Development of a Novel Flow Cytometry-Based System for White Blood Cell Differential Counts: 10-color LeukoDiff

Dongjin Park, M.D.1, Jiyoung Chang, M.D.1, Jimin Kahng, M.D.1, Hunhee Park, Ph.D.2, Irene Jo, M.D.1, Yonggoo Kim, M.D.1, and Kyungja Han, M.D.1

1Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; 2Department of Clinical Laboratory Science, Ansan University, Ansan, Korea

Background: Flow cytometry (FCM) is commonly used to identify many cell populations. We developed a white blood cell (WBC) differential counting system for detecting abnormal cells using FCM incorporating 10 colors and 11 antibodies in a single tube, called “10-color LeukoDiff,” and evaluated its performance.

Methods: Ninety-one EDTA-anti-coagulated peripheral blood samples from 76 patients were analyzed using 10-color LeukoDiff. We compared 10 color LeukoDiff results with the results of manual differential count (manual diff). WBCs were classified into 17 cell populations: neutrophils, total lymphocytes, T lymphocytes, B lymphocytes, CD5 and CD19 co-expressing lymphocytes, natural killer cells, total monocytes, 16+ monocytes, eosinophils, immature granulocytes, basophils, myeloblasts, B-blasts, T-blasts, myeloid antigen-positive B-blasts, CD19- plasma cells, and 19+ plasma cells.

Results: The correlations between the 10-color LeukoDiff and manual diff results were strong (r>0.9) for mature neutrophils, lymphocytes, eosinophils, immature granulocytes, and blasts and moderate for monocytes and basophils (r=0.86 and 0.74, respectively). There was no discrepancy in blast detection between 10-color LeukoDiff and manual diff results. Furthermore, 10-color LeukoDiff could differentiate the lineage of the blasts and separately count chronic lymphocytic leukemic cells and multiple myeloma cells.

Conclusions: The 10-color LeukoDiff provided an accurate and comprehensive WBC differential count. The most important ability of 10-color LeukoDiff is to detect blasts accurately. This system is clinically useful, especially for patients with hematologic diseases, such as acute leukemia, chronic lymphocytic leukemia, and multiple myeloma. Application of this system will improve the development of FCM gating strategy designs.

Key Words: Flow cytometry, Manual differential count, 10-color LeukoDiff, Blasts, Immature granulocytes

INTRODUCTION

Automated hematology analyzers are useful for white blood cell (WBC) differential counts, especially for differentiating mature neutrophils (mNE), lymphocytes (LY), monocytes (MO), eosinophils (EO), and basophils (BA) [1]. However, these analyzers may have problems in identifying abnormal cells, including blasts (BL) and immature granulocytes (IG). In such cases, “flag” messages are used to show the presence of abnormal cells and to inform the user of a potential inaccuracy in the differential count [2].
Therefore, when the presence of abnormal cells, such as circulating BL is suspected, a microscopic examination with manual differential count (manual diff) is required [3]. Manual diff has traditionally been considered a reference method [4]; however, it is labor-intensive, time-consuming, and vulnerable to error [5]. To overcome these disadvantages, several attempts have been made to use flow cytometry (FCM) in WBC differential counting [5-8]. A FCM WBC differential counting method using a five-color and six-antibody reagent cocktail was recently introduced [6]. This method could successfully identify various cell populations, but it showed lower sensitivity and specificity than manual diff in detecting important immature cells, such as BL and IG, and it failed to identify specific cell populations, such as chronic lymphocytic leukemia (CLL) cells [6].

Using an extended number of antibodies would improve the sensitivity and specificity of FCM-based WBC differential counting, since the method is based on the immunological recognition of cell lineage-specific antigens [9, 10]. We developed a system for detecting abnormal cells using 10 colors and 11 antibodies in a single tube with three-laser FCM, called “10-color LeukoDiff.” To evaluate its performance, its results were compared with those obtained from manual diff.

### METHODS

#### Patients and samples

In this retrospective study, 91 fresh EDTA-anti-coagulated residual blood samples from 76 patients (45 males and 31 females; age range, 13–77 years; median age, 53 years) of Seoul St. Mary’s Hospital, Seoul, Korea, were used. There were 36 samples from 26 patients with acute myeloid leukemia (AML), five samples from four patients with acute promyelocytic leukemia, nine samples from eight patients with B-acute lymphoblastic leukemia (B-ALL), one sample from a patient with T-ALL, one sample from a patient with chronic lymphocytic leukemia (CLL), five samples from five patients with chronic myelogenous leukemia (CML), nine samples from eight patients with non-Hodgkin’s malignant lymphomas, one sample from a patient with Hodgkin’s lymphoma, eight samples from seven patients with myelodysplastic syndrome (MDS), three samples from two patients with multiple myeloma (MM), and 13 samples from patients with other hematologic and non-hematologic diseases. This study was conducted from March 2014 to February 2015 and approved by the Institutional Review Board (2010-0186-000) of Seoul St. Mary’s Hospital.

#### Manual diff

Two trained hematology technicians with over 15 years of experience in diagnostic hematology laboratories performed the manual diff on 200 cells, and the average of the results was used. They counted mNE, LY, MO, EO, BA, BL, IG, (including myelocytes, metamyelocytes, and promyelocytes), plasma cells (PC), and nucleated red blood cells (nRBCs).

#### 10-color LeukoDiff

10-color LeukoDiff used a three-laser Navios flow cytometer (Beckman Coulter, Indianapolis, IN, USA) and Kaluza analysis software (Beckman Coulter). For photomultiplier tube (PMT) voltage calibration, each tube stained with a single antibody was run, and after all 10 tubes were run, the Navios automatically set up using an automatic instrument setting software provided by the manufacturer, Flowset Pro software (Beckman Coulter); the calibration was saved as a protocol. Subsequently, we used this saved protocol until any of the antibodies were changed. The compensation between colors was automatically set using Navios auto setup wizard. The antibodies (Beckman Coulter) were premixed, and the volume of each antibody is shown in Table 1.

The premixed reagent was stored at 4°C and used for one week. Before conducting this study, we had confirmed that the photomultiplier tubes (PMT) were stable for one week. After all 10 tubes were run, the Navios automatically run, and after all 10 tubes were run, the Navios automatically set up using an automatic instrument setting software provided by the manufacturer, Flowset Pro software (Beckman Coulter); the calibration was saved as a protocol. Subsequently, we used this saved protocol until any of the antibodies were changed. The compensation between colors was automatically set using Navios auto setup wizard. The antibodies (Beckman Coulter) were premixed, and the volume of each antibody is shown in Table 1.

The premixed reagent was stored at 4°C and used for one week. Before conducting this study, we had confirmed that the photomultiplier tubes (PMT) were stable for one week.

| Antibody | Cellular expression | Fluorescence | Amount (µL) |
|----------|--------------------|--------------|-------------|
| CD5      | TL, TBL            | PC5.5        | 5           |
| CD13     | MO, NE, EO, BA, IG, MyeloBL | PE          | 2.5         |
| CD15     | NE, EO             | PE           | 2.5         |
| CD16     | Inflammatory MO, NE | APC700      | 2.5         |
| CD19     | B LY, B BL         | APC          | 5           |
| CD33     | MO, NE, EO, BA, IG, MyeloBL | PE          | 2.5         |
| CD34     | BL, HPC            | ECD          | 7           |
| CD36     | MO, erythrocytes, platelets | FITC     | 2.5         |
| CD45     | LCA                | APC750       | 3           |
| CD138    | Plasma cell        | OCS15        | 2.5         |
| CD203c   | BA                 | PC7          | 5           |
| Total amount |                |              | 42.5        |

Abbreviations: NE, neutrophil; LY, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophil; IG, immature granulocyte; BL, blast; HPC, hematopoietic progenitor cells; LCA, leukocyte common antigen; PC5.5, Phycoerythrin-Cyanin 5.5; PE, R-Phycoerythrin; PB, Pacific Blue; APC, Allophycocyanin; APC700, APC-Alexa Fluor 700; ECD, PE-Texas Red; FITC, Fluorescein isothiocyanate; APC750, APC-Alexa Fluor 750; OCS15, Orange Cytognos 515; PC7, Phycoerythrin-Cyanin 7.
Gating strategy for subpopulations

The gating strategy was established from the 10-Color LeukoDiff dot plot A (Fig. 1) of the whole nucleated cell (WN). In plot A, the N area contains nRBCs, platelets, and RBC debris, and we excluded the N area, including nRBCs. From the WN gate, we created orientating gates (A), (B), and (C) so that we could not identify the nRBCs. As described in Table 2, the first orientating (A) gate could identify mNE, MO, EO, IG, BL, and PC. From the second orientating (B) gate, partial mN, BA, and BL could be identified. The third orientating (C) gate could identify B, T-LY, NK, and BL. The BL lineage could be detected using CD34 co-expressing My BL by using CD13 and 33. B BL, T BL, myeloid antigen-positive B BL (My+B BL), CD19-PC (19-PC), and 19+PC (Table 1).

The total WBC count was determined using an XE-2100 automated hematology analyzer (Sysmex, Kobe, Japan). With the 10-color LeukoDiff method, the cut-off of normal and abnormal (including IG, BL, and PC) cell populations was set at 1%.

Statistical analysis

Binomial envelope scatter plots and linear regression analysis
for normal cell populations (neutrophil (NE), LY, MO, EO, and BA) were performed to compare the results of 10-color LeukoDiff and manual diff. Binomial XY scatter plots and linear regression analysis were also conducted for the abnormal cell populations, including IG, BL, and PC. Receiver operating characteristic (ROC) curves for IG and BL were analyzed, and their sensi-

**Fig. 1.** 10-color LeukoDiff plots of whole nucleated cell (WN) (A), orientating gate (B), neutrophils (C, D, and E), lymphocytes (T, B, NK, 5+19+) (F, G, and H), monocytes (I and J), eosinophils (K), immature granulocytes (L), chronic myelogeneous leukemia (M), basophils (N and O), acute myeloid leukemia (P), blasts (Q and R), myeloblasts (S), B blasts (T), myeloid antigen-positive B blasts (U), and 19-plasma cells (V, W, X, and Y) in peripheral blood samples. Abbreviation: NK, natural killer cells.
Activity and specificity were calculated when the cut-off value was set at 1%. All statistical analyses were performed using MedCalc 15.0 (MedCalc Software, Ostend, Belgium), and the level of statistical significance was set at $P < 0.05$.

## RESULTS

Among the 91 samples, manual diff revealed BL in 37 samples and IG in 34 samples. The correlation coefficients between the manual diff and 10-color LeukoDiff in leukopenia, normal WBC count, and leukocytosis samples are shown in Table 3.

### mNE, LY, MO, EO, BA, and IG

The correlation of mNE counts between manual diff and 10-color LeukoDiff was strong ($r = 0.95$, $P < 0.001$) for all 91

---

### Table 2. Cellular expression profiles and FCM gate strategies of 17 cells populations in peripheral blood

| FCM color code | Cell population | Cell subpopulation | Gate | Description |
|----------------|-----------------|--------------------|------|-------------|
| 1              | NE, mature      | mNE                | (A)  | CD15+CD16+  |
| 2              | LY (Total)      | B LY + T LY + NK   |      | CD15+CD16+  |
| 3              | T LY            | T Ly               | (C)  | CD16+CD36-  |
| 4              | B LY            | B LY               | (C)  | CD16+CD36-  |
| 5              | NK cell         | NK                 | (C)  | CD16+CD36-  |
| 6              | 5+19+LY         | 5+19+              | (C)  | CD16+CD36-  |
| 7              | MO (total)      | MO                 | (A)  | CD16+CD36-  |
| 8              | 16+ MO          | 16+MO              | (C)  | CD16+CD36-  |
| 9              | EO              | EO                 | (A)  | CD15+CD16-CD45+|
| 10             | IG              | IG                 | (A)  | CD15+CD16-CD45+|
| 11             | BA              | BA                 | (B)  | CD203c+CD15-|
| 12             | My              | My BL              | (B)  | CD19+CD34+/-|
| 13             | B BL            | B BL               | (B)  | CD19+CD34+/-|
| 14             | T BL            | T BL               | (B)  | CD19+CD34+/-|
| 15             | My+B BL (My+B BL) | My+B BL       | (B)  | CD19+CD34+/-|
| 16             | 19+PC           | 19+PC              | (A)  | CD19+CD34+/-|
| 17             | 19-PC           | 19-PC              | (A)  | CD19+CD34+/-|

Abbreviations: FCM, flow cytometry; mNE, mature neutrophil; LY, lymphocyte; NK, natural killer; MO, monocyte; EO, eosinophil; My, myeloblast; IG, immature granulocyte; BA, basophil; BL, blast; PC, plasma cell; My+ B BL, myeloid antigen-positive B blast.

### Table 3. Correlation coefficients between the manual differential counts and 10-color LeukoDiff results according to total leukocyte counts

| Cell population | Total WBC* |
|-----------------|------------|
| Leukopenia (N=24) | Normal (N=38) | Leukocytosis (N=29) |
| mNE             | 0.94       | 0.94       | 0.95       |
| LY              | 0.84       | 0.93       | 0.94       |
| MO              | 0.94       | 0.83       | 0.86       |
| EO              | 0.94       | 0.97       | 0.99       |
| BA              | 0.52       | 0.59       | 0.91       |
| IG              | 0.42       | 0.85       | 0.98       |
| BL              | 0.99       | 0.99       | 0.99       |

*Leukopenia, WBC < 4.00 x 10^9/L; Normal, WBC = 4.00–10.00 x 10^9/L; Leukocytosis, WBC > 10.00 x 10^9/L; all correlations were significant at $P < 0.05$.

Abbreviations: WBC, white blood cells; mNE, mature neutrophil; LY, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophil; IG, immature granulocyte; BL, blast.
Fig. 2. Binomial scatter plots and correlations between manual differential count and 10-color LeukoDiff results for neutrophils (A), lymphocytes (B), monocytes (C), eosinophils (D), basophils (E), immature granulocyte (F), blasts (G), and plasma cells (H).
samples, with a linear relationship of $y=0.859x+1.822$ (Fig. 2). Correlation coefficients varied according to the NE counts: $r=0.93$ in the case of neutropenia ($<1.50 \times 10^9/L$), $r=0.95$ for normal NE count ($1.50–7.50 \times 10^9/L$), and $r=0.91$ for neutrophilia ($>7.50 \times 10^9/L$).

The correlation of LY counts between manual diff and 10-color LeukoDiff for all 91 samples was strong ($r=0.92$, $P<0.001$), with a linear relationship of $y=0.930x+1.466$ (Fig. 2). The specific characteristics of LY cases were as follows: there were two lymphocytosis cases with LY $>10.00 \times 10^9/L$, including one CLL case and one B-ALL case. The CLL case was of a 64-year-old male with a WBC count of $23.99 \times 10^9/L$. The manual diff results showed 3% mNE, 93% LY, 3% MO, and 1% BA, and the 10-color LeukoDiff results showed 8% mNE, 90% LY, and 2% MO. The CD5$^+$CD19$^+$ co-expressing cells accounted for 71% in 10-color LeukoDiff (Fig. 1H). The B-ALL case was of a 29-year-old male with a WBC count of 157.64 $\times 10^9/L$. The manual diff results showed 5% mNE, 20% LY, 1% MO, and 74% BL, and the results for 10-color LeukoDiff were 2% mNE, 6% LY, 1% MO, and 91% BL. Compared with the manual diff method, there was a 14% difference in the LY count and a 17% difference in the BL count (data not shown). In this case, the BL population was CD34-positive, which confirmed that the results of 10-color LeukoDiff were correct.

There was a strong correlation ($r=0.86$; $P<0.001$; $y=0.962x+2.982$) between the manual diff and 10-color LeukoDiff for the MO counts of all 91 samples (Fig. 2). However, when the MO count was $<1.00 \times 10^9/L$ (74 cases), the correlation coefficient was reduced to $r=0.59$, and when it was $>1.00 \times 10^9/L$ (17 cases), it was increased to $r=0.97$.

The correlation of EO counts between the two methods was very strong ($r=0.98$; $P<0.001$; $y=1.007x+0.350$) for all 91 samples (Fig. 2). The correlation coefficient varied according to the EO count. When the EO count was $>0.50 \times 10^9/L$ (eosinophilia), the correlation coefficient was 0.98, whereas it was 0.88 in other cases.

The correlation was moderate for the BA count between the two methods ($r=0.74$; $P<0.001$; $y=0.632x+0.0360$; Fig. 2). In four basophilia cases ($>0.50 \times 10^9/L$), the correlation coefficient was 0.77; however, in samples with $<0.50 \times 10^9/L$ BA, the correlation coefficient was the lowest, at 0.57. The 10-color LeukoDiff result was lower than the manual diff result in 25 cases.

There was a very strong correlation for the IG count ($r=0.97$; $P<0.001$; $y=1.135x+0.641$) for all 91 samples (Fig. 2). The sensitivity of 10-color LeukoDiff was 97.1%, and the specificity was 94.7% at the cut-off value of 1% (Table 4). Furthermore, there were no significant differences in the average comparison. In ROC curve analysis, the area under the curve (AUC) was 0.871. In the manual diff, IG was detected in 34 samples, ranging from 1% to 73%. The 10-color LeukoDiff detected IG ranging from 1% to 85%. There was a significant difference in the correlation coefficient, which increased with the increase in the WBC count (Table 3). This pattern was also applicable to mNE, LY, BA, EO, and IG, but not to MO and BL (Table 3). There were three cases, in which IG was not observed with manual diff but was detected with only 10-color LeukoDiff. All of these cases showed the presence of BL in both the second manual diff and 10-color LeukoDiff. Additional microscopic examinations showed a significant number of IG gathered at the edge of the slides. The concordance results of IG are summarized in Table 4.

### BL and PC

The two methods showed a strong correlation for BL count ($r=0.99$; $P<0.001$; linear regression, $y=0.990x+0.617$) for all 91 samples (Fig. 2). There was no difference in the correlation coefficients according to WBC counts (Table 3). Thirty-four samples showed BL in the manual diff method, ranging from 1% to 94%. When the cut-off value of BL was set to 1% in 10-color LeukoDiff, BL was detected in all 34 cases. There was no discrepancy between 10-color LeukoDiff and manual diff in detecting BL. Binomial scatter plots of correlations between the two methods are shown in Fig. 2. There was also no significant difference between the methods in the average comparison. The sensitivity and specificity of 10-color LeukoDiff were both 100% at the 1% cut-off. The concordance results of BL are summarized in Table 4.

Two patients showed PC in the manual diff; they were diagnosed as having MM and Waldenstrom’s macroglobulinemia, respectively. The 10-color LeukoDiff was performed repeatedly.

### Table 4. Concordance between manual differential count and 10-color LeukoDiff for IG, BL, and PC

| 10-color LeukoDiff | Manual differential count |
|-------------------|---------------------------|
| Observed          | Not observed              |
| IG detected       | 33                        | 3                         | 36 |
| IG not detected   | 1                         | 54                        | 55 |
| BL detected       | 34                        | 0                         | 34 |
| BL not detected   | 0                         | 57                        | 57 |
| PC detected       | 2                         | 0                         | 2  |
| PC not detected   | 1                         | 88                        | 89 |

Abbreviations: IG, immature granulocyte; BL, blast; PC, plasma cell.
on the sample of the patient diagnosed as having MM. The first measurement revealed a count of 15% mNE, 5% LY, and 80% PC, while the second measurement revealed 16% mNE, 5% LY, and 79% PC. This finding was quite acceptable in terms of reproducibility. Although three cases showed PC in the manual diff, they showed a remarkably strong correlation \( r=1.00; P<0.001 \); linear regression, \( y=1.030x−0.0641 \) (Fig. 2). There were no significant differences in the average comparison. Interestingly, there was one discrepant case (the patient diagnosed as having Waldenstrom’s macroglobulinemia) between the two methods, in which approximately 6% of the PC was observed in only manual diff. We investigated the manual slide and 10-color LeukoDiff data to determine the plasmacytoid cell lineage, and no CD138-expressing cells were observed in 10-color LeukoDiff. It suggested that these cells should be regarded as LY rather than as PC.

**DISCUSSION**

Several recent studies have discussed the application of FCM for WBC differential counts [7, 8, 11, 12]. We validated 10-color LeukoDiff FCM, which is time-saving in comparison with previous FCM methods, with a hands-on time of only 20 minutes for analyzing samples from 20 cases.

The key to this method lies in the identification of 17 different cell populations using 10 colors and 11 antibodies in a single tube. The ability to analyze more antigens on the cell membrane increases the accuracy of FCM differential counts. Analyzing the same number of antibodies in a single tube, rather than in multiple tubes, markedly increases the power of cell differentiation due to the ability to uncover the presence of multiple antigen-expressing cells. Our newly developed protocol for a gate strategy for the detection of 17 cell populations using 11 antibodies greatly reduces the need for additional adjustment of LY, EO, IG, and BL gates, which was required in previous methods [7, 8].

We used several gates for counting each type of WBC. For example, the BL population was defined by CD45 expression and the SS plot using the relative position of other granulocytes (i.e., mNE, BA, EO), MO, and LY determined in previous studies [8, 11]. However, the morphology of BL is highly variable, and some BL are found out of the range of this region, while other cells are also frequently contaminated in this region [6, 8]. Therefore, we searched for BL in all three large gates and then excluded mature cells using the antigen expression profile in a step-by-step manner. Most BL would be included in the (B) gate, although other BL located in the (A) and (C) gates could be detected using CD34. The total BL count represents the sum of BL in the three gates. Other types of WBC populations, such as mNE, MO, and BA, were also calculated from the sum of two or more large gates. Theoretically, there was no missed cell type.

The correlation of the 10-color LeukoDiff with manual diff was overall quite strong. There were three discrepant cases, in which IG was not counted in the manual diff but was detected in 10-color LeukoDiff. Through additional microscopic inspection, IG aggregations were observed at the edge of the slide. Therefore, we confirmed that the 10-color LeukoDiff results were correct; 10-color LeukoDiff counted not only the normal LY subpopulation but also CD5+CD19+ CLL cells using CD5 and CD19. Because there are many conditions associated with lymphocytosis, it is important to be able to suggest CLL based on routine complete blood count. In addition, 10-color LeukoDiff reports LY subset results. Therefore, this method will be useful in various clinical settings, such as for monitoring immune status before and after bone marrow and organ transplantsations [13-17].

The MO count was derived from the (A) and (C) gates and divided into CD16+ MO and CD16- MO populations, because their clinical significance has been reported [18]. The EO count was derived from only the (A) gate, because EO shows high side scatter and is predicted to reside in the (A) gate. The BA count was derived from the (B) and (C) gates. The correlation of the 10-color LeukoDiff BA count with the manual diff BA count was weaker than that of other cell populations possibly because their count was very low.

The most important aspect of 10-color LeukoDiff is its ability to detect BL more accurately and to further differentiate the BL lineages. There were 37 cases showing BL in the manual diff with various hematologic malignancies, such as AML, ALL, CML, MDS, and CLL. The 10-color LeukoDiff showed BL in all of these cases, but none in the remaining 54 cases. There was no discrepancy in BL detection between the manual diff and the 10-color LeukoDiff counts. Furthermore, the 10-color LeukoDiff count showed the BL lineage, which is very important for the diagnosis of hematologic malignancies [19-22]. The 10-color LeukoDiff reports BL lineages as My BL, B BL, T BL, and My+B BL. Although this method cannot completely replace FCM analysis of BL, it is helpful for making an initial diagnosis in patients showing BL. This discrimination is valuable for monitoring patients with MM.

Although 10-color LeukoDiff requires various types of antibodies and skilled workers to carry out the complex operations and
quality control, the technology could be simplified by employing a premixed reagent and by developing an analysis algorithm for automation in the future.

A limitation of our study was that we could not compare our data with those obtained with an automated hematology analyzer. Application of this method might have improved the understanding of the variable expression of CD markers among cells and the development of FCM gating strategy designs. In summary, 10-color LeukoDiff provided accurate and extended WBC differential counts. The system has considerable clinical value, especially for monitoring patients with hematologic diseases, such as acute leukemia, CLL, and MM.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgements

This work was supported by the Technology Innovation Program (No: 10049771, Development of Highly-Specialized Platform for IVD Medical Devices) funded by the Ministry of Trade, Industry & Energy, Korea.

REFERENCES

1. Meintker L, Ringwald J, Rauh M, Krause SW. Comparison of automated differential blood cell counts from Abbott Sapphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex. XE-2100 in normal and pathological samples. Am J Clin Pathol 2013;139:641-60.
2. Eliertsen H, Vøllestad NK, Hauge TA. The usefulness of blast flags on the Sysmex XE-5000 is questionable. Am J Clin Pathol 2013;139:633-40.
3. Barnes PW, McFadden SL, Machin SJ, Simson E, International Consensus Group For Hematology. The International Consensus Group for Hematology review: suggested criteria for action following automated CBC and WBC differential analysis. Lab Hematol 2005;11:83-90.
4. Novis DA, Walsh M, Wilkinson D, St Louis M, Ben-Ezra J. Laboratory productivity and the rate of manual peripheral blood smear review: a College of American Pathologists-Q-Probes study of 95,141 complete blood count determinations performed in 263 institutions. Arch Pathol Lab Med 2006;130:596-601.
5. Kim AH, Lee W, Kim M, Kim Y, Han K. White blood cell differential counts in severely leukopenic samples: a comparative analysis of different solutions available in modern laboratory hematology. Blood Res 2014;49:120-6.
6. Faucher JL, Lacronique-Gazaille C, Frelit E, Trimoreau F, Donnard M, Bordessoule D, et al. “6 markers/5 colors” extended white blood cell differential by flow cytometry. Cytometry A 2007;71:934-44.
7. Jo Y, Kim SH, Koh K, Park J, Shim YB, Lim J, et al. Reliable, accurate determination of the leukocyte differential of leukopenic samples by using Hematoflow method. Korean J Lab Med 2013;31:131-7.
8. Kahng J, Kim Y, Kim M, Oh EJ, Park YJ, Han K. Flow cytometric white blood cell differential using CytoDiff is excellent for counting blasts. Ann Lab Med 2015;35:28-34.
9. Cossarizza A, De Biasi S, Gibellini L, Bianchini E, Bartolomeo R, Nasi M, et al. Cytometry, immunology, and HIV infection: three decades of strong interactions. Cytometry A 2013;83:680-91.
10. Ratei R, Karawajew L, Lacombe F, Jagoda K, Del Poeta G, Kraan J, et al. Discriminant function analysis as decision support system for the diagnosis of acute leukemia with a minimal four color screening panel and multiparameter flow cytometry immunophenotyping. Leukemia 2007;21:1204-11.
11. Allou K, Vial JP, Bene MC, Lacombe F. The routine leukocyte differential flow cytometry Hematoflow method: a new flagging system for automatic validation. Cytometry B Clin Cytom 2015;88:375-84.
12. Im M, Chae H, Kim T, Park HH, Lim J, Oh EJ, et al. Comparative quantitative analysis of cluster of differentiation 45 antigen expression on lymphocyte subsets. Korean J Lab Med 2011;31:148-53.
13. Buhlmann L, Buser AS, Cantoni N, Gerull S, Tichelli A, Gratwohl A, et al. Lymphocyte subset recovery and outcome after T-cell replete allogeneic hematopoietic SCT. Bone Marrow Transplant 2011;46:1357-62.
14. Fernandez-Ruiz M, Silva JT, Lopez-Medrano F, Allende LM, San Juan R, Cambra F, et al. Post-transplant Monitoring of NK cell counts as a simple approach to predict the occurrence of opportunistic infection in liver transplant recipients. Transpl Infect Dis 2016;18:552-65.
15. Fujimaki K, Maruta A, Yoshida M, Kodama F, Matsuizaki M, Fujisawa S, et al. Immune reconstitution assessed during five years after allogeneic bone marrow transplantation. Bone Marrow Transplant 2001;27:1275-81.
16. Rueff J, Medinger M, Heim D, Passweg J, Stern M. Lymphocyte subset recovery and outcome after autologous hematopoietic stem cell transplantation for plasma cell myeloma. Biol Blood Marrow Transplant 2014;20:896-9.
17. van de Berg PJ, Hoevenaars EC, Yong SL, van Donselaar-van der Pant KA, van Tellingen A, Florquin S, et al. Circulating lymphocyte subsets in different clinical situations after renal transplantation. Immunology 2012;136:198-207.
18. Ziegler Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007;81:584-92.
19. Kalina T, Flores-Montero J, Lecrevisse Q, Pedreira CE, van der Velden VH, Novakova M, et al. Quality assessment program for EuroFlow protocols: summary results of four-year (2010-2013) quality assurance rounds. Cytometry A 2015;87:145-56.
20. Matarraz S, Almeida J, Flores-Montero J, Lecrevisse Q, Guerri V, Lopez A, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908-75.
21. Zare H, Bashashati A, Kridel R, Aghaeepour N, Haffari G, Connors JM, et al. Automated analysis of multidimensional flow cytometry data improves diagnostic accuracy between mantle cell lymphoma and small lymphocytic lymphoma. Am J Clin Pathol 2012;137:75-85.