Automated counting of nucleated red blood cells in blood samples of newborns

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Summary
Nucleated red blood cells (NRBC) in blood samples interfere with the white blood cell (WBC) count on many types of automated haematology analysers. This makes it necessary to correct the WBC count by counting NRBC microscopically. This report describes the evaluation of two analysers, the Cell-Dyn 4000 and the Sysmex XE-2100, which use new techniques to recognize and enumerate NRBC. We conclude that both the Cell-Dyn 4000 and the Sysmex XE-2100 give an accurate WBC count in the presence of NRBC. Furthermore, they can enumerate NRBC correctly when compared with microscopic observation.

Keywords
Nucleated red blood cell, automated haematology analysers, white blood cell, Cell-Dyn 4000, Sysmex XE-2100

Introduction
The presence of nucleated red blood cells (NRBC) in the peripheral blood may indicate pathological erythropoiesis or may be physiological in newborns and very young children. When present with immature cells of the granulocyte series, leukaemia, neoplasia, cardiac malfunction and/or infections (Schwartz & Stanbury, 1954) may be inferred. NRBC alone are found mainly in situations such as thalassemia major, haemolytic anaemia’s, extreme blood loss or severe hypoxia. NRBC may also be seen in extramedullary erythropoiesis. In these conditions, NRBC must be identified and counted correctly, even at low numbers. In newborns and very young children, the presence of NRBC is physiological, indicating immaturity of the bone marrow or persistent splenic erythropoiesis. Identification and counting of NRBC is important, as their presence interferes with the counting of white blood cells (WBC) in automated analysers. This may lead to an erroneous result and wrong diagnosis and treatment, especially in neonatal patients with sepsis and low WBC counts.

Until recently, most laboratories have used manual review procedures to detect and estimate the number of NRBC and correct the leucocyte count. The stained blood film is examined and 100 or 200 WBC are counted together with the number of observed NRBC and results are expressed in NRBC per 100 WBC (National Committee for Clinical Laboratory Standards, 1992). This method is time-consuming, subjective and statistically imprecise, due to the small number of cells counted.

Both the Cell-Dyn 4000 (Abbott Laboratories, Santa Clara, CA, USA) and the Sysmex XE-2100 (TOA, Kobe, Japan) are able to detect and quantify NRBC. To verify their ability to count NRBC accurately, we collected blood samples from our neonatal department and compared the results of both automated haematology analysers with the results obtained by microscopic examination of the stained blood films.

Materials and methods
Blood was obtained from patients in the department of neonatology, collected by heel prick into microtainers containing K3EDTA. Blood samples were processed within 4 h (National Committee for Clinical Laboratory Standards, 1992). Samples with less than 0.5% NRBC on both analysers were not reviewed microscopically because such low percentage has no effect on the result of the WBC counting.

All samples with a positive signal for the presence of NRBC were remeasured on the other analyser, and a blood
smear was made. The blood smears were stained by the May Grünwald–Giemsa method and NRBC per 200 WBC were counted by two experienced technologists. The results were reported as NRBC per 100 WBC. Microscopy was used as the reference method.

The Cell-Dyn 4000 uses an argon laser in combination with two fluorescence channels to measure NRBC. The reagent lyses the membrane of NRBC and mature erythrocytes, while WBC are left intact. The fluorochrome propidium iodide binds to the nuclear DNA of the NRBC and WBC. The combination of cell size and fluorescence intensity permits the separation of NRBC from the leucocyte population. The absolute NRBC count and the proportion of NRBC per 100 WBC are reported (Cell-Dyn 4000 system operator’s manual, 1999). Measurements were performed in the ‘extended lyse mode’ because erythrocytes in newborns are relatively resistant to the lysis agents.

The Sysmex XE-2100 uses a semiconductor laser. The cell membranes of NRBC and mature erythrocytes are lysed, while the WBC become permeable, allowing quick influx of the dye, but remain intact. The nuclear material of NRBC and WBC is stained with a polymethine-based dye.

Passing and Bablok regression (Passing & Bablok, 1983) was used to calculate the agreement between the various methods. Bland and Altman (1995) plots were used to determine the deviation of the automated methods from the reference method.

**Results**

The number of NRBC in the measured samples ranged from 0 to 414 per 100 WBC, using the reference method, with an average of 20. It was possible to perform measurements on both analysers and to obtain blood films in 139 samples. An additional 15 samples were used for the correlation study of microscopy vs. the Smax XE2100 (XE) because there was not enough blood for measurements on both instruments. Passing and Bablok (1983) analysis yielded correlation coefficients of 0.972, 0.980 and 0.995 respectively for comparisons of microscopy with the XE2100, microscopy with Cell-Dyn 4000 (CD) and for the XE2100 with the CD4000 (Table 1).

In the Bland and Altman (1995) plots (Figure 1), the mean differences from the microscopic count were −1.4 and −1.7 respectively for the XE2100 and CD4000. For the different ranges (1–10, 10–100 and > 100 NRBC by the microscopic count) the mean differences for the XE2100 were −0.8, −5.4 and 3.4 respectively. For the CD4000, the mean differences were 0.1, 4.5 and −21.5 respectively.

**Discussion**

The clinical importance of identification and enumeration of NRBC is linked to diagnostic significance of the presence of these cells in the peripheral blood. This can mask leukopenic sepsis or falsely elevate the WBC, particularly in newborn patients. Various companies have searched for methods to establish the presence of NRBC. Most current haematology analysers flag for the possible presence of NRBC. In positive cases, a microscopic count should be

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**Table 1. Counting of NRBC: summary of Passing and Bablok (1983) results of two haematology analysers (XE: XE2100 and CD: CD4000) and microscopy (M)**

<table>
<thead>
<tr>
<th></th>
<th>M vs. XE</th>
<th>M vs. CD</th>
<th>XE vs. CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>139</td>
<td>154</td>
<td>139</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.972</td>
<td>0.980</td>
<td>0.995</td>
</tr>
<tr>
<td>Slope</td>
<td>0.995</td>
<td>0.867</td>
<td>0.965</td>
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<tr>
<td>Y-intercept</td>
<td>−1.2</td>
<td>0.5</td>
<td>1.2</td>
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**Figure 1. Microscopy vs. automated analysers. (a) Microscopy vs. Sysmex XE-2100 (XE); mc, microscopic count (n = 154). (b) Microscopy vs. Cell-Dyn 4000 (CD); mc, microscopic count (n = 139).**
performed to correct the WBC count before reporting the result. This procedure is time consuming and can only be performed by experienced technologists, as the evaluation of blood smears in newborns is often difficult. Slide preparation may be difficult because of the high RBC count in newborns. The presence of damaged WBC may also interfere with the quantitation of NRBC per 100 WBC.

Previously, efforts were made to detect and count NRBC with the aid of antibodies (CD71, CD45 etc.) in combination with flow cytometry (Borowitz et al., 1993; Pattanapanyasat et al., 1994; Tsuji et al., 1999). Although these methods were shown to provide reliable results, there were some major drawbacks. Specialized flow cytometry skills were needed and the method was expensive and time consuming. Furthermore, relatively large volumes of blood were needed for these flow cytometric determinations which could pose problems in newborns and very young children.

For efficiency and economy, automated NRBC counting is the method of choice, especially in laboratories with many samples from newborns. The results of the present study show that both the CD4000 and XE2100 can detect NRBC reliably, the results showing good correlation with microscopic NRBC counts, and enabling accurate enumeration of WBC.

References