Flow Cytometric Reticulocyte Counting

Parallel Evaluation of Five Fully Automated Analyzers: An NCCLS-ICSH Approach

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Key Words: Reticulocyte counting; Automated analyzers

Abstract

We performed a parallel evaluation of 5 automated reticulocyte analyzers. The guidelines were those proposed by the National Committee for Clinical Laboratory Standards and the International Council for Standardisation in Haematology. Duplicate analyses were performed for 225 healthy subjects and 115 patients affected by various diseases.

The reference intervals were different for each method (ADVIA 120, 27-125 × 10^3/μL [27-125 × 10^9/L]; CELL DYN 4000, 25-108 × 10^3/μL [25-108 × 10^9/L]; GEN-S, 20-85 × 10^3/μL [20-85 × 10^9/L]; SE 9500 RET, 23-95 × 10^3/μL [23-95 × 10^9/L]; and VEGA RETIC, 30-130 × 10^3/μL [30-130 × 10^9/L]). The comparisons of percentage counts with the microscopic reference method were satisfactory for all automated methods. However, a tendency to overestimate at low counts was noted. This progressively increased from the SE 9500 RET to the VEGA RETIC. The imprecision was excellent for all the methods at normal and high concentrations. However, a tendency to overestimate at low counts was noted. This progressively increased from the SE 9500 RET to the VEGA RETIC. The imprecision was excellent for all the methods at normal and high concentrations. When compared with the microscopic reference, the analyzers showed satisfactory sensitivity at low counts and excellent sensitivity at high counts. The overall agreement varied from 74.8% for the GEN-S to 86.1% for the SE 9500 RET.

Automated reticulocyte counting, made possible several years ago by means of multipurpose or dedicated flow cytometers, recently has been extended to common hematology analyzers, initially in semiautomation and later in complete automation. The advantages derived from automation are high throughput, reduced imprecision (essentially owing to the high number of cells counted), and the production of further clinically useful parameters, such as the immature reticulocyte fraction and reticulocyte indices.

Problems still exist that essentially depend on the differing sensitivities of the dyes used to stain the RNA of reticulocytes, on the technology used to identify positive cells (fluorescence, light scattering, absorbance), and on the software that is more or less capable of separating reticulocytes from erythrocytes (since there is a physiologic continuum between these populations) and from other cells, such as platelets or nucleated RBCs (NRBCs). All this results in counts that sometimes disagree with one another depending on the method, with the consequent need for reference intervals that are method specific. A further limit is due to the different performances on samples with severe reticulocytopenia, but rarely do these have implications for clinical decision making, as in patients with reticulocytopenia. The lack of calibration material that is stable over time and that can be used by all methods contributes to the interpretive uncertainties regarding the comparison of methods and the agreement of the results of the various commercially available systems.

The use of fresh blood, the ideal material for reticulocyte counts, as a calibrator has some limitations such as the following: (1) the poor stability over time of the concentration of reticulocytes, which undergo progressive maturation;
(2) the lack of a universally accepted reference method of measuring reticulocyte concentrations; and (3) the exacting calibration procedures that are strictly manufacturer dependent and that cannot easily be modified by the user.

Two standards have been published: (1) H44-A,4 jointly prepared by the National Committee for Clinical Laboratory Standards (NCCLS) and the International Council for Standardisation in Haematology (ICSH), proposes guidelines for the evaluation of automated methods of reticulocyte counts. (2) The ICSH standard5 proposes a microscopic reference method for reticulocyte counts.

The aim of our study was, through the use of the aforementioned guidelines, to evaluate the analytic performance of the 5 most recently available automated hematology analyzers by a parallel study in a single institution. Five analyzers were studied. Particular attention was given to the performance on samples with very low reticulocyte concentrations.

Materials and Methods

Analyzers

Five hematology analyzers with the option of reticulocyte counts in complete automation were evaluated over 6 weeks, in a parallel study at the Clinical Pathology Laboratory of the Geriatric Hospital of Padua, Padua, Italy. The analyzers studied were the following: CELL DYN (CD) 4000 (software version 7.5, Abbott, Santa Clara, CA); VEGA RETIC (currently called PENTRA 120; software version 3.16, ABX, Montpellier, France); ADVIA 120 (software version 1.16, Bayer Diagnostic Division, Tarrytown, NY); GEN-S (software version 1D, Coulter-Beckman, Hialeah, FL); and SE 9500 RET (previously called SE-4000 (software version 7.5, Abbott, Santa Clara, CA); H44-A criteria, were selected for calculation of the reference intervals for tested analyzers. The subjects were males and females 3 to 50 years old. From these subjects, only 126 samples were used for the manual microscopic reference method. An additional 115 patients affected by various diseases or conditions (bone marrow transplantation, hemolytic and posthemorrhagic anemias, bone marrow aplasia, acute and chronic inflammations, neoplastic diseases, nutritional anemias before and during treatment, myeloproliferative and lymphoproliferative diseases) were examined.

To study the behavior of the analyzers on samples with reticulocyte counts near zero, samples were collected from 46 patients who had undergone intensive chemotherapy for acute leukemia or were in the aplastic phase after bone marrow transplantation. None of these patients had received transfusions during the 5 days before sample collection. Since some potential interfering substances on reticulocyte counts are known to exist,4 the behavior of the analyzers was verified in the presence of the following substances: thrombocytes (n = 6; range, 850-1,310 × 10^9/L [850-1,310 × 10^9/L]); giant platelets (n = 8); microcytic RBCs (n = 5, range, 400-5,000/µL [0.4-5.0 × 10^9/L]); macrocytic RBCs (n = 5, range, 58-67 fL); dimorphic RBC population (n = 4); lymphocytes (n = 3; range, 54,000-93,000/µL [54.0-93.0 × 10^9/L]); NRBCs (n = 4; range, 400-5,000/µL [0.4-5.0 × 10^9/L]). All samples were collected by venipuncture using 5-mL tripotassium EDTA–evacuated tubes (Vacutainer, Becton Dickinson, Rutherford, NJ). For patients with bone marrow aplasia, samples were taken from leftover blood collected for routine CBC counts. The wedge films were prepared within 2 hours after collection for microscopic assessment. All samples were processed within 4 hours using the various analyzers.

Microscopic Assessment

The new blue methylene method was used (Sigma Diagnostics, St Louis, MO), and reticulocytes were counted according to the indications of the ICSH.5 Briefly, 2 sets of trained clinical pathologists proceeded with the counts, blinded, on each of 2 different films for each sample. With a 2-headed microscope, all the erythrocytes and reticulocytes observed in several fields (with edge rule) were counted for each film until a count of at least 1,000 RBCs was reached.

Table I
Analytic Methods of Reticulocyte Counters*

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Dye</th>
<th>Technology</th>
<th>Collected Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVIA 120</td>
<td>Oxazine 750</td>
<td>Absorbance</td>
<td>−40,000</td>
</tr>
<tr>
<td>CELL DYN 4000</td>
<td>CD4K 530</td>
<td>Fluorescence</td>
<td>50,000</td>
</tr>
<tr>
<td>GEN-S</td>
<td>New methylene blue</td>
<td>Light scattering</td>
<td>32,000</td>
</tr>
<tr>
<td>SE 9500 RET</td>
<td>Auramine O</td>
<td>Fluorescence</td>
<td>−30,000</td>
</tr>
<tr>
<td>VEGA RETIC</td>
<td>Thiazole orange</td>
<td>Fluorescence</td>
<td>32,000</td>
</tr>
</tbody>
</table>

* See text for proprietary information.
The mean value obtained by both sets of observers was used for comparison with the automated methods.

**Statistical Analysis**

Every sample was analyzed in duplicate and in random order. The absolute value \((\times 10^9/L)\) was provided by the various analyzers, while for the reference method it was obtained by multiplying the percentage values by the RBC count given by the routine hematology analyzer. The mean values obtained for each method were compared for a total of 256 samples comprising healthy subjects and patients.

The comparison with the manual reference method was performed for all samples and separately for the subgroup of the reticulocytopenic samples. This comparison was presented graphically with 95% confidence intervals, which represent the standard error of the parameter, and with linear regression statistics. The results obtained also were compared by nonparametric statistics based on ranks: first a Friedman test and subsequently a Wilcoxon 2-tailed test.

To verify interchangeability of the various automated methods, the reciprocal comparison was summarized in the regression and correlation statistics.

The imprecision was estimated with 2 methods: (1) with 1-way analysis of variance between duplicates of all samples analyzed divided into 3 subgroups (low, normal, high) selected after having established reference intervals for each method; and (2) with the imprecision profile extended to a wide range of values \((1.5-250 \times 10^3/\mu L [1.5-250 \times 10^9/L])\) by performing repeated analysis (8-10 counts) on several samples (15-20 samples) for each analyzer.

**Clinical Usefulness**

The clinical usefulness (ability to distinguish between normal and abnormal conditions) was evaluated on samples from the 115 patients using the criteria proposed by the NCCLS H20-A12 and by the ICSH. The absolute count was classified by reference and tests into normal, abnormal high, and abnormal low according to whether the results were above or below the reference interval.

**Linearity and Carryover**

Linearity was evaluated by mixing (in distinct experiments) a sample with a moderately elevated reticulocyte concentration (mean of 4 counts from 94.4 \(\times 10^3/\mu L [94.4 \times 10^9/L; 6.3\%]\) for the SE 9500 RET to 164 \(\times 10^3/\mu L [164 \times 10^9/L; 8.7\%]\) for the CD 4000) in decreasing proportion with an ABO-compatible reticulocytopenic sample (mean of 4 counts from 1.4 \(\times 10^3/\mu L [1.4 \times 10^9/L; 0.06\%]\) for the SE 9500 RET to 10.5 \(\times 10^3/\mu L [10.5 \times 10^9/L; 0.6\%]\) for the GEN-S). The counts were performed in duplicate for each dilution, and the mean value was used. The calculation of carryover was done in separate experiments for each instrument by analyzing a pair of samples: 1 sample at a high reticulocyte concentration (from 250 \(\times 10^3/\mu L [250 \times 10^9/L]\) for the GEN-S to 117 \(\times 10^3/\mu L [117 \times 10^9/L]\) for the VEGA RETIC) analyzed 3 times \((i_1, i_2, i_3)\), immediately followed by 1 sample at a low concentration (from 3.5 \(\times 10^3/\mu L [3.5 \times 10^9/L]\) for the CD 4000 to 12.4 \(\times 10^3/\mu L [12.4 \times 10^9/L]\) for the VEGA RETIC), also analyzed 3 times consecutively \((j_1, j_2, j_3)\), and using the formula \([\frac{(j_1-j_3)}{(i_3-j_3)}]\) \(\times 100\).

**Results**

**Reference Intervals**

The reference intervals in percentage and absolute values of the 5 tested methods and the reference method are given in Table 2I. Since the distributions are approximately log-normal, the intervals were calculated with a nonparametric method (the central 95% of the distribution). Note that it is possible to distinguish 2 groups: one consisting of the microscopic reference method, SE 9500 RET, and GEN-S with median values between 43 and 46 \(\times 10^3/\mu L [43-46 \times 10^9/L]\), and the other consisting of the ADVIA 120, the CD 4000, and the VEGA RETIC with median values between 57 and 60 \(\times 10^3/\mu L [57-60 \times 10^9/L]\).

The results agree with published results obtained with the microscopic method and with previous results.
for the SE 9500 RET (data referred to the R 1000 or 3000),\(^2\)\(^,\)\(^4\) the ADVIA 120 (formerly the H3),\(^1\)\(^,\)\(^4\) the GEN-S (previous data referred to MAXM or STKS),\(^2\) and the CD 4000.\(^1\)\(^3\) Data are not available for the VEGA RETIC.

**Comparison Between Methods**

The comparison between the manual reference method and tests according to the NCCLS H44-A recommendations\(^4\) is shown in **Figure 1A**, **Figure 1B**, **Figure 1C**, **Figure 1D**, and **Figure 1E**. The linear regression statistics are summarized in **Table 3**. **Figure 1F** shows the corresponding regression line of the 5 methods.

**Table 4** shows the results obtained with the Wilcoxon test in percentages and absolute counts.

Note that all methods have a tendency to overestimate, both in percentage and absolute values with respect to the microscopic method. This overestimation becomes negligible for the SE 9500 RET (in fact, it is the only method that gave differences that were not statistically significant) and increases progressively to its highest value (0.39%, \(17.41 \times 10^9 /\mu L\)) for the VEGA RETIC.

Even regression statistics confirm the existence of a constant positive intercept (from \(11.15 \times 10^3 /\mu L\) [\(11.15 \times 10^9 /L\)] for the SE 9500 RET to \(23.42 \times 10^3 /\mu L\) [\(23.42 \times 10^9 /L\)] for the VEGA RETIC) with a slope less than 1. This agrees with the tendency to overestimate at low values and to underestimate at normal and high values.

This behavior also was evident on the bias plot in **Figure 2**, which shows the comparison of the reticulocytopenic samples only (\(<10 \times 10^9 /L\)). The tendency was toward overestimation, with average differences of \(4.1 \times 10^3 /\mu L\) (\(4.1 \times 10^9 /L\)) for the SE 9500 RET, \(6.4 \times 10^3 /\mu L\) (\(6.4 \times 10^9 /L\)) for the CD 4000, \(6.6 \times 10^3 /\mu L\) (\(6.6 \times 10^9 /L\)) for the ADVIA 120, \(10.8 \times 10^3 /\mu L\) (\(10.8 \times 10^9 /L\)) for the GEN-S, and \(11.4 \times 10^3 /\mu L\) (\(11.4 \times 10^9 /L\)) for the VEGA RETIC.

Even if the overestimation could be due to difference between the “discrete” manual method, which measures the amount of a precipitate, and the automated methods, which use a “continuum” measure of RNA content, this cannot completely justify the difference between instruments. In fact, independent of the results obtained by the microscopic reference method, the concentrations obtained on the 10 lowest counts of aplastic marrow samples by each analyzer were included between 1.0 and \(2.0 \times 10^3 /\mu L\) (1.0-\(2.0 \times 10^9 /L\); mean, \(1.7 \times 10^3 /\mu L\) [\(1.7 \times 10^9 /L\)] for the SE 9500 RET; between 0.9 and \(2.6 \times 10^3 /\mu L\) (0.9-\(2.6 \times 10^9 /L\); mean, \(1.7 \times 10^3 /\mu L\) [\(1.7 \times 10^9 /L\)] for the CD 4000; between 1.2 and \(3.3 \times 10^3 /\mu L\) (1.2-\(3.3 \times 10^9 /L\); mean, \(2.3 \times 10^3 /\mu L\) [\(2.3 \times 10^9 /L\)] for the ADVIA 120; between 3.1 and \(6.0 \times 10^3 /\mu L\) (3.1-\(6.0 \times 10^9 /L\); mean, \(5.1 \times 10^3 /\mu L\) [\(5.1 \times 10^9 /L\)] for the GEN-S; and between 2.9 and \(7.1 \times 10^3 /\mu L\) (2.9-\(7.1 \times 10^9 /L\); mean, \(5.7 \times 10^3 /\mu L\) [\(5.7 \times 10^9 /L\)] for the VEGA RETIC. The analytic sensitivity was, therefore, particularly noteworthy for the SE 9500 RET and the CD 4000.

**Table 3**

**Comparison of Percentage Results for Reticulocyte Count: Linear Regression Statistics**

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of Samples</th>
<th>Intercept</th>
<th>Slope</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVIA 120</td>
<td>256</td>
<td>0.38</td>
<td>0.89</td>
<td>0.76</td>
</tr>
<tr>
<td>CELL DYN 4000</td>
<td>256</td>
<td>0.39</td>
<td>0.96</td>
<td>0.82</td>
</tr>
<tr>
<td>GEN-S</td>
<td>256</td>
<td>0.36</td>
<td>0.86</td>
<td>0.73</td>
</tr>
<tr>
<td>SE 9500 RET</td>
<td>256</td>
<td>0.20</td>
<td>0.84</td>
<td>0.80</td>
</tr>
<tr>
<td>VEGA RETIC</td>
<td>256</td>
<td>0.49</td>
<td>0.91</td>
<td>0.81</td>
</tr>
</tbody>
</table>

* See text for proprietary information.

**Table 4**

**Comparison of Methods: Analysis of Data by the Wilcoxon Test**

<table>
<thead>
<tr>
<th>Method</th>
<th>% × 10^9/L % × 10^9/L % × 10^9/L % × 10^9/L % × 10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVIA 120</td>
<td>Mean 1.18 58.51 1.52 58.85 1.38 52.76 1.20 47.80 1.56 64.80</td>
</tr>
<tr>
<td></td>
<td>Minimum 0.02 1.20 0.04 0.90 0.11 3.07 0.04 1.00 0.13 2.90</td>
</tr>
<tr>
<td></td>
<td>Maximum 8.66 251.60 8.25 269.00 6.65 220.49 6.86 237.40 7.23 278.55</td>
</tr>
<tr>
<td></td>
<td>Mean difference 0.25 11.12 0.35 11.46 0.20 5.37 0.02 0.42 0.39 17.41</td>
</tr>
<tr>
<td></td>
<td>SD of differences 0.65 24.76 0.57 21.89 0.69 27.22 0.54 20.65 0.57 25.03</td>
</tr>
<tr>
<td></td>
<td>P .01 .01 .01 .01 .01 .50 .75 .01 .01</td>
</tr>
</tbody>
</table>

* The values × 10^9/L are Système International units; traditional units are × 10^9/µL. See text for proprietary information.
Table 5 gives the regression statistics when methods were compared reciprocally to verify possible interchange. The results were better when the various automated systems were compared among themselves rather than against the microscopic reference method.

Imprecision

The coefficients of variation (CVs) are given in Table 6. The imprecision of all the methods decreased as the reticulocyte concentration increased and did not differ from results obtained in similar studies.1,2,14-16 It should be noted...
that all methods at all concentrations showed an imprecision less than that of the microscopic method.\textsuperscript{1,2,17} The results generally indicated a lower imprecision for the SE 9500 RET and the CD 4000, followed by the GEN-S and then the VEGA RETIC and ADVIA 120. The imprecision profile also showed analogous behavior, and, as seen in Figure 3, near moderate reticulocytopenia ($10^9$ \textmu L [$10^9$/L]); the CV was a minimum of 11% for

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
 & ADVIA 120 & CELL DYN 4000 & GEN-S & SE 9500 RET & VEGA RETIC & Reference \\
\hline
Intercept & 3.758 & 7.076 & 8.312 & 3.064 & 18.141 & \\
Slope & 0.930 & 0.975 & 1.050 & 0.856 & 0.852 & \\
$\hat{r}^2$ & 0.816 & 0.817 & 0.828 & 0.767 & 0.704 & \\
\hline
Intercept & 7.562 & 9.563 & 6.53 & 0.299 & 18.202 & \\
Slope & 0.877 & 0.934 & 1.094 & 0.904 & 0.858 & \\
$\hat{r}^2$ & 0.816 & 0.817 & 0.954 & 0.908 & 0.758 & \\
\hline
Intercept & 3.714 & 7.134 & 6.563 & 0.299 & 18.202 & \\
Slope & 0.838 & 0.954 & 1.094 & 0.904 & 0.858 & \\
$\hat{r}^2$ & 0.817 & 0.904 & 0.954 & 0.908 & 0.758 & \\
\hline
Intercept & 1.641 & 3.804 & 3.04 & 11.151 & \\
Slope & 0.789 & 0.834 & 0.766 & 0.773 & \\
$\hat{r}^2$ & 0.828 & 0.979 & 0.904 & 0.908 & \\
\hline
Slope & 0.897 & 0.977 & 1.096 & 1.096 & 1.096 & 0.873 & \\
$\hat{r}^2$ & 0.767 & 0.783 & 0.862 & 0.862 & 0.862 & 0.706 & \\
\hline
Slope & 0.827 & 0.954 & 0.766 & 0.766 & 0.766 & \\
$\hat{r}^2$ & 0.704 & 0.904 & 0.773 & 0.773 & 0.773 & \\
\hline
\end{tabular}
\caption{Simple Regression Analysis of Absolute Reticulocyte Count From 256 Samples$^*$}
\end{table}

$^*$ See text for proprietary information.
Linearity and Carryover

The linearity was excellent for all systems, both in absolute values (intercept between 0.585 and 1.09 × 10^9/L; slope between 0.993 and 1.005, and R^2 > 0.99) and in percentages. Figure 4 shows the corresponding linearity graph for the CD 4000 and for the GEN-S. The carryover calculated on the absolute concentrations was negligible and varied from 0.8% for the CD 4000 and the GEN-S to 1.8% for the SE 9500 RET, 1.9% for the ADVIA 120, and 3.5% for the VEGA RETIC.

Clinical Usefulness

Table 7 and Table 8 give the results for clinical usefulness for 115 patients by using only absolute concentrations. The values of sensitivity and the predictive value of a positive result were calculated separately for “low” and “high” samples; the calculation of specificity, of the predictive value of a negative test, and of concordance refer to the entirety of samples. The incorrect classification of patients concerns adjacent groups (low vs normal or high vs normal) with the exception of only 1 patient with beta-thalassemic trait and microcytic RBCs (mean corpuscular volume, 59 µm^3 [59 fL]) classified as high with the GEN-S (reticulocyte count, 137 × 10^9/L) and low (reticulocyte count, 7.1 × 10^9/L) with the reference method. Even the other methods showed a somewhat contained overestimation in this sample, placing the result in the normal group (SE 9500 RET, 51.5 × 10^9/L; CD 4000, 69 × 10^9/L; ADVIA 120, 80 × 10^9/L; VEGA RETIC, 88.6 × 10^9/L).

Discussion

The popularity that automated methods have obtained depends on the possibility of replacing manual visual counts, which in routine are subjective, highly imprecise, and tedious, and on the advantages that derive from a precise and objective count mainly at low values, necessary for the SE 9500 RET to a maximum of 25% for the VEGA RETIC.
diagnosis of hypoplastic anemias or for the early monitoring of erythropoietic regeneration of bone marrow. A further advantage of automated methods, not considered in this study, is the identification of other reticulocyte parameters, such as the immature reticulocyte fraction or the reticulocyte indices, such as the mean reticulocyte volume or the mean hemoglobin content, which have been shown to be useful in several clinical conditions.18,19

The increase in the number of methods available (the 5 instruments evaluated represent the most recent and sophisticated evolution of these methods) requires, however, that the results from the various methods agree with one another. Differences in counts are clearly evident in the College of American Pathologists’ survey3 in which the use of stabilized control material could justify some unevenness, especially for instruments based on light-scattering techniques.

It should be noted that even previous comparisons based on the use of fresh blood have shown some disagreement (H3 vs R 1000, MAXM vs R 1000) with the need for specific reference intervals.1,2,20 The present study confirms the diversity of the reference intervals, which partially overlap in 2 subgroups; the first consists of the microscopic reference method, the SE 9500 RET, and the GEN-S, and the

### Table 7
Clinical Usefulness: Distributional Classification (Absolute Counts) *

<table>
<thead>
<tr>
<th>Reference Method</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVIA 120</td>
<td>44</td>
<td>8 (2)</td>
<td>0</td>
<td>45 (1)</td>
<td>7 (1)</td>
<td>0</td>
<td>38</td>
<td>13</td>
<td>1</td>
<td>45</td>
<td>7</td>
<td>0</td>
<td>45 (7)</td>
</tr>
<tr>
<td>CELL DYN 4000</td>
<td>4</td>
<td>37 (5)</td>
<td>4 (1)</td>
<td>5</td>
<td>35</td>
<td>7</td>
<td>1</td>
<td>30</td>
<td>14 (1)</td>
<td>3</td>
<td>36</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>GEN-S</td>
<td>0</td>
<td>2 (1)</td>
<td>16 (11)</td>
<td>0</td>
<td>0</td>
<td>18 (1)</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>SE 9500 RET</td>
<td>48</td>
<td>47</td>
<td>20</td>
<td>48</td>
<td>42</td>
<td>25</td>
<td>39</td>
<td>43</td>
<td>33</td>
<td>48</td>
<td>43</td>
<td>24</td>
<td>49</td>
</tr>
<tr>
<td>VEGA RETIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Parentheses indicate number of samples with flag. See text for proprietary information.

1 Obtained by multiplying the percentage count of the reference method by the RBC count given by the routine hematology analyzer.
second consists of the CD 4000, the ADVIA 120, and the VEGA RETIC. Even more obvious is the difference in the median between these 2 subgroups.

The comparison (percentage values) of the various methods with the reference method showed generally satisfactory behavior (intercept between 0.2 and 0.49 × 10^9/µL, [0.2-0.49 × 10^9/L], slope between 0.84 and 0.96, and determination coefficient between 0.73 and 0.82). Detailed analysis, however, showed a tendency, which varied according to the method, to overestimate at low levels and underestimate, to a lesser extent, at high levels. These results were confirmed by data from previous studies, even when the comparison was based on different methods for microscopic counting.\(^1,2,20\) When the various automated methods were compared among themselves, better results were obtained than when they were compared with the microscopic reference method, without, however, strict agreement.

For diagnostic purposes, at elevated concentrations, the clinical sensitivity was excellent; the same was not true for low concentrations in which an overestimation by several systems reduced the sensitivity to nonoptimal conditions. When the reticulocyte was used to verify the severe reticulocytopenia in patients with bone marrow aplasia or to monitor early erythropoietic response, the interchange of the various methods could be misleading owing to the differences in

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**Table 8**
Assessment of Distributional Classification *

<table>
<thead>
<tr>
<th></th>
<th>ADVIA 120</th>
<th>CELL DYN 4000</th>
<th>GEN-S</th>
<th>SE 9500 RET</th>
<th>VEGA RETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low reticulocyte count</td>
<td>84.6 (71.9-93.1)</td>
<td>86.5 (74.2-94.4)</td>
<td>73.1 (59.0-84.4)</td>
<td>86.5 (74.2-94.4)</td>
<td>86.5 (74.2-94.4)</td>
</tr>
<tr>
<td>High reticulocyte count</td>
<td>88.9 (65.3-98.6)</td>
<td>100.0 (81.5-100.0)</td>
<td>100.0 (81.5-100.0)</td>
<td>100.0 (81.5-100)</td>
<td>83.3 (58.6-96.4)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low reticulocyte count</td>
<td>82.2 (65.3-98.6)</td>
<td>77.8 (62.9-88.8)</td>
<td>66.7 (51.1-80.0)</td>
<td>80 (65.4-90.4)</td>
<td>73.3 (58.1-85.4)</td>
</tr>
<tr>
<td>Positive value (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low reticulocyte count</td>
<td>91.6</td>
<td>93.8</td>
<td>97.4</td>
<td>93.8</td>
<td>91.8</td>
</tr>
<tr>
<td>High reticulocyte count</td>
<td>80.0</td>
<td>72.0</td>
<td>54.5</td>
<td>75.0</td>
<td>65.2</td>
</tr>
<tr>
<td>Negative result</td>
<td>78.7</td>
<td>83.3</td>
<td>69.8</td>
<td>83.7</td>
<td>76.7</td>
</tr>
<tr>
<td>Total agreement</td>
<td>83.4</td>
<td>85.2</td>
<td>74.8</td>
<td>86.1</td>
<td>80.8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are 95% confidence intervals. See text for proprietary information.
counts at these concentrations. For example, the reticulocyte concentrations in Figure 5 are for 2 patients during bone marrow aplasia after chemotherapy and at the first evidence of marrow response. While the increase in the values during the response phase was emphasized by all analyzers (with the exception of the VEGA RETIC for patient 1), strict concordance could be found only between the SE 9500 RET and the CD 4000; the ADVIA 120 showed intermediate behavior, and the GEN-S and VEGA RETIC showed discrete oscillations, even in the phase of complete aplasia. It follows that even when monitoring during the early post-aplasia phase, it is mandatory to use the same method (or at least methods with overlapping values), possibly chosen from those with the highest analytic sensitivity.

The imprecision of the automated methods was notably less for the manual-visual method mainly at low concentrations. The analytic goal for imprecision, which currently is widely shared, is based on biologic variability. Among the various clinical conditions, monitoring has the most restrictive criteria, with an imprecision goal of the CV percentage of 0.5 CVb or less (where CVb represents within-subjects biologic variability). In this case, the analytic imprecision adds 11.8% to the overall variability, but this value increases to 41.4% when the CV percentage is equal to the CVb. It therefore is obvious that the lower the imprecision compared with biologic variability, the lower the difference between successive measurements needed before the difference can be considered significant. The studies published on within-subjects variability do not provide concordant results; they range from 11% to 20%, therefore with different analytic goals (5.5% and 10%, respectively). These goals are reached or approximated only for high or normal reticulocyte concentrations and with better results from the CD 4000 and the SE 9500 RET. For concentrations below the reference interval these results are still distant from the analytical goals.

The need for low imprecision in tests used for reticulocytopenic patients can be justified in certain clinical applications, such as monitoring the early erythropoietic response after bone marrow transplantation or spontaneous or medically induced aplasia. In such cases, characterized by reticulocyte concentrations less than $10 \times 10^3/\mu L$ ($10 \times 10^9/L$), even small increases, with trends confirmed during the following days, are an indication of recovery of erythropoiesis by the bone marrow. It is, however, questionable whether similar analytic goals must be extended from normal and high reticulocyte concentrations to severe reticulocytopenia. In fact, at a concentration of $5 \times 10^3/\mu L$ ($5 \times 10^9/L$), the maximum variability allowed for a goal of 5.5% would be $0.250 \times 10^3/\mu L$ ($0.250 \times 10^9/L$), which is a negligible value when clinical decision making is concerned. It should be pointed out that some systems, like the GEN-S and the ADVIA 120, show an improvement over the previous semiautomated methods proposed by the same manufacturers as MAXM and STKS or H3, respectively. The precision profile, when examined over a wide range of concentrations and with a sufficient number of samples, presents a double advantage: (1) the possibility of knowing the imprecision at every concentration, most important since this is nonlinear; and (2) easy comparison because profiles of different analyzers can be plotted on the same graph. With this approach at normal or high concentrations the CV percentage is less than 10% for all systems. Differences, however, exist: the best results are from the SE 9500 RET.
and CD 4000. At lower values, these differences are amplified further.

Regarding clinical usefulness, while behavior is satisfactory when the reticulocyte concentration increases, it is less so when the concentration is low, with a sensitivity from 86.5% for the VEGA, the SE 9500 RET, and the CD 4000 to 84.6% for the ADVIA 120 and 73.1% for the GEN-S. Even the exclusion of flagged samples (which would have to undergo a careful microscopic count) does not improve the sensitivity, causing a decrease in overall efficiency since many correctly classified but flagged samples would be excluded.

The potential interfering substances studied did not demonstrate systematic effects on the counts. It should be noted that in 3 samples with microcytosis and 1 with microcytosis with a dimorphic population, the GEN-S gave an overestimation with respect to the microscopic count (from 33 to 130 × 10^9/L [33-130 × 10^9/L]) and the other instruments. In one of these samples, there was also an overestimation by the VEGA RETIC (87 × 10^9/L [87 × 10^9/L]) and the CD 4000 (60 × 10^9/L [60 × 10^9/L]). In one of the samples containing NRBCs, there was also an overestimation by the VEGA RETIC (43 × 10^9/L [43 × 10^9/L]), the GEN-S (43 × 10^9/L [43 × 10^9/L]), and the ADVIA 120 (131 × 10^9/L [131 × 10^9/L]). All instruments, however, always flagged the result and indicated the suspicion of NRBCs. Regarding the interference of NRBCs, our samples had modest concentrations; therefore, further study is necessary before reaching a final verdict. In conclusion, we can affirm that total automation of reticulocyte counts represents a definite improvement over microscopic counts and, in certain respects (greater precision), even over the previous semiautomated methods. Several problems remain unresolved: (1) the incomplete agreement of the counts with the consequent necessity of method-specific reference intervals; (2) the less-than-optimal analytic sensitivity for some systems; and (3) the difficulty using some analyzers to monitor early erythropoietic response when using only the reticulocyte count owing to the imprecision at low concentrations.

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