBCs. WBC membranes are not lysed and their cytoplasm is retained. The fluorescent polymethine dye in STROMATOLYSER-NR penetrates the cell membrane of WBCs and stains cytoplasmic organelles. Thus two clear populations emerge on the basis of differences in fluorescence intensity. Using forward scatter light signals, volumetric differences between nucleated cells and ghosts permit their clear distinction. (Figure 2)
- The larger the amount of nucleic acids in a cell, the stronger the side fluorescence signal on the X axis. The larger the size of a cell, the larger the forward scatter on the Y axis. NRBCs are separated from WBCs due to the difficulty of staining the cells which have been stripped into nuclei and contracted. (Figure 3)

Figure 2 - Stomatolyser-NR lysing and staining reaction.
Stomatolyser-NR lysing and staining reaction

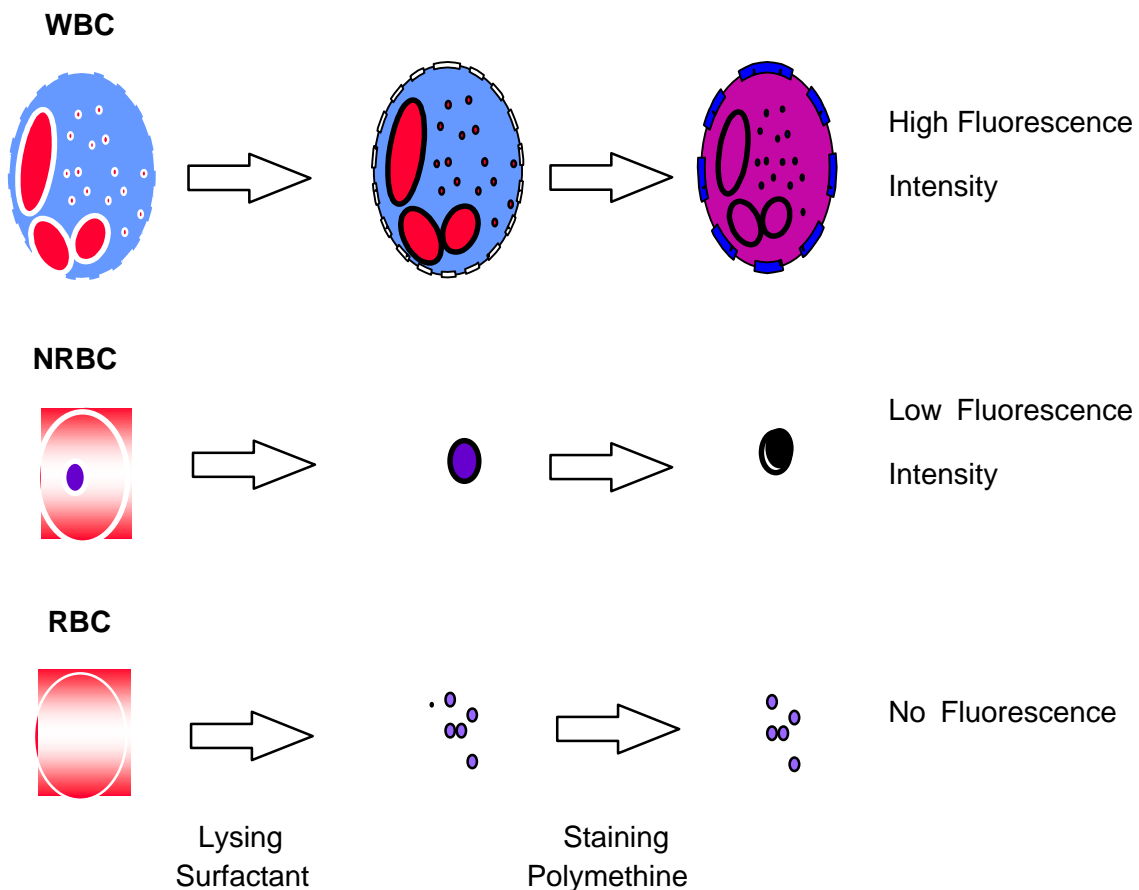
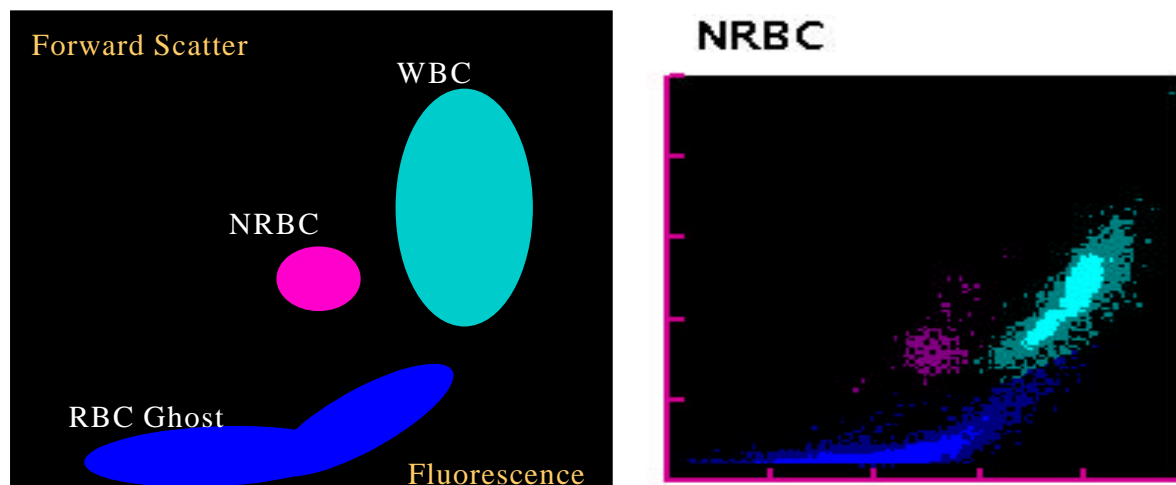


Figure 3 - NRBC CHANNEL



- When the analyser is not run in NRBC mode a NRBC? Flag is generated from the WBC/BASO channel. The displayed WBC value includes the NRBCs but an action message “Count NRBC-ch” appears to alert the user to perform an NRBC count.

METHODS

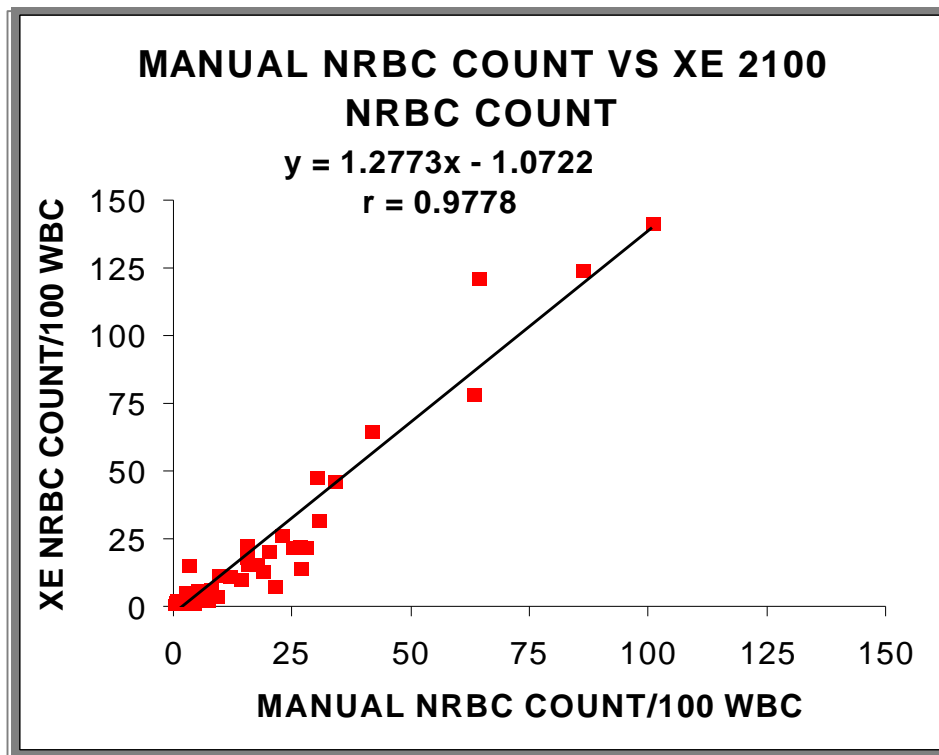
- The anticoagulant used was K₃EDTA in vacutainers; (BD Haemoguard) and all samples were processed within 4 hours of venesection.
- 100 apparently healthy normal controls and 149 samples selected by diagnosis in which one was likely to find NRBCs were analysed on the XE-2100 in the NRBC mode. The pathological samples were mostly from patients with haemaglobinopathies, neonates, haematology/oncology patients, HIV and malaria.
- The XE-2100 NRBC count per 100 WBCs was compared to a manual 400 cell differential count was performed according to NCCLS H20 - A protocol. 2 x 200 cell counts by at least two observers.
- To assess the performance of the NRBC? flag and action message to alert the user to perform a NRBC count when the analyser is not in NRBC mode a further 62 samples (27 without NRBCs and 35 with NRBCs) were analysed in NRBC mode to establish whether there were NRBCs present and then in CBC and DIFF mode. The presence or absence of the flag and action message were noted.
- Linearity, reproducibility, carryover and stability of the NRBC count were all assessed.

RESULTS

- From the apparently healthy normal controls, all samples were negative for NRBCs
- Of the 149 pathological samples 107 had NRBCs on the XE-2100 ranging from 0.1 - 855 per 100 WBCs. The lower limit of detection on the XE-2100 is 0.1 per 100 WBCs.
- The lower limit of detection for the manual count was 0.5 per 100 WBCs. From the 107 samples positive on the XE-2100, 29 (27%) were negative on the manual film, with a range of 0.1-1.4 per 100 WBCs.

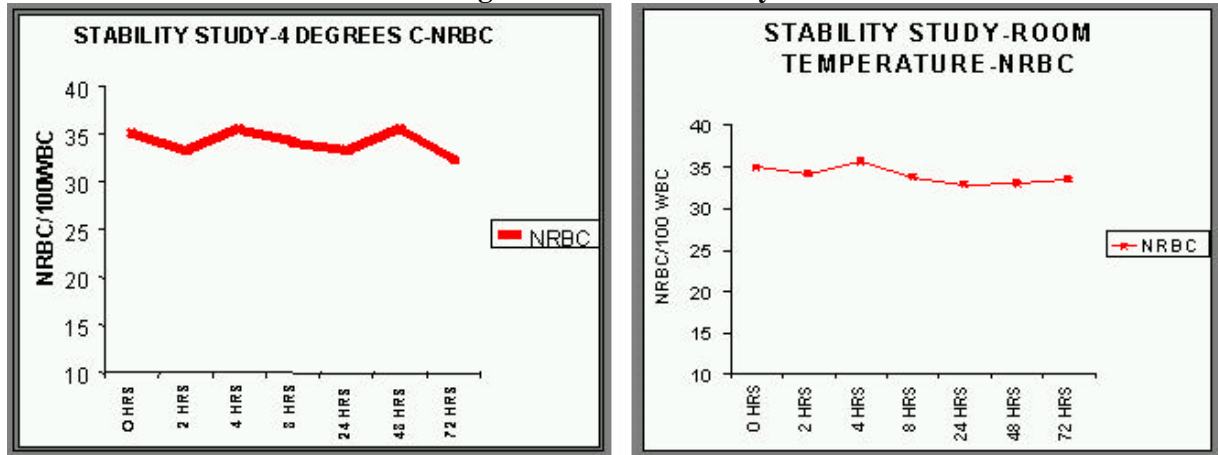
- Two samples had a negative NRBC count on the XE-2100 but cells were seen on the film, one from a patient with myelodysplastic syndrome, 1.5 NRBCs per 100 WBCs and the other from a neonate, 1 NRBC per 100 WBCs.
- Thus 76 samples were positive on the XE-2100 and manual count. The correlation for these samples can be seen in Figure 4. The two highest values at 255 manual and 192.6 on the XE-2100 and 898 manual and 855 on the XE-2100 were excluded to increase the statistical validity of the correlation value.
- There was no evidence of interference from red cell inclusion bodies such as Howell Jolly bodies, Pappenheimer bodies or malarial parasites.

Figure 4 - Correlation of manual and XE-2100 NRBC counts



- The NRBC? flag and action message was found to give a true positive or negative result in 93.5% of cases with a false positive flag in 5% and a false negative in 1.5%.
- No carryover for the NRBCs was detectable.
- Reproducibility on 10 consecutive measurements on a sample with a mean NRBC count of 63.91 gave a CV of 2.25%.
- Linearity showed an r^2 value of 0.99. The NRBC count showed excellent stability over 72 hours both at room temperature and 4°C. (Figure 5)

Figure 5 - NRBC stability



CONCLUSIONS

- The NRBC count reported by the XE-2100 gives excellent correlation with the manual reference counts.
- It is likely that the XE-2100 is more accurate and precise than the manual count due to the greater number of cells being counted and the lower limit of detection.
- The total WBC count and lymphocyte count are automatically corrected in samples with a positive NRBC count.
- An accurate NRBC count is important in the diagnosis and therapeutic monitoring of various clinical states such as sickle cell disease, β Thalassaemia major, neonates, haematological and other malignancies and megaloblastic anaemia.
- The ? NRBC flag and action message is sensitive and specific.
- The introduction of this new parameter will greatly reduce the number of manual differentials performed in the laboratory and the laborious correction of the total white cell count.

