Original Article

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# Evaluation of Red Blood Cell Indices and Reticulocyte Parameters for Thalassemia Carrier Screening Using the Dymind Automated Hematology Analyzer

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## Abstract:

**Objective:** The Dymind automated hematology analyzers, comprising the DF55, DH76, and DH615, were recently released. The appropriate cutoffs of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for hemoglobinopathy screening are related to the study population and automated analyzers. This study aimed to evaluate the Dymind automated hematology analyzers for screening thalassemia and common abnormal hemoglobin (Hb).

**Material and Methods:** Blood samples of known $\alpha^{0-}$thalassemia and β-thalassemia carriers were analyzed to establish the suitable cutoffs for each analyzer, derived from the receiver operating characteristic curve. These selected cutoffs were used for prospective validation for screening $\alpha^{0}$-thalassemia and β-thalassaemia, in combination with the dichlorophenolindophenol (DCIP) test for Hb E screening. Thalassemia genotypes were determined using Hb typing and Deoxyribonucleic acid (DNA) analyses.

**Results:** MCV 80 fL and MCH 27 pg, analyzed using the DF55, DH76, and DH615 analyzers, were the appropriate cutoffs. Using these cutoffs in combination with the DCIP test for screening $\alpha^{0}$-thalassaemia, β-thalassaemia, and Hb E revealed 100% sensitivity and 100% negative predictive value. Our study showed that a reticulocyte hemoglobin equivalent (Ret-He) less than 26 pg could be indicative of both the thalassemia trait and iron deficiency. Interestingly, the cutoff

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below 23.7 pg is more indicative of the $\alpha^0$–thalassemia trait. For the sample stability study, the MCV and MCH remained stable at 4 °C. Additionally, the MCH remained stable at room temperature.

**Conclusion:** Therefore, the combined MCV and MCH analyzed using the Dymind automated hematology analyzers with the DCIP test, together with Ret–He parameters from the DH615, are effective for screening thalassemia and common abnormal Hb in the Thai population.

**Keywords:** cutoff values, thalassemia screening, automated hematology analyzer, MCV, MCH, Ret–He

## Introduction

Hemoglobinopathies are genetic disorders that affect hemoglobin (Hb) production (thalassemia) or its molecular structure (abnormal Hb). Thalassemia and abnormal Hb are the most common congenital disorders and characterize a major public health concern in many areas of the world, especially Southeast Asia[1–3]. The common abnormal Hb disorders in Thailand are Hb E and Hb Constant Spring (Hb CS), with a prevalence of 50% and 8%, respectively. The objective of prevention and control programs in these areas was to diminish the incidence of the 3 severe thalassemia syndromes, including homozygous $\alpha^0$–thalassemia or Hb Bart's hydrops fetalis, homozygous β–thalassemia, and $\beta^0$–thalassemia/Hb E[1, 2]. Two alternative approaches have been proposed. One approach employs the use of a combined osmotic fragility test for screening $\alpha^0$–thalassemia and β–thalassemia carriers and a dichlorophenolindophenol (DCIP) test for identifying Hb E. Another approach uses the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) deduced from automated blood cell counters combined with DCIP[4–6]. Although the former approach is simple, interpreting the results is highly observer–dependent and difficult to standardize. Moreover, automated hematological analyzers have been widely developed for laboratories.

The MCV and MCH are commonly used to screen for abnormalities. Typically, an MCV of 80 fL and an MCH of 27 pg are recommended as cutoff values for thalassemia screening[6–8]. Individuals with MCV and MCH lower than the cutoffs require additional analysis, such as Hb typing and Deoxyribonucleic acid (DNA) analysis, to detect whether they are carriers of $\alpha^0$–thalassemia and β–thalassemia. Thus, the screening assay should have fewer or no false negatives. The number of false positives should also be minimized. However, previous reports have revealed that different automated blood cell analyzers demonstrate different MCV and MCH cutoffs in the Thai population. The best cutoff values for MCV and MCH were found to be 78 fL and 27 pg, respectively, using the URIT–2900 hematology analyzer[9]. Moreover, the appropriate cutoffs for the Coulter LH 780, Pentra ES–60, Sysmex XS–800i, and Sysmex XN–1000 were established to be 78 fL for MCV and 25 pg for MCH. The appropriate cutoffs for MCV and MCH for Cell–Dyn Ruby were 82 fL and 25 pg, respectively[10]. Therefore, the appropriate cutoffs for MCV and MCH are related to the study population and type of automated hematology analyzer.

In addition to thalassemia, iron deficiency (ID) is another cause of microcytic hypochromic red blood cells (RBCs). The diagnosis of ID has been based on biochemical analysis such as serum iron, transferrin, transferrin saturation, and ferritin. The reticulocyte Hb content (Ret–He) or mean reticulocyte hemoglobin content (MCHr), a laboratory parameter from automated hematological analyzers, serves as a valuable marker for evaluating recent iron utilization and erythropoiesis status[11–14].

The Dymind automated hematology analyzer (Dymind Shenzhen, China), comprising the 5-part hematology analyzers (DF55 and DH76) and a 6-part hematology analyzer (DH615), was recently developed. The impedance method is used for RBC counting using the Dymind automated hematology analyzer. Moreover, the MCV is measured directly and the MCH is derived from the formula: Hb/RBC count. However, the MCV and MCH values regarding the Dymind automated hematology analyzer have not been established. Therefore, this study aimed to evaluate the red cell indices and the appropriate cutoffs for MCV and MCH, provided by the Dymind automated hematology analyzer, especially the reticulocyte parameters. Ret-He was also analysed using the DH615 for screening thalassemia and common abnormal Hb in the Thai population.

## Material and Methods

### Subjects

The study subjects initially included 445 routine blood specimens from the Department of Pathology, Faculty of Medicine, Prince of Songkla University, in order to establish the appropriate cutoff values. Leftover blood samples were analyzed for RBC indices using the Sysmex XN3000 (Sysmex Co., Kobe, Japan). Blood specimens (32 samples) with different MCV and MCH were also included for the purpose of studying specimen stability. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Human Research Ethics Unit (HREU), Faculty of Medicine, Prince of Songkla University (Approval ID: REC.66-208-19-2).

### Laboratory investigation

The RBC indices were measured using the Dymind automated hematology analyzers, DF55, DH76, and DH615 (Shenzhen Dymind Biotechnology Co., Ltd. Guangming, Shenzhen, P.R. China). The impedance method was used for RBC counting. Hb concentration was detected using a cyanide-free colorimetric method. The MCV was measured directly and the MCH was derived from the formula: Hb/RBC count. The measurements were performed within 6 hours of blood collection[15]. The Dymind automated hematology analyzers were calibrated according to the manufacturer's instructions using appropriate commercial calibrators. Routine instrument checks were performed before running control and blood specimens. Three levels of quality control materials–low, normal, and high–were tested daily. For the sample stability study, blood specimens were stored at room temperature or refrigerated at 4 °C for 0–6 days before analysis. All samples were screened for Hb E using the DCIP test, as previously described[4,5,15]. The reticulocyte parameter, such as % reticulocyte, % immature reticulocyte fraction (%IRF), and Hb content (Ret-He), was measured using the Sysmex XN3000 (Sysmex Co., Kobe, Japan). A Ret-He of less than 27 pg indicated iron deficiency[13]. The reticulocyte parameter was also evaluated using the Dymind automated hematology analyzers, DH615. Hb analysis was achieved by automated capillary electrophoresis (CAPILLARYS 2, Sebia, France). β-thalassemia was diagnosed by Hb analysis using the following criteria: (1) β-thalassemia trait when the Hb types showed $A_2A$ with Hb $A_2$ >3.5%, (2) β-thalassemia disease when the Hb types showed $A_2FA$ or $A_2F$ patterns, (3) $\beta^0$-thalassaemia/Hb E disease when the Hb types showed an EF pattern, (4) $\beta^+$-thalassemia/Hb E disease when the Hb types showed an EFA pattern, (5) heterozygous Hb E when the Hb type showed an EA pattern, and (6) homozygous Hb E when the Hb type showed an EE pattern. Identification of the $\alpha^0$-thalassemia, such as –SEA, --THAI, --Chiang Rai, --South Africa, --FIL, --MED, and -(α)20.5, $\alpha^+$-thalassemia (-α3.7 and -α4.2 deletions), Hb CS, and Hb Paksé was routinely performed in our laboratory using PCR methods described previously[16,17].

### Statistical analysis

To determine the appropriate cutoff MCV, MCH, and Ret-He values for thalassemia screening, a receiver operating characteristic (ROC) curve was plotted using the MedCalc software (free trial version) (MedCalc Software, Inc., Mariakerke, Belgium). To validate the established cutoffs, the MCV and MCH values obtained from each analyzer were used in combination with the DCIP test and the MCV/DCIP and MCH/DCIP protocols. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. For the assessment of method comparisons, data distribution was assessed by the Shapiro-Wilk test. The Passing-Bablok linear regression analysis and Spearman correlations were used to determine the degree of correlation between the Sysmex XN3000 and the Dymind DH615 system. Bland-Altman difference plots were applied to assess the absolute differences. Correlation coefficients and biases for samples were determined. For statistical comparisons, p-value<0.05 was considered a significant difference.

## Results

A total of 445 routine blood specimens were analyzed. Deoxyribonucleic acid (DNA) analysis revealed that 208 had non-thalassemia without iron deficiency (Ret-He 31.89±2.87 pg), and 37 had iron deficiency (Ret-He <27 pg; 22.45±3.05). Seventy-six subjects were diagnosed with α-thalassemia, including $\alpha^{+}$-thalassemia (n=30), $\alpha^{0}$-thalassemia (n=25), and Hemoglobin Constant Spring (Hb CS) (n=21). Twenty-eight subjects were diagnosed with β-thalassemia with or without α-thalassemia. Seventy-eight subjects were diagnosed with the Hb E trait, including those without α thalassemia (n=71), those with $\alpha^{+}$-thalassemia(n=7), and those with $\alpha^{0}$-thalassemia (n=4). Three and 2 subjects were diagnosed with homozygous Hb E without α-thalassemia and homozygous Hb E with α-thalassemia, respectively. Nine subjects were diagnosed with $\beta^{+}$-thalassemia/Hb E (n=3) and $\beta^{0}$-thalassemia/Hb E with or without α-thalassemia (n=6). The hematological data of all blood specimens that characterized the genotype and phenotype are summarized in Table 1.

To identify the appropriate cutoffs for the MCV and MCH from the DF55, DH76, and DH615 Dymind automated hematology analyzers for thalassemia screening, ROC curves were plotted and calculated. The suitable MCV and MCH cutoff values for screening $\alpha^{0}$-thalassemia and β-thalassemia were 80 fL and 27 pg, respectively (Figure 1). The results showed that screening using MCV cutoffs provided a sensitivity and specificity of 91.95% and 68.24% for DF55, 95.97% and 67.23% for DH76, and 93.66% and 75.45% for DH615, respectively. MCH cutoffs revealed a sensitivity and specificity of 99.33% and 62.16% for DF55, 97.99% and 62.50% for DH76, and 100% and 67.41% for DH615, respectively. However, all the Dymind automated hematology analyzers could yield 100% sensitivity and 100% NPVs when the MCV and MCH obtained from each analyzer were used in combination with the DCIP test (Table 2). The distributions of the MCV and MCH from each sample group using the cutoff values of MCV 80 fL and MCH 27 pg are presented in Figure 2. All subjects with $\alpha^{0}$-thalassaemia, β thalassemia, Hb E trait with $\alpha^{0}$thalassaemia, β thalassemia/Hb E, and homozygous Hb E could be screened using these cutoff points. However, patients with $\alpha^{+}$-thalassemia, heterozygous Hb CS, Hb E trait, and Hb E trait with $\alpha^{+}$thalassemia typically had normal MCV and MCH values. Most Hb E carriers with normal MCV and MCH were also found to have α-thalassemia (Figure 2).

For the sample stability study, the blood specimens were stored at room temperature or refrigerated at 4 °C for 0–6 days before analysis. The MCV and MCH values were determined using the Dymind automated hematology analyzer. However, DH615 was evaluated for 0–5 days.

**Table 1** Hematological parameters of the study subjects measured using the Sysmex XN3000 hematology analyzers

| Subject group | No. | Parameters | | | | | | | | α or β genotype deviation | Hb typing |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RBC (x $10^{12}$/L) | Hb (g/dL) | Hct (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW (%) | Ret-He (pg) | | |
| Normal | 208 | 4.57 (0.69) | 12.96 (1.93) | 39.92 (5.49) | 87.49 (8.73) | 28.50 (2.69) | 32.43 (1.25) | 14.03 (2.50) | 31.89 (2.87) | αα/αα, β/β | $A_2A$ |
| IDA | 37 | 4.47 (0.57) | 10.11 (1.81) | 32.86 (6.77) | 73.79 (8.73) | 22.68 (3.42) | 30.62 (2.40) | 17.84 (2.67) | 22.45 (3.05) | αα/αα, β/β | $A_2A$ |
| Hb CS | 21 | | | | | | | | | | |
| -Hb CS trait | 19 | 5.07 (1.04) | 11.78 (2.05) | 37.92 (6.00) | 76.67 (10.07) | 23.88 (3.91) | 31.45 (1.56) | 15.08 (2.99) | 26.70 (4.15) | $-\alpha^{CS}\alpha/\alpha\alpha$ | $CSA_2A$ |
| -Hb CS trait with $\alpha^+$-thal trait | 1 | 5.71 | 10.20 | 35.70 | 62.50 | 17.90 | 28.60 | 20.40 | 21.10 | $-\alpha/\alpha^{CS}\alpha$ | $CSA_2A$ |
| -Homozygous Hb CS | 1 | 3.93 | 10.40 | 34.30 | 87.30 | 26.50 | 30.30 | 15.60 | 29.1 | $\alpha^{CS}\alpha/\alpha^{CS}\alpha$ | $CSA_2A$ |
| $\alpha^0$-thalassemia | 25 | | | | | | | | | | |
| -$\alpha^0$-thal trait | 24 | 5.33 (0.72) | 11.03 (1.32) | 35.61 (4.17) | 67.16 (4.96) | 20.80 (1.68) | 30.99 (1.28) | 17.86 (3.08) | 22.29 (2.62) | --/αα | $A_2A$ |
| -Hb H disease | 1 | 4.81 | 8.70 | 30.9 | 64.20 | 18.10 | 28.20 | 25.20 | 18.70 | --/-α | $A_2AH$ |
| β-thalassemia | 28 | | | | | | | | | | |
| -β-thal trait | 23 | 5.12 (0.74) | 10.45 (1.41) | 33.89 (4.18) | 66.67 (6.83) | 20.54 (2.36) | 30.80 (0.71) | 17.17 (2.46) | 22.74 (2.94) | $\beta^+/\beta$ or $\beta^0/\beta$ αα/αα | $A_2A$ |
| -β-thal trait with $\alpha^+$-thal trait | 2 | 5.93 (1.06) | 12.40 (1.41) | 41.15 (4.03) | 69.90 (5.66) | 21.05 (1.34) | 30.15 (0.49) | 16.60 (2.26) | 24.15 (0.21) | $\beta^+/\beta$ or $\beta^0/\beta$,-α/αα | $A_2A$ |
| -β-thal trait with $\alpha^0$-thal trait | 1 | 4.56 | 11.30 | 36.00 | 78.90 | 24.80 | 31.40 | 18.60 | 26.90 | $\beta^+/\beta$ or $\beta^0/\beta$,--/αα | $A_2A$ |
| -$\beta^+$-thal disease | 2 | 4.65 (0.99) | 9.95 (0.21) | 30.65 (0.35) | 65.95 (0.64) | 21.40 (0.00) | 32.45 (0.35) | 18.70 (2.26) | 23.30 (1.27) | $\beta^+/\beta^+$ or $\beta^+/\beta^0$, αα/αα | $A_2FA$ |
| Hb E trait | 71 | 4.80 (0.68) | 11.48 (1.64) | 35.81 (5.04) | 74.69 (4.72) | 23.95 (1.65) | 32.06 (1.02) | 15.19 (2.19) | 4.65 (0.99) | $\beta^E/\beta$, αα/αα | EA |
| Hb E trait with α+-thalassemia | 7 | | | | | | | | | | |
| - Hb E trait with α+-thal trait | 3 | 5.32 (0.07) | 12.77 (0.93) | 39.57 (3.45) | 74.40 (7.45) | 24.03 (2.04) | 32.30 (1.20) | 15.77 (3.75) | 27.00 (1.61) | βE/β, -α/αα | EA |
| - Hb E trait with homozygous α+-thal | 2 | 5.72 (0.46) | 12.40 (0.28) | 39.50 (0.42) | 69.30 (4.81) | 21.80 (2.26) | 31.40 (0.99) | 15.05 (0.63) | 25.05 (1.34) | βE/β, -α/-α | EA |
| - Hb E trait with Hb CS trait | 2 | 4.20 (0.54) | 10.70 (2.12) | 33.35 (4.74) | 79.40 (0.99) | 25.35 (1.77) | 31.95 (1.77) | 14.55 (3.61) | 28.15 (4.60) | βE/β, αCSα/αα | CSEA |
| Hb E trait with α0-thalassemia | 4 | | | | | | | | | | |
| -Hb E trait with α0-thal trait | 3 | 5.57 (0.99) | 11.63 (1.97) | 37.37 (6.57) | 67.07 (0.96) | 20.90 (0.36) | 31.17 (0.21) | 16.30 (0.87) | 24.00 (0.89) | βE/β, --/αα | EA |
| -EABart's disease | 1 | 5.51 | 8.80 | 27.10 | 49.20 | 16.00 | 32.50 | 23.20 | 16.40 | βE/β, --/-α | EABart's |
| Homozygous Hb E | 5 | | | | | | | | | | |
| -Homozygous Hb E | 3 | 4.84 (0.54) | 9.70 (0.66) | 30.27 (1.48) | 62.87 (5.28) | 20.13 (1.40) | 32.03 (0.60) | 17.67 (2.21) | 21.20 (1.51) | βE/βE, αα/αα | EE |

**Table 1** Continued

| Subject group | No. | Parameters | | | | | | | α or β genotype deviation | H typing [b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | RBC (x $10^{12}$/L) | Hb (g/dL) | Hct (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW (%) | Ret-He (pg) | |
| –Homozygous Hb E with α+–thal trait | 1 | 5.53 | 10.40 | 32.50 | 58.80 | 18.80 | 32.00 | 20.00 | 21.70 | βE/βE, –α/αα | EE |
| –Homozygous Hb E with homozygous α+–thal | 1 | 6.03 | 13.10 | 39.70 | 65.80 | 21.70 | 33.00 | 16.40 | 25.50 | βE/βE, –α/–α | EE |
| β-thal/Hb E | 9 | | | | | | | | | | |
| –β+–thal/Hb E | 3 | 4.81 (0.44) | 8.67 (1.33) | 27.63 (3.10) | 57.33 (1.42) | 17.97 (1.37) | 31.27 (1.64) | 20.20 (1.44) | 18.13 (2.49) | β+/βE, αα/αα | EFA |
| –β0–thal/Hb E | 4 | 3.84 (0.48) | 6.93 (0.44) | 22.00 (0.48) | 57.88 (6.87) | 18.13 (1.46) | 31.48 (1.65) | 29.73 (1.47) | 16.30 (0.92) | β0/βE, αα/αα | EF |
| –β0–thal/Hb E with Hb CS trait | 2 | 4.45 (0.08) | 7.75 (0.63) | 23.70 (0.71) | 53.25 (2.62) | 17.45 (1.77) | 32.70 (1.77) | 28.00 (0.71) | 16.55 (1.20) | β0/βE, αCSα/αα | EF |

Values are presented as mean (S.D.)

At 4 °C, our results revealed that the MCV of all Dymind automated hematology analyzers remained significantly stable for 6 days for DF55 and DH76 and 5 days for DH615 (Supplementary Figure 1A, 1C, and 1E). Only one sample with a high MCV remained stable for 3 days, followed by a trend toward higher values after 3 days in the DF55 and DH76 Dymind automated hematology analyzers (Supplementary Figure 1A and 1C). At room temperature, the MCV of all Dymind automated hematology analyzers significantly increased after 1 day (Supplementary Figure 1B, 1D, and 1F). In contrast, the MCH was more stable at both temperatures (Supplementary Figure 2).

For the assessment of method comparisons between the Sysmex XN3000 and the Dymind DH615 system, the comparison between reticulocyte parameters was performed by Passing–Bablok regression and Bland–Altman plots. The obtained Spearman's rank correlation (*r*) of % reticulocyte was 0.89 (95% CI: 0.854 to 0.903, p–value<0.0001). Passing–Bablok linear regression revealed a regression equation y=0.09+1.11x, y–intercept 0.09 (95% CI: 0.02 to –0.16) and slope 1.11 (95% CI: 1.06 to 1.17) (Figure 3A). Bland–Altman difference plots analysis of % reticulocyte showed a positive mean bias of 0.22 (95% CI: 1.19 to 0.25) (Figure 3B). Passing–Bablok regression showed Spearman correlation coefficients of % IRF was 0.88 (95% CI: 0.85 to 0.90, p–value<0.0001) and y=2.42+1.19x, y–intercept 2.42 (95% CI: 1.88 to 2.85) and slope 1.19 (95% CI: 1.13 to 1.26) (Figure 3C). The Ret–He comparisons results revealed Spearman correlation coefficients 0.93 (95% CI: 0.92 to 0.95, p–value<0.0001) and y=–7.55+1.45x, y–intercept –7.55 (95% CI: –8.90 to –6.51) and slope 1.45 (95% CI: 1.41 to 1.50) (Figure 3E). The Bland–Altman plot of % IRF and Ret–He demonstrated an average bias for each test were 4.3 (95% CI: 3.95 to 4.57) and 3.4 (95% CI: 3.18 to 3.64) (Figure 3D and 3F), respectively.

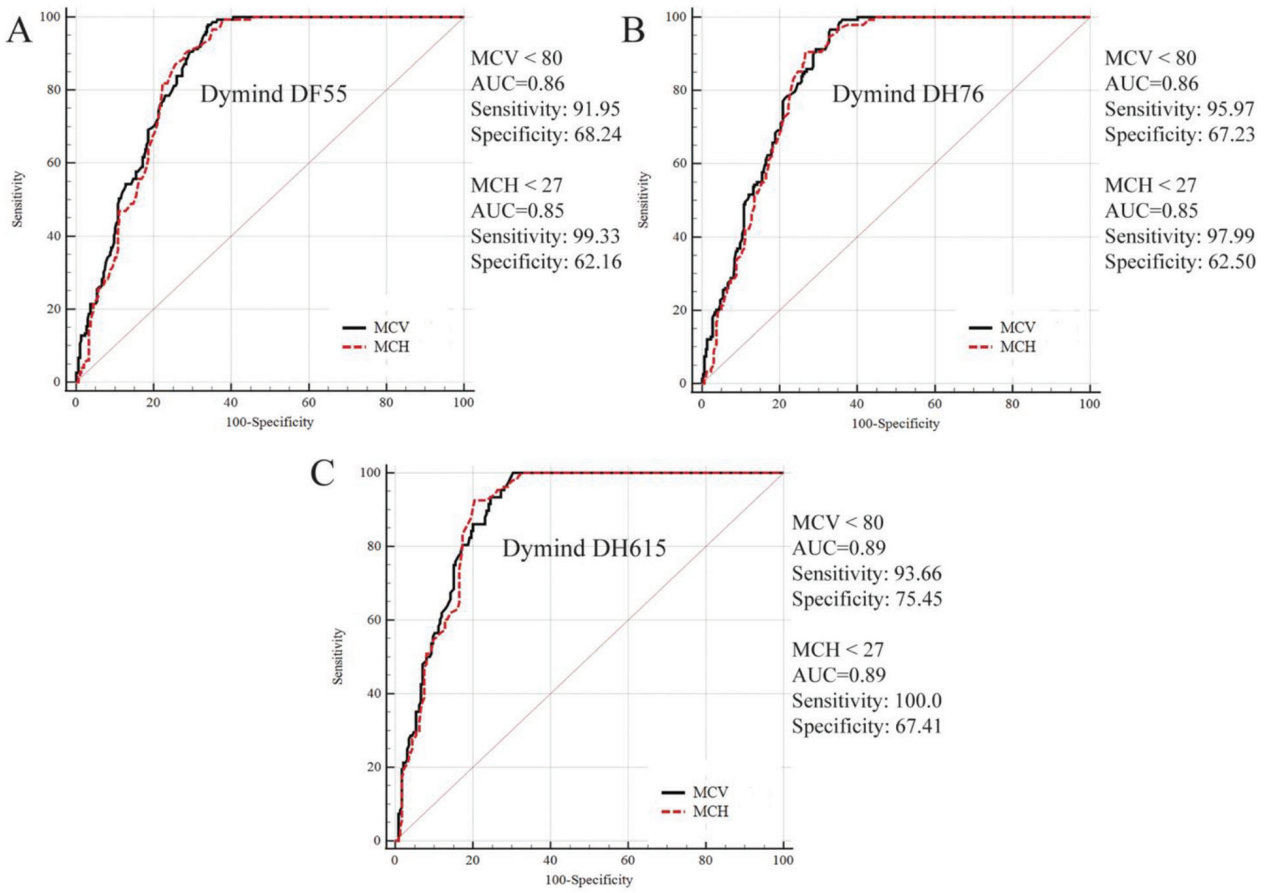
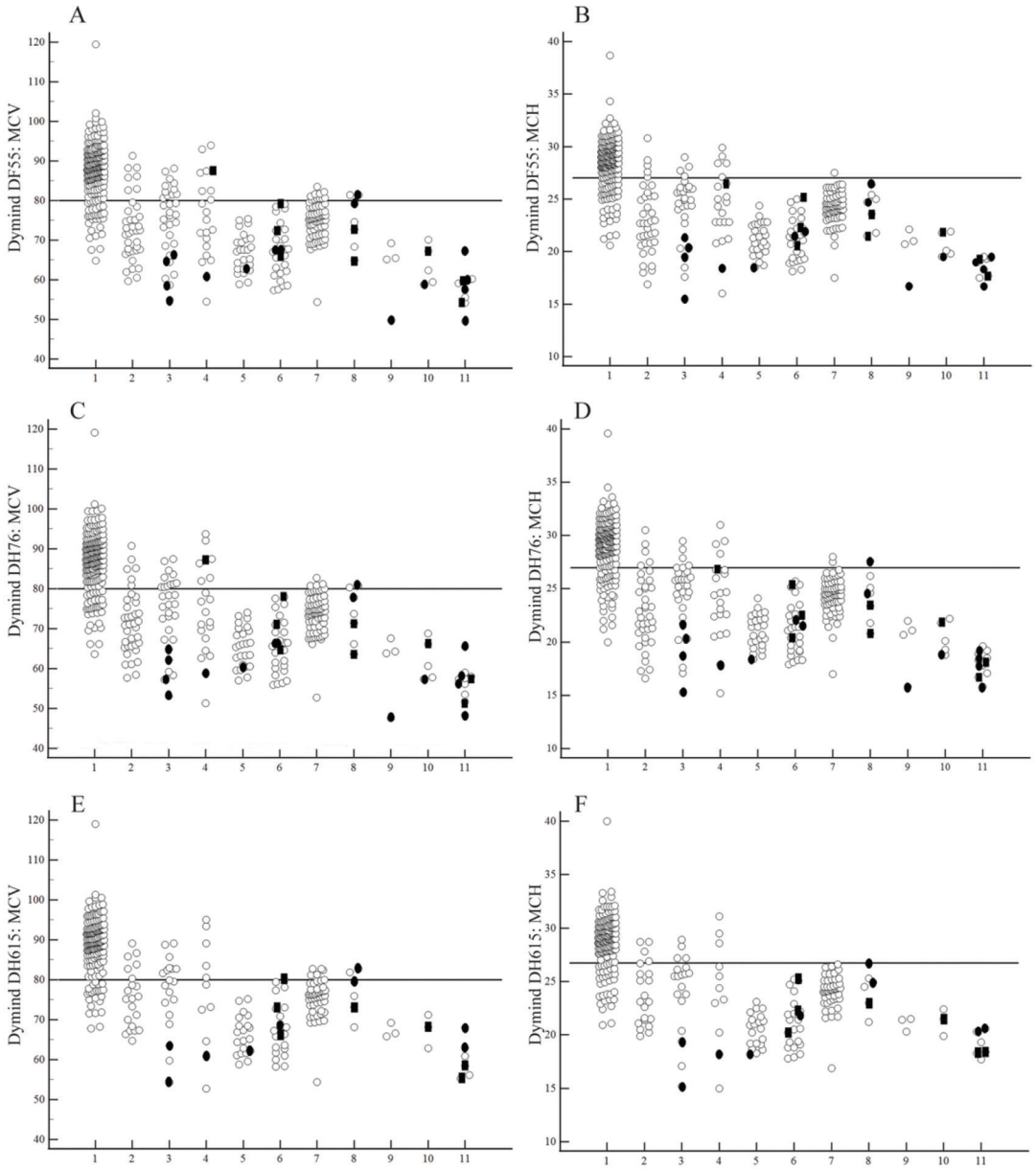



**Figure 1** The ROC curve of the MCV and MCH for abnormal hemoglobin, including Hb E and thalassemia screening using the DF55 (A), DH76 (B), and DH615 (C) Dymind automated hematology analyzers

**Table 2** Sensitivity and specificity of the screening for $\alpha^0$–thalassemia, β–thalassemia, and Hb E using the Dymind Hematology Analyzer (DF55, DH76, and DH615) with MCV (cutoff, 80 fL) or MCH (cutoff, 27 pg) and a combined MCV or MCH with DCIP test

| | DF55 | | DH76 | | DH615 | |
|---|---|---|---|---|---|---|
| | MCV or MCH | MCV or MCH and DCIP | MCV or MCH | MCV or MCH and DCIP | MCV or MCH | MCV or MCH and DCIP |
| Sensitivity (%) | 99.24 | 100 | 96.95 | 100 | 100 | 100 |
| Specificity (%) | 68.58 | 68.58 | 67.23 | 67.23 | 73.19 | 73.19 |
| Positive Predictive value (PPV) (%) | 58.30 | 58.48 | 56.70 | 57.46 | 52.27 | 52.27 |
| Negative Predictive value (NPV) (%) | 99.51 | 100 | 98.03 | 100 | 100 | 100 |




1: non-thalassemia(n= 208), 2: Iron-deficiency anemia (n=37), 3: $\alpha^+$-thalassemia[○: $\alpha^+$-thalassemia trait (n=26), ●: homozygous $\alpha^+$-thalassemia(n=4)] (n=30), 4: Hb CS [○: Hb CS trait (n=19), ●: Hb CS trait with $\alpha^+$-thal trait (n=1), ■: Homozygous Hb CS (n=1)], 5: $\alpha^0$-thalassemia[○: $\alpha^0$-thalassemiatrait (n=24), ●: Hb H disease (n=1)], 6: β-thalassemia[○: β-thal trait (n=23), ●: $\beta^+$-thal disease (n=2), ■: β-thal trait with $\alpha^+$-thal trait (n=2) and β-thal trait with $\alpha^0$-thal trait (n=1)], 7: Hb E trait (n=71), 8: Hb E trait with $\alpha^+$-thalassemia[○: Hb E trait with $\alpha^+$-thal trait (n=3), ●: Hb E trait with Hb CS trait (n=2), ■: Hb E trait with homozygous $\alpha^+$-thal (n=2)], 9: Hb E trait with $\alpha^0$-thalassemia[○: Hb E trait with $\alpha^0$-thal trait (n=3), ●: EABart's disease (n=1)], 10: Homozygous Hb E [○: Homozygous Hb E (n=3), ●: Homozygous Hb E with $\alpha^+$-thal trait (n=1), ■: Homozygous Hb E with homozygous $\alpha^+$-thal (n=1)], 11: β-thal/Hb E [○: $\beta^+$-thal/Hb E (n=3), ●: $\beta^0$-thal/Hb E (n=4), ■: $\beta^0$-thal/Hb E with Hb CS trait (n=2)

**Figure 2** Scatter plots of the MCV and MCH values of subjects with known thalassemia genotypes for ROC curve construction in the DF55 (A, B), DH76 (C, D), and DH615 (E, F) Dymind automated hematology analyzers

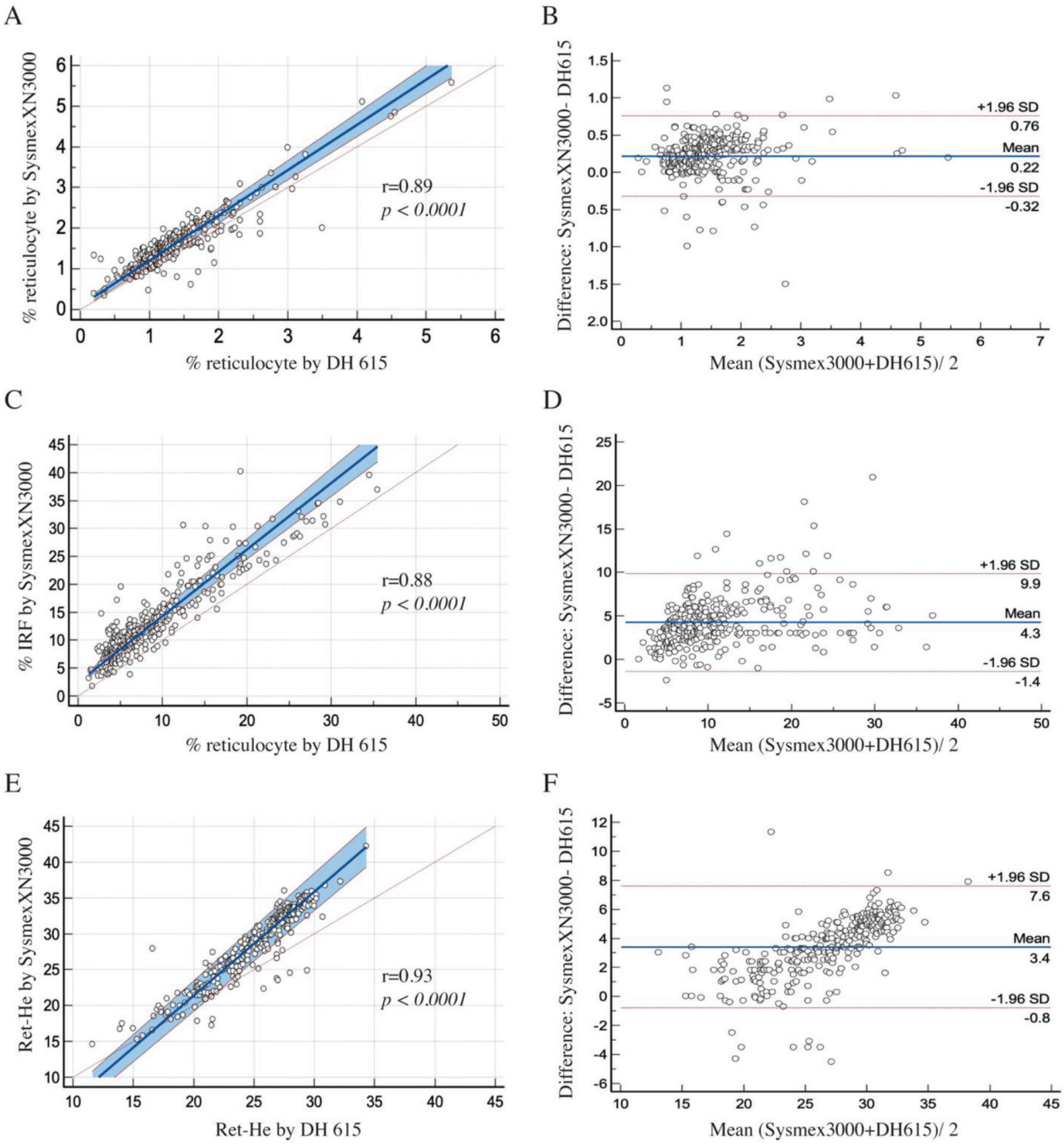



**Figure 3** Comparison of % reticulocyte, % IRF, and Ret-He parameter on the Sysmex XN3000 and Dymind DH615. Passing and Bablok regression and Spearman correlations analysis for (A) % reticulocyte, (C) % IRF and (E) Ret-He. Bland-Altman difference plots of Sysmex XN3000 and Dymind DH615 results for (B) % reticulocyte, (D) % IRF and (F) Ret-He, respectively

The % reticulocyte in Hb CS trait (p-value=0.0001) and β-thalassemia trait (p-value=0.0026) was significantly higher than normal (Figure 4A). The % IRF in the IDA and thalassemia trait was significantly increased compared to the normal (p-value=0.0001), except for the $\alpha^0$-thal trait (p-value=0.1389) (Figure 4B). While Ret-He in the IDA and thalassemia trait was significantly lower than normal (Figure 4C). Then, we analyzed cutoff to maximize the Ret-He diagnostic level for an IDA diagnosis using the Dymind DH615 by ROC analysis. With a Ret-He cutoff level of 25.4 pg, iron deficiency could be diagnosed with a sensitivity of 91.9% and a specificity of 89.0%. The area under the curve (AUC) for Ret-He was 0.957 (95%CI: 0.92-0.98) (Figure 5A). The appropriate cutoff for discriminating IDA and thalassemia trait from the normal group was 26 pg with a sensitivity of 87.97% and a specificity of 82.93% (Figure 5B). Moreover, with a Ret-He cutoff level of 23.7, the $\alpha^0$-thal trait could be discriminated from the normal group with a sensitivity of 88.52 % and a specificity of 98.17 % (Figure 5C).

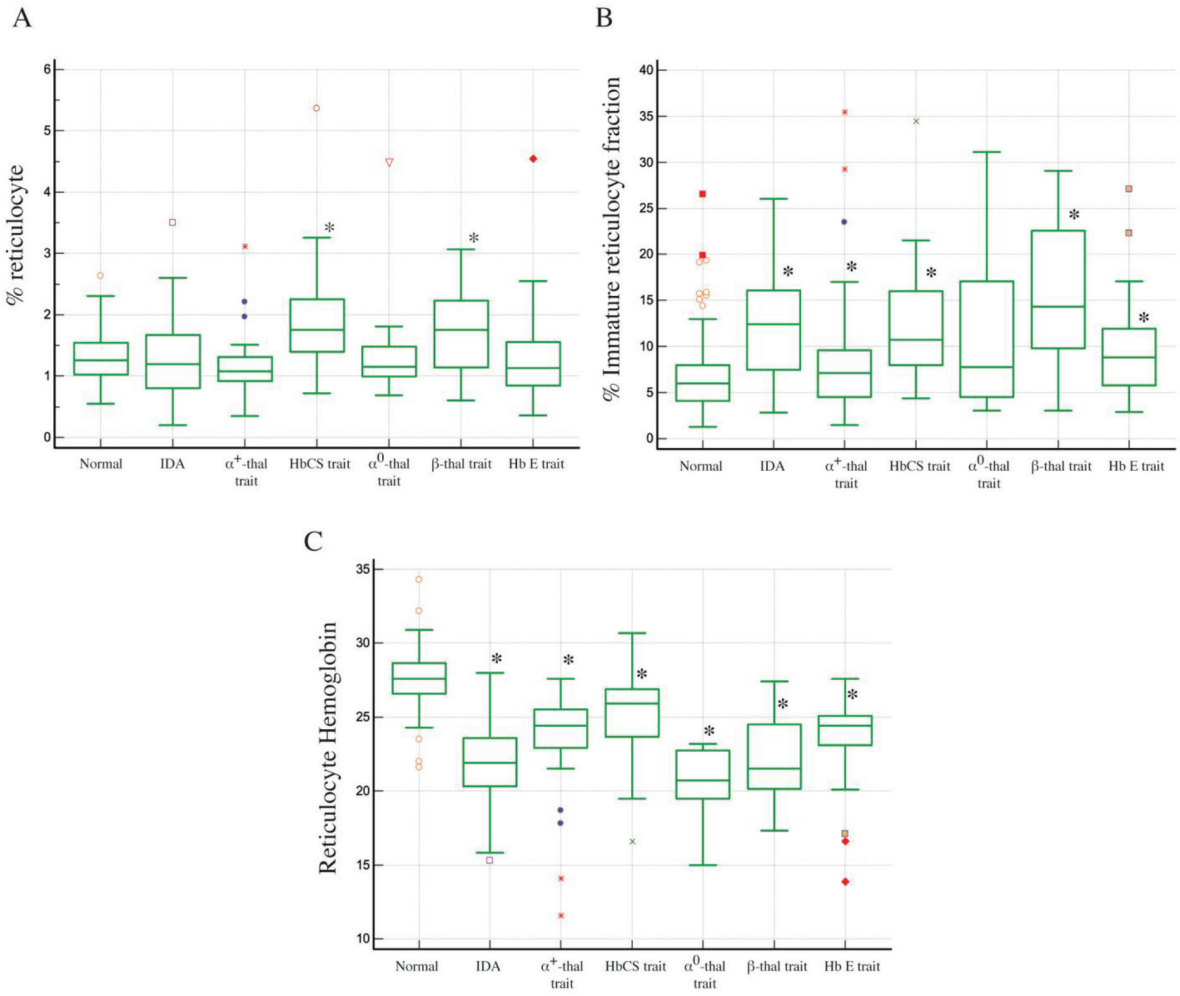



**Figure 4** (A) % reticulocyte, (B) % IRF, and (C) Ret-He parameter in normal, iron deficiency anemia (IDA), $\alpha^+$-thalassemia($\alpha^+$-thal), hemoglobin CS trait (HbCS trait), $\alpha^0$-thalassemia(α-thal), β-thalassemia trait (β -thal trait) and hemoglobin E trait (HbE trait)

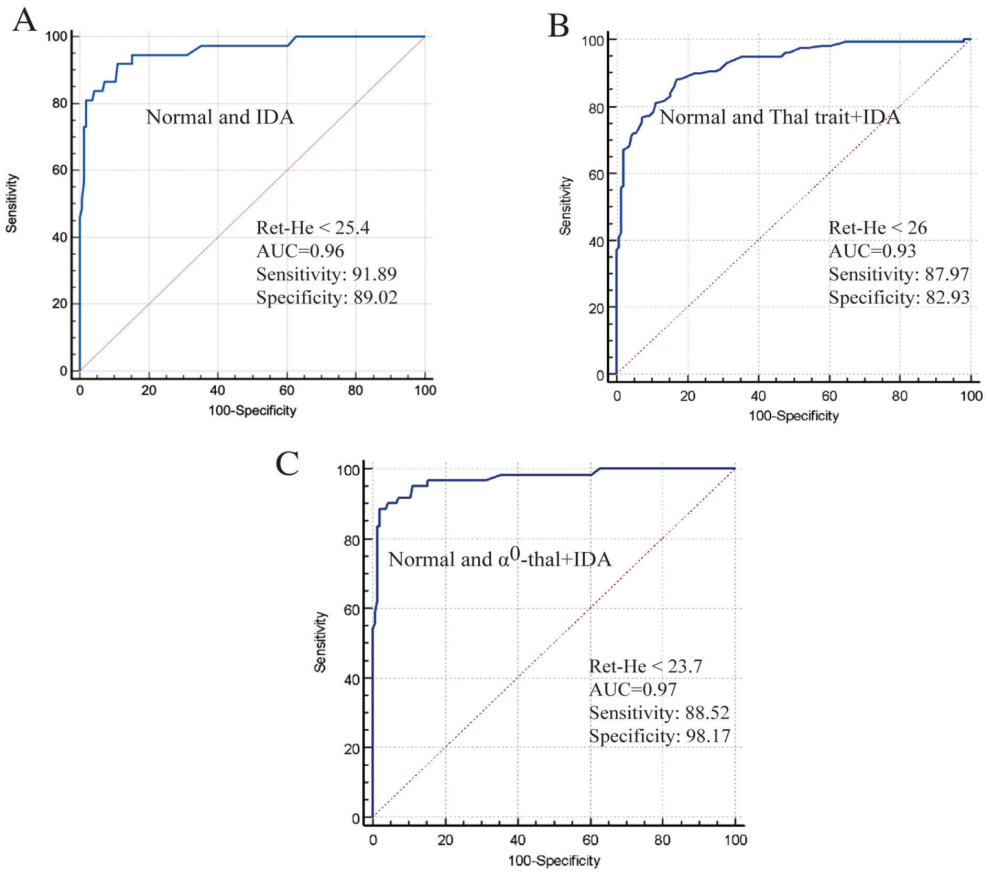



**Figure 5** The ROC curve of the Re-He for diagnosis of (A) iron deficiency anemia (IDA), (B) Thalassemia trait + IDA, and (C) α0-thalassaemia+ IDA

## Discussion

The non-purpose of thalassemia screening is to identify the carriers of thalassemia and abnormal Hb in order to evaluate the risk of a couple having a severely affected infant. The MCV and MCH values from hematology analyzers are frequently used to screen for abnormalities. The Department of Medical Sciences, Ministry of Public Health, Thailand, recommends an MCV <80 fL and an MCH <27 pg for screening $\alpha^0$-thalassemiaand β-thalassemia carriers[7,8]. However, the discrepancy in the cutoff values in different hematology analyzers has been reported[9,10]. Consequently, each automated hematology analyzer should be validated before being used for screening hemoglobinopathies in any population. This study established the cutoff MCV and MCH values for the new automated hematology analyzers: DF55, DH76, and DH615. Our results showed that the same cutoffs, 80 fL for MCV and 27 pg for MCH, as per the Thailand national guidelines, could be appropriate for all Dymind analyzers. This point provides more than 93% sensitivity for MCV and 97% sensitivity for MCH. This cutoff can cover all targeted carriers, including carriers of $\alpha^0$-thalassemiaand β-thalassemia.

Our findings indicate that thalassemia screening using the MCH value is more sensitive than using the MCV value. Although the 3 Dymind analyzers presented with similar cutoffs, our study found that the MCH value for DH615 was lower than that for DF55 and DH76. Thus, we observed that the MCH cutoff value for DH615 was more highly sensitive and covered more targeted carriers than the DF55 and DH76. For field applications, the DF55 is the most suitable because it is portable and easy to operate. The general guidelines for abnormal Hb (including Hb E and thalassemia) screening in this region state that either the MCV or MCH should be used in combination with the DCIP test for Hb E[4,5]. Our data revealed that, following the combination of MCV or MCH and DCIP test, all Dymind automated hematology analyzers could yield 100% sensitivity and 100% NPVs because all samples with Hb E had positive outcomes in the DCIP test. This result confirms that the MCV and MCH obtained from the hematology automation and DCIP tests are essential for screening abnormal Hb, including Hb E and thalassemia. The limitations of this study include the lack of iron study profiles, including serum iron, ferritin, and TIBC, to discriminate between iron deficiency and normal Hb typing.

In clinical hematology, the Ret-He is increasingly used as a parameter for assessing the functional iron available for erythropoiesis[11-14]. In Dymind DH615, we proposed the discrimination of thalassemia carriers by using MCV and MCH, together with Ret-He parameters (Figure 6). MCV and MCH are used as the first screening tools for the thalassemia trait. MCV >80 fL and MCH >27 pg displayed non-clinically significant thalassemia. They were then analyzed using the Ret-He parameter. The Ret-He value of less than 26 pg can be indicative of iron deficiency and thalassemia carriers. This value was reported in different automated blood cell analyzers. The appropriate cutoffs for the Sysmex XN-3000 and XN 9000 were 27 pg[13] and 31.2 pg[18], respectively. Moreover, the cutoff for the Mindray BC-6800Plus automatic blood analyzer was 26.7 pg[11]. Our study showed that a Ret-He value of less than 26 pg can be indicative of both the thalassemia trait and iron deficiency anemia. However, while Ret-He provides a useful marker for identifying conditions related to impaired hemoglobin synthesis, it lacks the specificity to differentiate between these 2 common causes of microcytic anemia. Our study shows that a threshold below 23.7 pg is more indicative of $\alpha^0$-thalassemia trait. These findings have important clinical implications and highlight the potential of Ret-He as an accessible marker for early thalassemia screening.

For the sample stability study, we found that the values of the MCV and MCH were more stable at 4 °C than at room temperature. In contrast, the MCH value was more stable than the MCV at room temperature. Therefore, the use of MCH rather than MCV is suggested because the MCH has higher sensitivity and is more stable than the MCV.

## Conclusion

Our results demonstrate that the Dymind automated hematology analyzer (DF55, DH 76, and DH615), when used in combination with the DCIP test, is suitable for first screening $\alpha^0$-thalassaemia, β-thalassemia, and Hb E in the Thai population. Ret-He parameters were also used in the discrimination of thalassemia carriers in DH615. Only the analytical performance of DH76 automated hematology has been reported[19]. Further studies are required to address the analytical performances of DF55 and DH615.

### Authors' Contributions

Conception and design: TP, WT, KS.

Analysis and interpretation of the data: TP, WT, NS, ST, KS.

Drafting of the article: KS.

Critical revision of the article for intellectual content: TP, WT, KS.

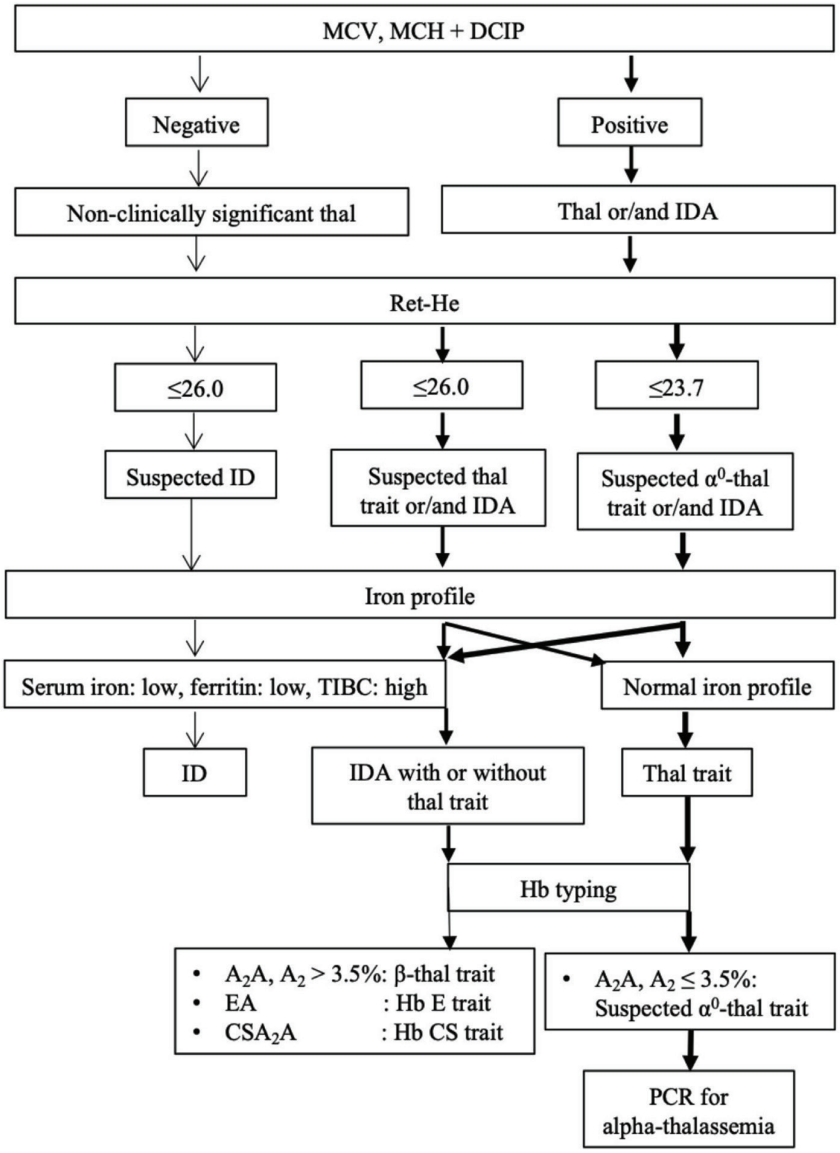



**Figure 6** Diagnostic algorithm for screening thalassemia carriers using MCV and MCH, together with Ret-He parameters in Dymind DH615

Final approval of the article: TP, WT, NS, ST, NT, KSr, SK, YN, CS, KS.

Provision of study materials or patients: NS, ST, NT, KSr, SK, YN, CS.

Statistical expertise: TP, WT, KS.

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## Conflict of interest

The authors declare that they have no competing interests.

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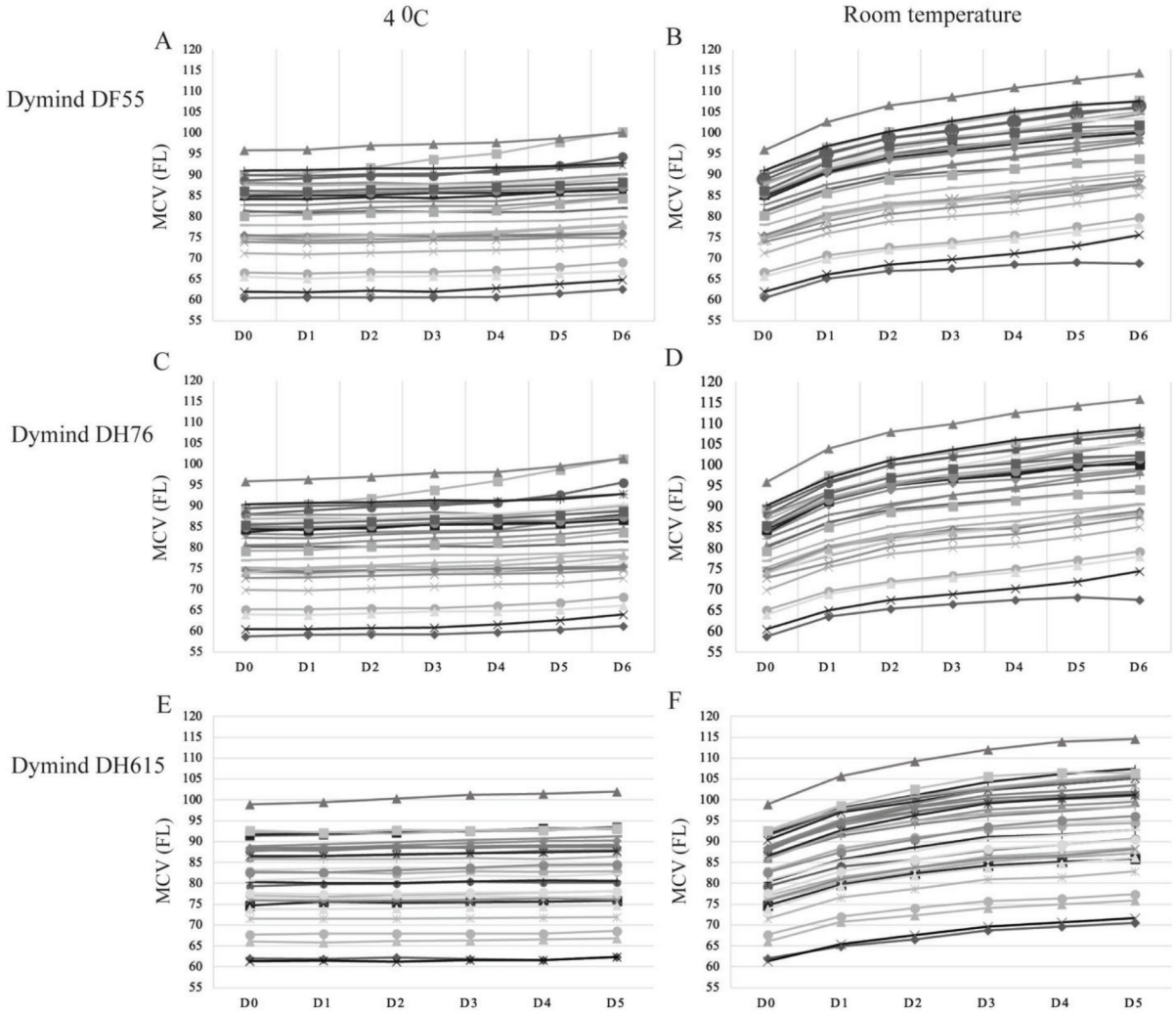
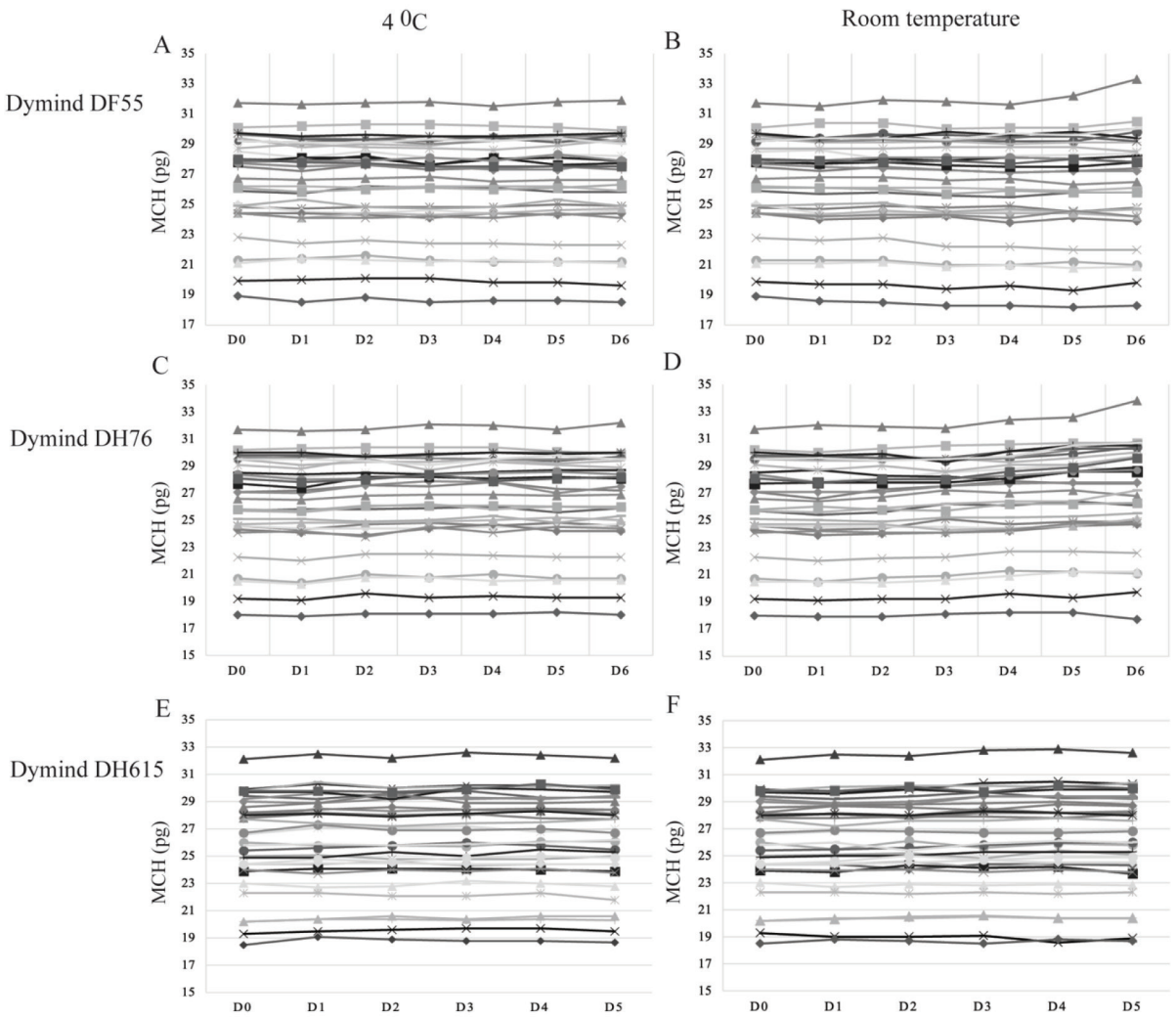



**Supplementary Figure 1** Results of the sample stability study for MCV values. Blood specimens with known MCV were stored at 4 °C and room temperature, respectively in the DF55 (A, B), DH76 (C, D) and DH615 (E, F) Dymind automated hematology analyzers




**Supplementary Figure 2** Results of the sample stability study for MCH values. Blood specimens with known MCH were stored at 4 °C and room temperature, respectively in the DF55 (A, B), DH76 (C, D) and DH615 (E, F) Dymind automated hematology analyzers