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Reference Intervals for a Complete Blood Count Determined on different Automated Haematology Analysers: Abx Pentra 120 Retic, Coulter Gen-S, Sysmex SE 9500, Abbott Cell Dyn 4000 and Bayer Advia 120

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We processed 317 samples from healthy adult volunteers for a complete blood count, including leukocyte differentials and reticulocyte parameters, through five new-generation haematology analysers: Abx Pentra 120 Retic, Coulter Gen-S, Sysmex SE 9500, Abbott Cell Dyn 4000 and Bayer Advia 120. From these data non-parametric 2.5–97.5 percentile reference intervals were calculated for all parameters on all analysers.

Some differences were found compared with previously reported reference intervals. Reference intervals for platelet parameters and reticulocytes agreed with these usually accepted. For red blood cell parameters, including haemoglobin and haematocrit, and white blood cell count, including absolute white blood cell differentials, our calculated reference intervals were in agreement with less frequently cited earlier reports, but were lower compared to the usually accepted reference intervals. Clin Chem Lab Med 2002; 40(1): 69–73

Key words: Reference intervals; Haematology analyser.

Abbreviations: ICSH, International Council for Standardisation in Haematology; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; K_3 EDTA, tri-potassium ethylenediaminotetraacetate; LUC, Large Unstained Cells; MCV, mean cellular volume; MPV, mean platelet volume; NCCLS, National Committee for Clinical Laboratory Standards.

Introduction

The complete blood count and differential leukocyte count are widely used in clinical practice and appropriate reference intervals are essential for the interpretation of patients' results. Analysers which were used to establish reference intervals in previous studies are being replaced by instruments employing newer technologies and providing extra parameters. Some recently introduced haematology analysers include a reticulocyte mode which offers an absolute reticulocyte count and reticulocyte maturation parameters (1). The present study was undertaken to determine reference intervals in a group of adults of West European ancestry using five new-generation automated haematology analysers.

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Subjects, Materials and Methods

Subjects and samples

Our study protocol complied with the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS)(2–4) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (5). The institutional committee for medical ethics approved the study protocol, and all volunteers gave informed consent. Volunteers were recruited by advertisements at the participating hospitals, at the local university and in two local newspapers. They had to reside in Antwerp and were asked to refrain from participation if they were aware of pre-existing illnesses or if they were taking medication. Infection was an exclusion criterion and blood donation habits were noted. Blood was collected in the morning after a 15 min recumbent rest, with the Sarstedt blood collection system (Sarstedt Monovettes; Sarstedt, Essen, Belgium). Tri-potassium ethylenediaminotetraacetate (K_3 EDTA) served as an anticoagulant. Samples were immediately brought to the laboratory and analysed within 4 hours.

Analysers

Complete blood counts, including a white blood cell differential and a reticulocyte count, were performed using five new-generation haematology analysers: Pentra 120 Retic (formerly Vegaretic, Abx, Montpellier, France), Gen-S (Coulter Electronics, Miami, USA), SE 9500 (Toa Medical Electronics Co, Kobe, Japan), Cell Dyn 4000 (Abbott Diagnostics, Santa Clara, USA) and Advia 120 (Bayer, Tarrytown, USA). All analysers were calibrated and maintained according to the manufacturer's instructions. Calibration of the different analysers was carried out using calibrator Minocal™ for Pentra 120 Retic, S-CAL® for Gen-S, SCS- 1000 for SE 9500, Cell Dyn HemCal™ for Cell Dyn 4000 and Advia SETpoint™ for Advia 120. The different analysers were not adjusted to one another. During the entire study commercial three-level controls were tested daily on all analysers. This study followed a complete instrument performance evaluation and comparison. Following the guidelines of the International Council for Standardisation in Haematology (ICSH) (6), all analysers were tested for linearity, reproducibility and carry-over and they were compared with the Coulter STKS, our routine laboratory analyser. Bias was calculated as the difference between the target value of the mid-level control and the mean of the daily results of that control. Imprecision was tested by running the same sample from a healthy volunteer sixteen times. The coefficient of variation of the 16 runs was calculated.

Since every sample had to be tested on all instruments, there was a time delay between measurements on the different analysers. To avoid any influence of this time delay, all samples were rotated in a strict sequence so that every analyser in turn received a series of samples first.

Statistics

The RefVal program (7) was used to calculate the non-parametric 2.5–97.5 percentile reference intervals for all parameters on each analyser. This program includes practical approaches and formulas recommended by the IFCC (5). Reference intervals for different subclasses (males and females) were separated according to NCCLS guidelines (2). The standard normal deviate test (z value) was used to evaluate the significance of the difference between subclass means. Standard deviations of two subclasses were also compared. If the larger standard deviation exceeded the smaller 1.5 fold, separate reference intervals for the two subclasses were calculated regardless of the z value. No statistical comparison between the reference intervals of the different analysers was made.

Results

Three hundred and seventeen apparently healthy individuals (142 men and 175 women) with a mean age of 39 years (range 19–60) were enrolled in the study. Forty seven volunteers had a history of blood donation (26 men and 21 women). Pentra 120 Retic, Gen-S, SE 9500, Cell Dyn 4000 and Advia 120 analysers analysed a total of 308, 312, 316, 317 and 317 samples, respectively. An identical group of 308 samples was analysed on all instruments.

All instruments had imprecision, linearity, carry-over and comparability characteristics agreeing with the manufacturers' specifications. Results of bias and imprecision are presented in Table 1 and are in agreement with earlier studies (8–13).

The calculated reference intervals for red blood cell and platelet parameters are presented in Table 2. According to NCCLS criteria (2), reference intervals for red blood cell count, haemoglobin concentration and haematocrit were defined according to gender. The male and female reference intervals are presented in Table 2. Blood donors were not excluded since blood donation habits did not influence our reference intervals for red cell parameters. To evaluate whether some volunteers had a degree of iron deficiency, we excluded all volunteers with low iron and ferritin and recalculated the reference intervals for red blood cell count, haemoglobin concentration and haematocrit. These recalculated intervals were similar to our initially calculated intervals (data not shown). Reference intervals for reticulocyte count and reticulocyte maturation fractions are presented in Table 3. Separate male and female reference intervals were calculated for absolute reticulocyte count.

Reference intervals for white blood cell count and

Table 1 Bias (%) and imprecision (CV; %) of the complete blood count on different analysers.

	Pentra 120 Retic		Gen-S		SE 9500		CD 4000		Advia 120	
	Bias	Imprecision	Bias	Imprecision	Bias	Imprecision	Bias	Imprecision	Bias	Imprecision
White blood cell count	0.9	1.5	1.9	0.5	1.4	1.6	2.5	2.0	0.6	1.9
Red cell count	0.2	0.9	1.3	0.4	1.7	0.7	1.1	0.7	1.6	0.5
Haemoglobin	1.5	0.9	0.6	0.1	0.7	0.3	1.4	0.3	1.6	0.5
MCV	0.1	0.5	0.2	0.5	0.3	0.2	0.8	0.2	0.1	0.1
Platelet count	2.7	3.1	2.8	2.5	0.5	1.4	4.3	2.5	0.8	2.7

Table 2 Reference intervals for red blood cell and platelet parameters.

	Pentra 120 Retic	Gen-S	SE 9500	CD 4000	Advia 120
Red cell count F (10^{12}/l)	3.74–4.73	3.70–4.69	3.65–4.59	3.60–4.69	3.74–4.80
Red cell count M (10^{12}/l)	4.16–5.59	4.14–5.56	3.98–5.47	4.06–5.58	4.16–5.63
Haemoglobin F (g/l)	107–141	109–142	110–144	108–142	113–145
Haemoglobin M (g/l)	126–158	130–16.0	129–16.4	129–159	133–162
Haematocrit F (l/l)	0.32–0.42	0.33–0.41	0.32–0.41	0.31–0.41	0.34–0.44
Haematocrit M (l/l)	0.38–0.47	0.38–0.47	0.35–0.46	0.36–0.47	0.39–0.49
RDW (%)	14.4–19.6	11.6–14.9	12.2–14.8	10.4–13.0	13.1–15.0
MCV (fl)	80.0–97.0	81.4–95.1	82.4–97.3	81.1–96.0	83.2–99.8
MCH (pg)	26.3–32.6	26.9–32.9	28.0–34.2	26.9–32.9	27.3–33.0
MCHC (g/l)	321–345	325–348	332–369	325–355	318–349
Platelet count (10^{9}/l)	139–329	142–325	142–340	155–366	149–362
MPV (fl)	6.8–10.0	7.6–10.7	9.4–12.9	6.9–10.6	6.4–9.7

F: female population; M: male population; RDW: red cell distribution width; MCV: mean cellular volume; MCH: mean cellular haemoglobin; MCHC: mean cellular haemoglobin concentration; MPV: mean platelet volume.

Table 3 Reference intervals for reticulocyte count (10^9/l and %) and reticulocyte maturation fractions (%).

	Pentra 120 Retic	Gen-S	SE 9500	CD 4000	Advia 120
Reticulocyte count F (10^9/l)	22–95	24–73	16–66	21–98	19–64
Reticulocyte count M (10^9/l)	31–130	30–90	16–70	30–110	29–69
Reticulocyte count (%)	0.61–2.16	0.61–1.79	0.44–1.55	0.61–2.24	0.50–1.40
Ret low	NC	NC	84.6–97.1	IRF 0.14–0.35	88.3–98.0
Ret medium	NC	NC	2.6–13.8	IRF 0.14–0.35	1.5–10.7
Ret high	NC	NC	0–2.4	IRF 0.14–0.35	0–2.0

F: female population; M: male population; Ret low, Ret medium and Ret high: percentage reticulocytes with low, medium or high fluorescence, respectively; IRF: immature reticulocyte fraction: ratio of immature reticulocytes to all reticulocytes (value specific to CD 4000 technology); NC: not calculated

Table 4 Reference intervals for white blood cells.

	Pentra 120 Retic	Gen-S	SE 9500	CD 4000	Advia 120 *
White blood cell count (10^9/l)	3.48–10.05	3.56–10.03	3.45–9.76	3.70–10.10	3.46–9.78
Neutrophils (%)	39.6–72.5	40.5–74.9	40.2–74.7	39.3–73.7	43.0–74.8
Lymphocytes (%)	18.8–47.4	16.8–46.7	17.6–47.6	18.0–48.3	16.4–45.0
Monocytes (%)	4.6–13.5	4.4–12.9	4.0–11.3	4.4–12.7	2.6–8.2
Eosinophils (%)	0.9–7.0	0.4–6.8	0.9–8.4	0.6–7.3	0.4–6.6
Basophils (%)	0.3–1.8	0.2–1.4	0.0–1.5	0.0–1.7	0.3–1.5
Neutrophils (10^9/l)	NC	1.66–7.16	1.56–7.081	1.63–6.96	1.65–6.81
Lymphocytes (10^9/l)	NC	1.00–2.88	0.98–2.85	1.09–2.99	0.94–2.62
Monocytes (10^9/l)	NC	0.24–0.76	0.20–0.64	0.24–0.79	0.15–0.51
Eosinophils (10^9/l)	NC	0.03–0.41	0.06–0.46	0.03–0.44	0.03–0.38
Basophils (10^9/l)	NC	0.01–0.07	0.00–0.08	0.00–0.08	0.02–0.08

NC: not calculated; * includes LUC: 2.0–5.4% and 0.10–0.31 10^9/l

white blood cell differential are given in Table 4. To compare these results with earlier reports, white blood cell differentials are presented as absolute numbers as well as percentages.

Discussion

We studied blood samples from 317 healthy adult volunteers on five new-generation haematology analysers and calculated reference intervals for the complete blood count, including leukocyte differential and reticulocytes. The importance of some newer parameters provided by these analysers, such as reticulocyte maturation fractions, is still under study and the calculation of reference intervals is an important initial step towards the validation process (1).

Following NCCLS recommendations (2) separate reference intervals for males and females had to be calculated for red blood cell count, haemoglobin concentration, haematocrit and absolute reticulocyte count. For all other parameters the reference intervals were calculated for the total population. Blood donation habits did not influence the reference intervals for red cell count, haemoglobin concentration and haematocrit (data not shown).

A number of factors may contribute to differences between reference intervals reported in different studies; these include characteristics of the studied volunteers (14), the analytical methods and the manner in which reference intervals were calculated (5).

During our initial instrument evaluation all analysers were proved to have a good analytical performance. All instruments performed well on daily quality controls. The inaccuracy and imprecision are presented in Table 1. We believe there is no reason to question the validity of these results from the analytical point of view.

Our calculated reference intervals for red blood cell count, haemoglobin concentration and haematocrit were lower compared to these usually accepted (15, 16), but were similar to the reference intervals presented in older, less frequently cited studies (17, 18). The reference intervals reported by Kelly *et al.* (17) and Davis *et al.* (18) are within our calculated 90% confidence intervals for red blood cell count, haemoglobin concentration and haematocrit, whereas reference intervals reported in Wintrobe's Clinical Hematology are outside and above our confidence intervals (data not shown). Our reference intervals for the mean cellular volume (MCV) are similar to both recent and older reports (15, 17). We correlated our reference intervals with those from two small studies where healthy volunteers were enrolled and samples were analysed using Cell Dyn 4000 (n=74) and Advia 120 (n=39) analysers. The reference intervals for red cell parameters from these two studies are similar to our results (data

not shown). In elderly people, especially in men above 70 years of age, haemoglobin concentration, red blood cell count and haematocrit decline (17). Our study population had a balanced age distribution and the results were not influenced by a higher proportion of the oldest age group. Following NCCLS guidelines, all samples were collected after a 15 min recumbent rest and with restricted use of a tourniquet (3). The strict control of these pre-analytical factors might explain our lower red cell reference intervals compared with some other studies. We realise that these ideal pre-analytical conditions cannot always be met in daily routine practice.

Our calculated platelet reference intervals are in agreement with both earlier and recently published reports (15, 16, 19). Platelet count in females is higher compared to males but the difference is small and there are no arguments to define separate intervals (19). The mean platelet volume (MPV) shows time-dependent changes due to swelling of platelets in the presence of anticoagulant, especially EDTA (20). Since samples rotated in a strict order between analysers, we do not believe a systematic time delay could result in the higher MPV reference interval for SE 9500. Rather, the difference in MPV reference intervals is methodology-dependent since aperture-impedance or flow cytometric methods give MPV differences of up to 40% (21).

In a recent evaluation of flow cytometric reticulocyte counting Butarello *et al.* also calculated reticulocyte reference intervals for the same five analysers (22). Although we calculated separate male and female reference intervals for the absolute reticulocyte count, we found similar reticulocyte reference intervals, except for the upper reference limit using Advia 120. In the above mentioned study the upper reference limit for Advia 120 was above 100×10^9/l, whereas in our study it was lower than 80×10^9/l. The upper reticulocyte reference limit for the H*3 haematology analyser (66.9×10^9/l), another Bayer instrument, reported by d'Onofrio and co-workers (23), was also lower compared to the study of Buttarello *et al.* (22). The difference observed in reticulocyte reference interval on Advia 120 reported in these studies needs further evaluation. We presented reference intervals of reticulocyte maturation fractions for SE 9500, Cell Dyn 4000 and Advia 120 analysers. An increase in the most immature reticulocyte fraction is known to correlate with some clinical conditions (1). Since the analysers have a different, arbitrary base to define these maturation fractions, we simply present these results without any further remarks.

Our reference intervals for white blood cells are lower compared to standard reports (15, 16). Reference intervals for the white blood cell differential presented as percentages of white blood cells are in agreement with standard reports, but reference intervals for the absolute white blood cell differential count are lower, following the lower count of white blood cells. Our white blood cell and white blood cell differential intervals are similar to those found by Davis *et al.* and Bain *et al.* (18, 24). In the latter study on white cell count in females of different ethnic origin Bain mentioned the importance of comparing patients' results with the appropriate reference intervals before starting unnecessary investigations in healthy "neutropenic" individuals. Based on the results collected during the National Health and Nutrition Examination Surveys (NHANES) One and Two, van Assendelft reported that there was a strong indication that the white blood cell count decreased over the one decade separating the two surveys (25). Although different studies found a gender-dependent difference in white blood cell intervals, especially in neutrophil count (26), the observed difference between male and female white blood cell count in our study was too small to calculate separate subclass reference intervals. Reference intervals for the different haematology parameters were similar for all instruments except for white blood cell differential. In contrast to the other analysers, Advia 120 has an additional, sixth cell cluster in its white blood cell differential (11). This cluster of Large Unstained Cells (LUC) possibly harbours cells that on other analysers are found in the lymphocyte and monocyte cell clusters, leading to a lower reference interval for monocytes on Advia 120. For this reason we also presented our calculated reference interval for LUC on Advia 120.

The statistical method for the calculation of reference intervals used in our study was based on NCCLS and IFCC recommendations (2, 5). This non-parametric method is used in most studies (17, 19, 22–24), although some studies also applied a parametric method (18, 24). The different statistical method could give slightly different results but would not entirely explain our lower calculated reference intervals for red blood cell parameters and for white blood cells.

We reported reference intervals on five haematology analysers. The most important findings of this study are the lower reference intervals for red blood cell parameters and for white blood cells. Although such lower reference intervals have been presented earlier using older technology, they are lower than generally accepted. These results should be considered before important investigations to evaluate "anaemia" and "leukopaenia" are initiated.

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