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Scand J Clin Lab Invest 2004; 64: 385–398

A multicentre study of reference intervals for haemoglobin, basic blood cell counts and erythrocyte indices in the adult population of the Nordic countries

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Nordin G, Mårtensson A, Swolin B, Sandberg S, Christensen NJ, Thorsteinsson V, Franzson L, Kairisto V, Savolainen E-R. A multicentre study of reference intervals for haemoglobin, basic blood cell counts and erythrocyte indices in the adult population of the Nordic countries. Scand J Clin Lab Invest 2004; 64: 385–398.

Eight haematological quantities were measured in EDTA anticoagulated venous blood specimens collected from 1826 healthy male and female individuals between 18 and 90 years of age in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). The samples, collected between November 1999 and November 2001 as part of the Nordic Reference Interval Project (NORIP), were analysed on 12 different types of modern automated haematology instruments currently in use among the 60 laboratories participating in the

Abbreviations and nomenclature: The following non-standard abbreviations are used in relation to the IFCC-IUPAC nomenclature system for properties and units in clinical laboratory sciences (http://www.ifcc.org/divisions/sd/c-npu.htm): B-Haemoglobin or B-Hb, is used for haemoglobin concentration in blood, either ''Blood-Haemoglobin; mass concentration'' or ''Blood-Haemoglobin (Fe); substance concentration'' (NPU02319) as distinguished by the unit. B-Erythrocytes, or B-Erc, is used for the concentration of erythrocytes in blood, that is ''Blood-Erythrocytes; number concentration'' (NPU01960). B-EVF is used for the erythrocyte volume fraction or ''packed cell volume'', that is ''Blood-Erythrocytes; volume fraction'' (NPU01961). B-MCV is used for the mean corpuscular volume of the erythrocytes, that is ''Blood-Erythrocytes; entitic volume'' (NPU01944). Erc-MCHC is used for mean corpuscular haemoglobin concentration, either ''Erythrocytes-Haemoglobin; mass concentration'' or ''Erythrocytes-Haemoglobin (Fe); substance concentration'' (NPU02321) as distinguished by the unit. Erc-MCH is used for mean corpuscular haemoglobin content, either ''Erythrocytes-Haemoglobin (Fe); entitic mass'' or ''Erythrocytes-Haemoglobin; entitic amount of substance'' (NPU02320) as distinguished by the unit. B-Trc is used for concentration of thrombocytes or platelets in blood, that is ''Blood-Thrombocytes; number concentration'' (NPU03568). B-Lkc is used for the concentration of leukocytes, that is ''B-Lkc; number concentration'' (NPU02593).

DOI 10.1080/00365510410002797

study. Non-parametric reference intervals (between 2.5 and 97.5 percentiles) have been calculated for B-Haemoglobin (females 117 – 153 g/L, males 134 – 170 g/L), B-Erythrocytes (females $3.94 - 5.16 \times 10^{12}$/L, males $4.25 - 5.71 \times 10^{12}$/L), B-EVF (females 0.348 – 0.459, males 0.395 – 0.500), B-MCV (82 – 98 fL), Erc-MCH (27.1 – 33.3 pg), Erc-MCHC (317 – 357 g/L), B-Trc (females 165 – 387×10^9/L, males $145 \times 348 \times 10^9$/L) and B-Lkc ($3.5 - 8.8 \times 10^9$/L). Partitioning of data according to age and gender was done according to a standardized procedure. For most variables the calculated reference intervals corresponded well with older and less well-defined reference intervals. The mean concentration of B-Haemoglobin increased by 0.08 g/L per year of age in women, and decreased by 0.1 g/L per year of age in men. B-Haemoglobin increased with body mass index in both men and women. Smoking increased the mean of B-Lkc by 1.1×10^9/L and regular use of alcohol increased the mean of B-MCV by 0.8 fL. The influence of these factors was small overall and did not promote specific reference intervals.

Key words: Haematology; leukocyte count; normal values; platelet count; red blood cell count

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INTRODUCTION

Reference intervals in haematology

Basic blood cell counts are the most frequently used laboratory investigations in health care. In the Nordic countries the reference intervals for blood cell counts vary considerably from country to country and from one laboratory to another, without any justifiable reason. Some laboratories have determined their own reference intervals while others have adapted values from the literature.

The manufacturers of cell counters usually recommend the end-user to estimate a local reference range, and not to trust the intervals suggested by the manufacturers. Published reference intervals in haematology are either based on data from limited and sometimes poorly described reference populations and consequently show large uncertainties for the reference limits, or are produced with older cell-counting instruments no longer on the market. Studies on reference intervals in haematology published in recent years have often concerned data on important subsets of populations such as the elderly, children or pregnant women, but not on the healthy adult population. Thus there were reasons to organize a new general reference interval study for cell counters currently in use.

A reference interval should be traceable to a reference population, as well as to the measurement procedure and its calibration. According to a European directive [1] the manufacturers of instruments for *in vitro* diagnostics (IVD) are responsible both for the metrologic traceability of the measurement results and for providing the reference interval. In general clinical chemistry this is normally accomplished with storable reference materials and reference measurement procedures of higher metrological orders. In haematology, other challenges are often met, because, with the exception of the concentration of haemoglobin, native samples must be measured fresh, and cannot be reanalysed after storage [2 – 4].

To be useful as a reference interval, the reference individuals ought to be representative of the general population. The Nordic population is rather homogeneous from an ethnic point of view, with low incidence of major haematological disorders, such as thalassaemia, facilitating the selection process of reference individuals. The aim of the present project was therefore to establish common reference intervals in the Nordic countries for B-Haemoglobin, B-Erythrocytes, B-EVF, B-MCV, Erc-MCH,

Erc-MCHC, B-Trc and B-Lkc, by using locally collected specimens measured on the local blood cell counters from various manufacturers. In addition, we sought to investigate the effect of different instruments and different lifestyle factors on the reference values. The study was carried out in connection with the Nordic reference interval project (NORIP) in the five Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). The intention of NORIP was to invite Nordic laboratories to recruit healthy reference persons and measure basic clinical chemistry variables. Subsequently, the project was extended to include basic haematology properties.

MATERIALS AND METHODS

The general organization of the NORIP project, the reference persons recruited for the project and the questionnaire completed by each participant are described elsewhere [5]. The haematological variables were measured in samples from 1826 reference individuals, i.e. a subset comprising approximately 60% of the total number of reference persons in NORIP. The subjects were subjectively healthy adult men and non-pregnant women recruited for the general clinical chemistry project. None of the results from any of the individuals was excluded, except for outliers, as described under *Statistics*. The majority of results are based on samples collected by laboratories in Finland and Sweden. Descriptive data for the reference population are presented in Table I.

In the study, 60 laboratories participated between November 1999 and November 2001. Venous blood was collected from the reference persons after at least 15 min in sitting position, as described elsewhere. The samples for the haematology were collected in tubes containing salts of ethylendiaminotetraacetate (EDTA) and analysed within 4 h after sampling as single measurements at the local laboratory.

Effect of sampling tubes

The mean cell number concentrations and the mean haemoglobin concentrations of the results reported from laboratories using tubes with dry EDTA were higher compared with those that used tubes with liquid EDTA. Consequently, from the sites using liquid EDTA, all values were therefore corrected in accordance with the volume of liquid EDTA in the test tube. The dilution factors varied between 1.2 and 3.8% depending on the different brands of EDTA tubes with liquid EDTA. It was assumed that the volume of the EDTA solution was distributed solely in the extra cellular phase, thereby effecting the number concentrations and the concentration of haemoglobin and B-EVF, but not the values for B-MCV, Erc-MCH and Erc-MCHC. Thus, a possible effect of type of EDTA on B-MCV has not been taken into consideration in the calculations.

The instruments used were all calibrated and run according to the manufacturer's instructions. No common control sample or reference substance was offered to the participants. The participating instruments were categorized into

TABLE I. Description of the reference individuals (n = 1826) that consist of a subset of the participants in the NORIP study.

Population	Females	Males
N	960	866
Median age (range)	48 (18 – 91)	46 (18 – 90)
Number of persons from Denmark/Finland/Iceland/Norway/Sweden	116/442/45/19/338	113/412/33/12/296
Characteristics of reference individuals		
Fasting ≥ 10 h prior to sampling	83%	78%
Sampling before 09.00 h	63%	64%
Regular smokers	13%	15%
Regular user of alcohol	38%	52%
Regular physical activity	11%	22%
Active blood donor	18%	22%
Obesity (BMI > 30)	5%	4%
User of oestrogen	35%	

BMI = body mass index.

TABLE II. The instruments, in alphabetical order, used by participants were categorized into 12 major groups. The majority of instruments belong to one of seven large instrument groups (in italics) named according to the right column.

Instrument name	No. of reference individuals	Name of major instrument group used in this report
ABX Pentra	50	
Bayer Advia	*313*	*Bayer Advia*
Bayer H-1, H-1 Junior, H-2, H-3	*328*	*Bayer H*
Cell-Dyn 1300, 1400, 1500, 1600, 1700, 2000	9	
Cell-Dyn 3200	74	
Cell-Dyn 4000	*280*	*Cell-Dyn 4000*
Cell-Dyn CS 3000, 3500	*167*	*Cell-Dyn 3500*
Cobas Argos, Helios, Vega	31	
Coulter SPlus II-VI, STKR, STKS, MaxM, Onyx, GenS	*234*	*Coulter big*
Coulter T540, T660, T850, TC10, Js, MD8, MD18, MDII 18, AcT	67	
Sysmex K 800, K-1000, K-4500, M-2000, KX-21	*170*	*Sysmex small*
Sysmex NE 1500, NE 8000, SE 9000, SF 3000, XE 2100	*103*	*Sysmex big*
Total	*1826*	

12 different instrument groups as presented in Table II. Instruments were named according to the Clinical Chemistry Methods guide from Labquality, Helsinki, Finland. Data from the seven major instrument groups each representing measurements on more than 100 reference individuals were used in a separate analysis of variation between instrument groups. The remaining instrument groups consisted of cell counters used by a restricted number of laboratories and were thus excluded for the description of the variation between instruments. However, there was no evidence that results from these instruments deviated from those of the other instruments, and none of the results from any of the instrument systems were excluded from the final calculation of the reference intervals.

Data for B-Hb, Erc-MCHC, Erc-MCH which were reported as substance concentration (mmol/L) and substance content (fmol), respectively, were converted into mass concentration (g/L) and mass content (pg) assuming the relative molecular weight of 16 500 g/mol for haemoglobin. Obvious reporting blunders, such as mixing of different units (g/dL and g/L for the concentration of haemoglobin, and the unit L/L and % for B–EVF), were identified and corrected before any calculations.

Without the common stable and commutable reference materials with which dependent bias could be estimated, the results from the measurements with the different instrument systems have to be compared with the overall mean of all results. Bias from the overall mean was judged against a maximum allowable bias derived from analytical quality specifications based on estimates of within-subject coefficient of variation (CV) and between-subject CV. Maximum allowable bias was calculated using the algorithm: $0.25 \times \sqrt{CV^2_{within} + CV^2_{between}}$. Based on published data [6] on representative CV_{within} and $CV_{between}$, the maximal allowable bias was consequently estimated to approximately $\pm 1\%$ for B-MCV, Erc-MCH and Erc-MCHC, $\pm 2\%$ for B-Hb, B-Erc, B-EVF, and $\pm 6\%$ for B-Trc and B-Lkc. These figures are suggested to be the maximal allowable bias from the overall mean for an instrument group, under which circumstances common reference interval can be applied.

Statistics

Data were reported by participating laboratories to the central NORIP database, and extracted to a secondary database. The laboratories were instructed to report the results with the same number of digits as is done for normal clinical data.

After correction for pre-analytical dilution by liquid EDTA and reporting blunders, outliers were defined as results deviating by >5 standard deviations (SD) from the initial mean value. In this way, 25 values were excluded in a first round. The procedure was

repeated once again and 3 more values were excluded. No other criteria for exclusion were applied.

All parametric calculations were performed with Microsoft Excel 2000, partly with help of WinSTAT for Excel, an add-in macro. Calculations of reference interval limits were made as non-parametric estimates of 2.5 and 97.5 percentile values with RefVal 4.0 [7]. Partitioning into subgroups by age and gender were applied as described by Lahti *et al*. [8] with the exception that non-parametric instead of parametric calculations were used. Partitioning was not done when the percentages of the subgroup distributions outside the 2.5 and 97.5 percentiles of the common distribution were between 0.9% and 4.1%.

Multiple stepwise linear regressions were used to investigate the impact of various lifestyle factors. Age and body mass index (BMI) were treated as continuous variables, while gender, smoking habits, alcohol habit, fasting, morning sampling (that is sampling before 09.00 h.), physical activity and former blood donation were coded to bimodal responses (''Yes/No'', allocated values either 1 or 0). The effects were investigated with a significance level of $p < 0.05$. The graded response to the question of alcohol habits was interpreted as either ''no'' (0 units of alcohol per week) or ''yes'' (1–21 or >21 units per week). Smoking habits were interpreted as either ''no'' (0 cigarettes per day) or ''yes'' (1–5 cigarettes per day or >5 cigarettes per day).

The participants reported their data in the same number format as they normally report clinical data. Results for some properties were frequently reported with only two digits. For B-MCH and B-MCV it was not possible to calculate the 2.5 and 97.5 percentile limits with sufficient certainty because of many tied values. Therefore random digits between 0 and 9 were added as substitutes for decimal figures. A value of 5 (mean of the random digit variables) was subtracted from all data in order to keep the mean value unchanged. With this procedure the percentile limits could be located more precisely than was possible with the original data.

RESULTS

The mean values from the major instrument groups for the reference persons are shown in Table III. Comparisons of the distributions of results from the major instrument systems are also illustrated in Figure 1, where the standard normal deviates for the cumulative distribution have been calculated (Figs 1a–h). The lines correspond to the maximum allowed biases (± 1, ± 2 and $\pm 6\%$ deviation respectively) from the overall mean.

With few exceptions, the distributions of the quantities measured with the major instrument groups were within the limit for allowable bias (see Material and Methods). Compared to the allowable bias of $\pm 1\%$ the small Sysmex instruments showed B-MCV and Erc-MCH values just below the limit while the Cell Dyn 4000 instruments showed mean values slightly above the limits (Figs 1d and e). Although within the allowable limits the small Sysmex instruments showed slightly higher B-Erc counts, and the Cell-Dyn 4000 instruments slightly lower counts.

TABLE III. Mean values of the reported data in NORIP for the major haematology instrument groups.

		Mean for NORIP reference individuals							
Instrument group	N	B-Hb g/L	B-EVF L/L	B-Erc $\times 10^{12}$/L	B-MCV fL	Erc-MCH pg	Erc-MCHC g/L	B-Lkc $\times 10^{9}$/L	B-Trc $\times 10^{9}$/L
Bayer Advia	313	143.3	0.422	4.73	89.2	30.4	340	5.69	261
Bayer H	328	142.7	0.426	4.74	90.1	30.1	334	5.55	238
Cell-Dyn 4000	280	143.0	0.425	4.66	91.2	30.7	337	5.73	248
Cell-Dyn 3500	168	142.7	0.421	4.74	89.6	30.2	336	5.91	248
Coulter big	234	142.7	0.419	4.64	90.3	30.8	341	5.69	242
Sysmex small	170	143.4	0.425	4.78	88.7	30.0	337	5.50	237
Sysmex big	103	142.8	0.426	4.78	89.5	30.0	335	5.43	245
All data	1826	142.9	0.424	4.72	90.0	30.4	337	5.68	247

NORIP = Nordic Reference Interval Project.

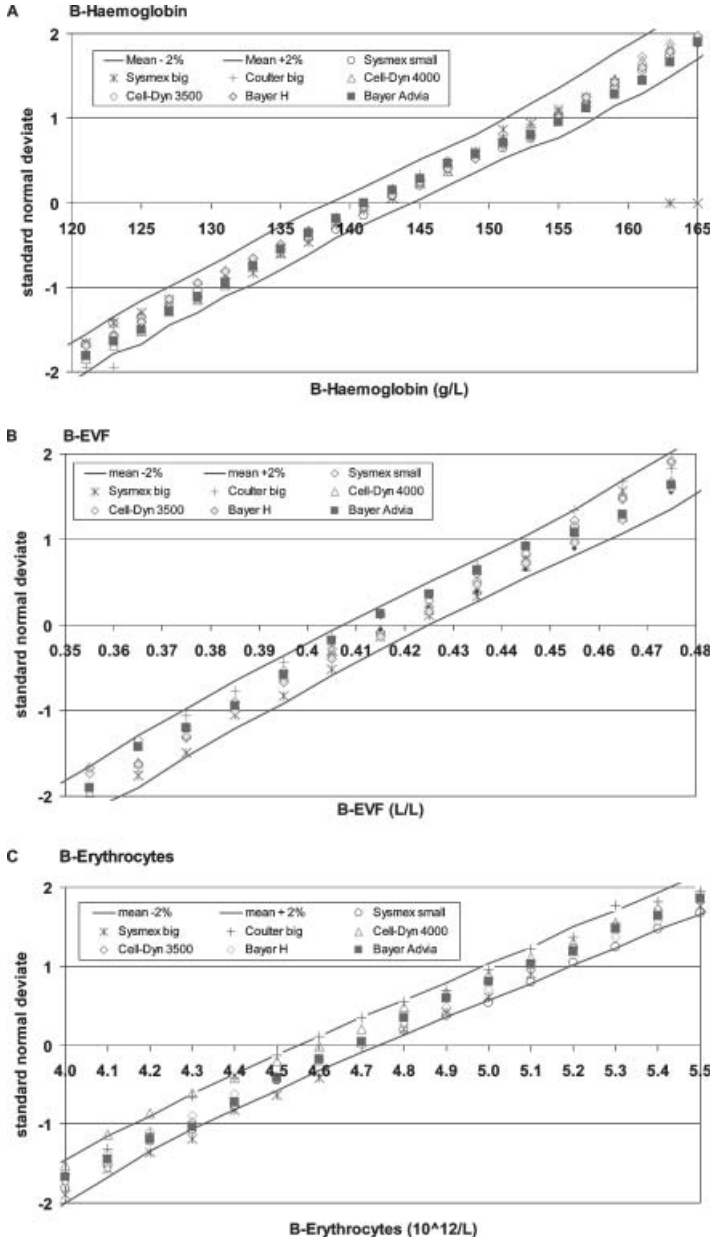

FIG. 1. Standard normal deviates calculated for the cumulated distribution of reported results from the Nordic Reference Interval Project (NORIP) population. Values on the x-axis denote mean values of the result class. The suggested limits of allowable bias denoted by unbroken lines, corresponding to $\pm 1\%$ (B-MCV, Erc-MCH, Erc-MCHC), $\pm 2\%$ (B-Haemoglobin, B-Erythrocytes, B-EVF (erythrocyte volume fraction) and $\pm 6\%$ (B-Leukocytes and B-Thrombocytes) respectively. When the results follow a normal distribution, the standard normal deviate is a straight line. Thus, the results of B-Leukocytes (Fig. h) are not distributed normally. Before calculation, the variables B-MCV and Erc-MCH were provided with random decimal digits (see text). Data points from instruments exceeding the acceptance limits are connected with broken lines, while data points within the acceptance limits are not connected with lines.

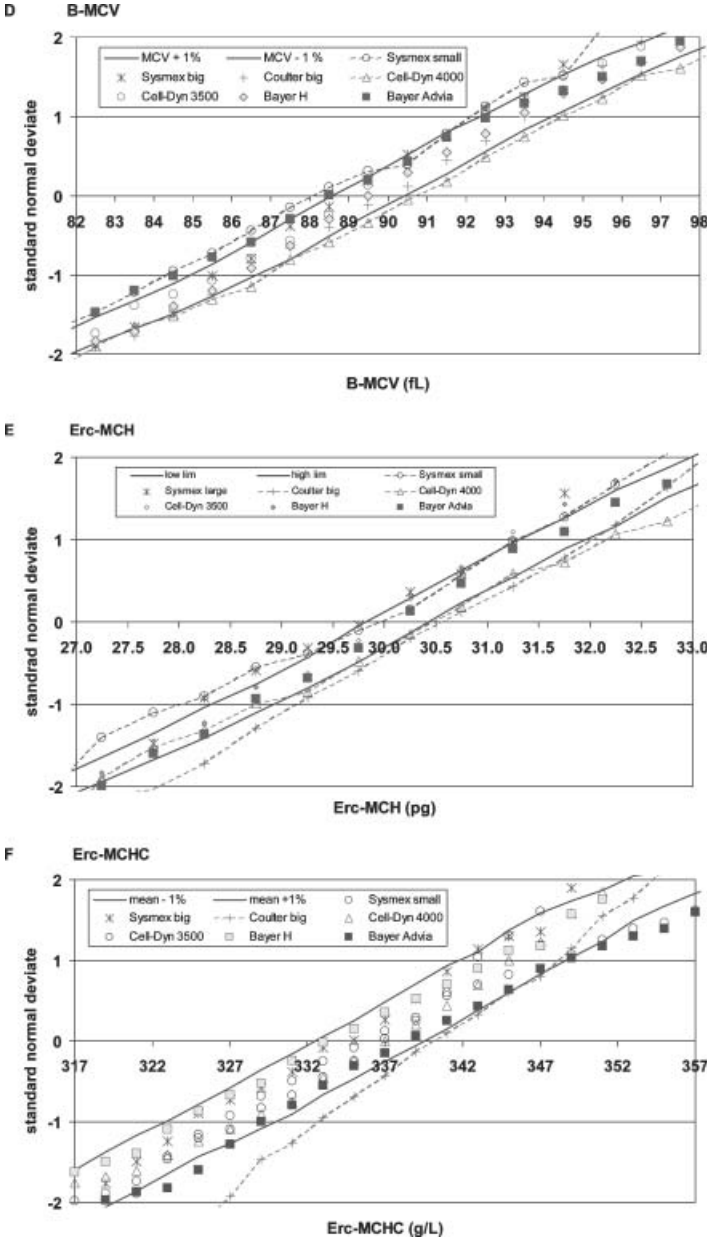

FIG. 1. (Continued.)

The big Coulter systems gave results slightly above the limit for Erc-MCH, and clearly above the limit for Erc-MCHC, especially in the low range of the distribution (Figs 1e and f).

The mean B-Trc count in the reference population measured with Bayer Advia systems was approximately 6% higher than the survey mean, which is in the zone of the maximum allowable deviation between instrument systems in the project.

Influence of lifestyle factors on the reference intervals

Several biological and lifestyle factors are known to influence inter- and intra-individual variation for the haematological quantities. The

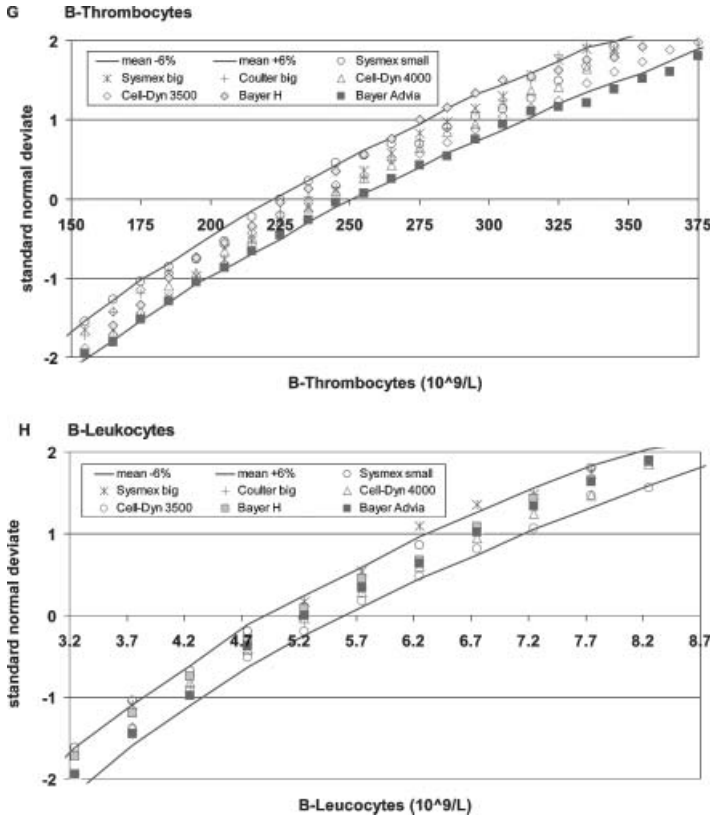

FIG. 1. (Continued.)

questionnaire used for the reference persons in NORIP covered some of these lifestyle factors [5]. The impacts of these factors on the haematological variables observed from this study are summarized in Table IV.

Some of the haematological quantities were statistically significantly related to age. One example is that the concentration of B-Hb increased with age in females by 0.08 g/L per year, but decreased by 0.10 g/L per year in males. BMI was positively related to B-Lkc, B-Hb, B-Erc (males), B-EVF and B-Trc (females) and negatively related to B-MCV and Erc-MCH.

As expected, sampling in the fasting state was associated with lower leukocyte counts while smoking was associated with higher leukocyte counts. B-Lkc was approximately 1×10^9/L higher in smokers compared to non-smokers. Smoking had also a positive effect on B-MCV independently of the expected effect on B-MCV exerted from alcohol use (+1.6 fL and +0.8 fL, respectively, Table IV). Smoking increased the mean concentration of B-Hb in women (+4 g/L) and the concentration of B-Trc in males ($+11 \times 10^9$/L).

When treating smokers as a subgroup compared with non-smokers, several of the distributions fulfilled the criteria for subgroup partitioning. In the final calculations of the suggested reference values, however, only gender and age have been considered when applying the rules for partitioning [8]. For Erc-MCH and Erc-MCHC statistically significant effects of gender were found (Table IV), but the partitioning criteria were not fulfilled. The partitioning criteria for gender were only fulfilled for B-Hb, B-EVF, B-Erc and B-Trc.

For 1291 of the reference individuals, data on serum iron were available as part of the NORIP project. It was therefore possible to estimate the possible impact on reference limits due to iron deficiency in this subgroup. If 37 individuals with serum iron <9 mmol/L had been

TABLE IV. Regression coefficients for the factors with independent significant ($p < 0.05$) correlation to the investigated blood cell variables in a stepwise multiple regression. Age and BMI were treated as continuous variables. Responses to questions regarding alcohol habits were interpreted as either "no" (0 units of alcohol per week) or "yes" (either 1–21 or >21 units per week). Smoking habits were interpreted as "no" (0 cigarettes per day) or "yes" (either 1–5 cigarettes per day or >5 cigarettes per day). The estimated time of fasting (h) before sampling was transformed to either fasting for less than 10 h or fasting for a longer period. Sampling times were divided into either sampling before 09.00 h or sampling at 09.00 h and later. Non-responders are excluded from the calculations. The complete questionnaire is presented in [5].

		Explanatory variables included in the model									
Quantity, gender (unit)	Constant	Gender (M = 1, F = 0)	Age (years)	BMI	Smoke (yes = 1, no = 0)	Alcohol (yes = 1, no = 0)	Fasting 10 h (yes = 1, no = 0)	Sampling before 09.00 h (yes = 1, no = 0)	Oestrogen (yes = 1, no = 0)	Physical activity (yes = 1, no = 0)	Former blood donor (yes = 1, no = 0)
B-Haemoglobin, F (g/L)	123.19	n.t.	0.0791	0.3311	4.09						
B-Haemoglobin, M (g/L)	147.01	n.t.	−0.1052	0.3771					n.t.		
B-EVF, F (L/L)	36.20	n.t.	0.0279	0.1112	1.31						
B-EVF, M (L/L)	43.13	n.t.	−0.0173	0.0937					n.t		
B-Erc, F (10^{12}/L)	3.97	n.t.		0.0233					−0.07		
B-Erc, M (10^{12}/L)	4.84	n.t.	−0.0069	0.0186					n.t.	−0.06	
B-MCV (fL)	91.02		0.0677	−0.1954	1.58	0.81			n.t.		
Erc-MCH (pg)	30.90	0.43	0.0148	−0.0621	0.52				n.t		
Erc-MCHC (g/L)	337.45	3.56	−0.0734				1.39		n.t		
B-Trc, F (10^9/L)	207.97	n.t.		2.5057			−10.22				
B-Trc, M (10^9/L)	240.79	n.t.	−0.2056		11.46				n.t		
B-Lkc (10^9/L)	4.59			0.0573	1.09		−0.50		n.t.		−0.20

n.t. denotes "not tested", that is the variable is not included in the analysis for that particular analyte (e.g. the responses to the question of use of oestrogen are only included in quantities for the female gender).

BMI = body mass index.

TABLE V. Suggested reference 95% reference intervals for eight variables in haematology based on measurements of samples from 960 females and 866 males. The lower and upper limits are given with 90% confidence intervals. Some reference limits suggested elsewhere are shown for comparison.

Analyte	Gender subgroup	NORIP low limit (90% CI)	NORIP high limit (90% CI)	Laurell [9]	Tsang[b] [10]	Bain[c] [11]	Beutler[h] [12]
B-Haemoglobin	Females	7.1 (7.03 – 7.15)	9.3 (9.21 – 9.45)				
(mmol/L)[a]	Males	8.1 (8.00 – 8.18)	10.3 (10.12 – 10.42)				
B-Haemoglobin (g/L)	Females	117 (116 – 118)	153 (152 – 156)	115 – 147[e]	122 – 161	118 – 148	123 – 153
	Males	134 (132 – 135)	170 (167 – 172)	131 – 163[e]	131 – 175	133 – 167	140 – 175
B-EVF (L/L)	Females	0.348 (0.347 – 0.357)	0.459 (0.453 – 0.466)	0.37 – 0.44	0.36 – 0.47	0.36 – 0.44	0.36 – 0.45
	Males	0.395 (0.388 – 0.398)	0.500 (0.497 – 0.502)	0.39 – 0.49	0.39 – 0.51	0.39 – 0.50	0.42 – 0.50
B-Erc (10^{12}/L)	Females	3.94 (3.89 – 3.95)	5.16 (5.12 – 5.30)	3.7 – 4.9	4.0 – 5.4	3.88 – 4.99	4.1 – 5.1
	Males	4.25 (4.20 – 4.30)	5.71 (5.65 – 5.77)	4.1 – 5.4	4.2 – 5.9	4.32 – 5.66	4.5 – 5.9
Erc-MCV (fL)		82 (81.5 – 82.4)	98 (97.7 – 98.3)	82 – 102	80.0 – 99.0[d]	82 – 98	80 – 96
Erc-MCH (fmol)[a]		1.64 (1.63 – 1.65)	2.02 (2.01 – 2.03)				
Erc-MCH (pg)		27.1 (26.9 – 27.3)	33.3 (33.2 – 33.5)	28 – 35	27.0 – 34.0[d]	27.3 – 32.6	27.5 – 33.2
Erc-MCHC (mmol/L)[a]		19.2 (19.15 – 19.33)	21.6 (21.52 – 21.70)				
Erc-MCHC (g/L)		317 (316 – 319)	357 (355 – 358)	320 – 360		316 – 349	334 – 355
B-Lkc (10^9/L)		3.5 (3.38 – 3.53)	8.8 (8.5 – 9.2)	4.0 – 10.0	3.9 – 9.5[d]	3.7 – 9.5[f]	4.4 – 11.3
B-Trc (10^9/L)	Females	165 (159 – 173)	387 (375 – 403)	125 – 340	163.0 – 414.0	188 – 445[g]	172 – 450
	Males	145 (138 – 149)	348 (334 – 358)	125 – 340	153.0 – 382.0	168 – 414[g]	172 – 450

NORIP = Nordic Reference Interval Project; IFCC = International Federation of Clinical Chemistry and Laboratory Medicine; IUPAC = International Union of Pure and Applied Chemistry.

[a]The reference intervals for the haemoglobin components are given both as mass concentration and substance concentration, the latter recommended by IFCC/IUPAC.

[b]Technichon H2. Fasting values. Type of EDTA not specified. Confounding factors (drugs, alcohol, tobacco) excluded. Reference persons 49 – 97 years of age.

[c]Coulter S and S Plus IV, Technicon Hemalog 8 and H2. Dry EDTA.

[d]Values in table for males. Females: Erc-MCV: 80.0 – 97.0 fL, Erc-MCH: 27.0 – 33.0, B-Leukocytes: $3.6 – 9.4 \times 10^9$/L.

[e]Value for male and females <60 years. Females >60 years: 113 – 153 g/L, males >60 years 122 – 166 g/L.

[f]Values in table for males. Females: $3.9 – 11.1 \times 10^9$/L.

[g]Impedance counting. Dry EDTA. Values from several other sources also cited in the reference.

[h]Type of EDTA not specified.

excluded, the lower limit of the reference interval for B-Hb would have been increased by less than 1 g/L compared to the reference interval for all data. The upper limit had, as expected, not been affected (data not shown).

The finally calculated reference intervals as well as some established reference intervals are shown in Table V.

DISCUSSION

General discussion

For the cell-counting quantities, the instrument manufacturers provide their customers with tailor-made products to be used for proper calibration of the instruments. With the exception of B-Haemoglobin, it is not possible to use a common and native reference material to estimate the agreement between different haematology instruments. In this study the degree of agreement has therefore been evaluated by identifying clinically relevant discrepancies between the population distributions reported from different sites and measurement systems.

In general, the reference intervals suggested in the present study were in good agreement with the recently published data [9–12]. One deviation from many previously described reference intervals was the somewhat lower reference limit for B-EVF in females. It is not clear whether this discrepancy might be explained by differences in techniques. A correction for trapped plasma in the estimation of B-EVF was earlier recommended [13], at which time the reference intervals 0.37–0.47 L/L (females) and 0.40–0.54 L/L (males) were accepted. A new procedure based on measurements of B-Hb and Erc-MCHC, thus avoiding the problem of trapped plasma, has now been recommended as the reference method for B-EVF [14], but the possible effects on reference intervals are unclear.

Another difference compared to previously described reference interval studies is the lower reference limit for leukocyte counts (3.5×10^9/L). When data from the different participating sites were compared, the lowest mean counts for B-Lkc were found for Finnish females compared to other subgroups (approximately 0.6×10^9/L lower than the overall mean value). Sampling in the fasting state results in a lower leukocyte count (Table IV). A partial explanation for the lower leukocyte counts in Finnish women might be that a slightly higher fraction of specimens was collected in the fasting state, compared to specimens from women in the other countries. No significant difference was observed between leukocyte counts in Finnish men and leukocyte counts from other subgroups.

The impacts of lifestyle factors, e.g. smoking, are not often systematically described in older studies of reference intervals. It is therefore difficult to determine whether discrepancies compared to older reference interval studies depend on different influences of lifestyle factors in the reference populations, or on differences in measurement procedures.

The lifestyle factors explain only a minor part of the total inter-individual variation, and are, in addition, difficult to control for in practice. Therefore, no attempt has been made to create unique reference intervals for subpopulations according to lifestyle factors. The NORIP reference population should be regarded as a representative mixture of the general population also with respect to common lifestyle factors.

It should be noted that these reference intervals do not replace or affect any clinical decision limits, which should be clearly distinguished from reference intervals.

Sampling tubes containing either dry EDTA or EDTA in solution are used among laboratories and have also been used in this study. A solution of EDTA has the advantage of easier mixing with the blood specimens, but when the tube is not filled appropriately with blood, the dilution is appreciable. However, by coating the inside of the tubes with EDTA the problem with poor mixing has been solved. The International Council for Standardization in Haematology (ICSH) now recommends the use of dry dipotassium EDTA [15] as an anticoagulant in the sampling tubes. For this reason we encourage the use of tubes with dry dipotassium EDTA, and have for this study converted all results reported from sites using test tubes with liquid EDTA with the dilution factor, to the expected levels achieved in tubes with dry EDTA. The proposed reference intervals are therefore valid only when sampling tubes with dry EDTA are used.

The variation in results between instrument groups was for most of the constituents small and within the allowable bias (Fig. 1 and

Table III). In a few cases an instrument group had a bias slightly larger than the criteria set. However, these small biases did not justify separate reference intervals for these instrument groups. The instrument variation, therefore, has only a limited widening effect on the reference intervals and common reference intervals were set for all instrument types.

The instruments used by laboratories participating in this study do not exactly reflect the instruments in general use in Nordic health care. The proportion of smaller instruments might be somewhat lower than that seen among participants in external quality assurance (EQA) schemes, because the participants in the NORIP study were mainly larger laboratories. However, no major differences between smaller and larger haematology instruments were identified.

There are no absolute rules on how to decide the number of digits needed to report laboratory results. In this paper the following strategy has been followed to decide the number of digits by which the suggested new reference intervals are expressed in Table V: the normal reference interval should describe the 2.5 and 97.5 percentile limits. To do this with reasonable exactness, three significant digits have to be used for some of the properties, although for clinical practice, haematology results with three digits may seem unnecessarily precise. For other properties, two significant digits are sufficient.

Comments to specific properties

For B-Haemoglobin the different instrument systems agree well in, for example, external quality assurance, confirming the uniform calibration of the measurements. The distribution of results from the NORIP population reported from the various instrument systems in use also agrees very well, supporting the assumption that the samples collected at the different sites in the NORIP project, actually are comparable. The mean level of B-Haemoglobin was positively related to BMI ($+0.3 - +0.4$ g/BMI units, Table IV). Independent from this effect of BMI, the mean level of haemoglobin decreased by age in men, while it increased by age in women. However, age did not fulfil the criteria for partitioning in subpopulations. In the NORIP there were only a small number of results from persons above 75 years of age. It cannot be excluded that outcome of the partitioning criteria had been different for, for example, B-Haemoglobin if the proportion of results from elderly persons had been higher. A significant decline in B-Hb has recently been presented [16] for males in the age group $70 - 88$ years.

Iron deficiency was rare in the investigated reference population and the impact on the calculated reference intervals low.

The inverse deviations for B-MCV and B-Erc noted for the small Sysmex instruments and Cell-Dyn 4000, explain why the B-EVF, as a variable calculated from B-MCV and B-Erc, does not deviate from the overall mean for any of the systems. B-MCV is often claimed to be instrument dependent. It is suggested that possible instrument effects, together with possible effects of different types of EDTA, should be further evaluated, e.g. in the form of external quality assurance.

The mean values of B-MCV increased with age and decreased slightly with increasing BMI. In addition, the well-known effects of both tobacco and alcohol on group mean values for B-MCV were confirmed [17]. For the interpretation of individual results from patients, the impact of these factors is negligible.

Conventionally, reference intervals for the thrombocyte counts are given without separation into gender, although a slight difference has been observed earlier [18]. In this material slightly higher concentrations of thrombocytes were found in females. According to the partitioning criteria, the difference in the lower end is large enough to motivate gender-specific reference intervals.

Clinical impact

Properly defined reference intervals will result in better interpretation of laboratory results. Patients are often examined in different institutions and their data are transferred between these institutions. The risk of misinterpretation of the results and the need for unnecessary retesting will decrease when common reference intervals are used. Likewise, the interpretation of results collected in multicentre trials will be facilitated.

It should be underlined that reference levels are not clinical decision limits, and that existing decision limits do not necessarily have to

change, owing to the change in reference intervals. Existing decision limits and thresholds for second-line testing, might for other reasons, however, have to be revised. Decision levels remain to be defined for specific purposes by the laboratories together with the clinicians. The purpose of this study has not been to suggest or decide on such decision limits.

This study has been restricted to healthy men and non-pregnant healthy women. For other major groups such as children, pregnant women and very old people, other ways have to be found to identify appropriate and useful reference intervals.

ACKNOWLEDGEMENTS

We express our gratitude to all reference individuals for sample donation and to the participating laboratories for help with sample collection and for performing the measurements.

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Received: 22 April 2004
Accepted: 3 May 2004

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