

Normal Ranges of Advanced Clinical Parameters on XN-2000 and Transference of Reference Ranges from XE-2100 to XN-2000

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The advent of automation and constant refinement of technology in the field of hematological cell counters has led to introduction of newer parameters on CBC analyzers. Many advanced clinical parameters (ACP) are being put into routine clinical use and their clinical evaluation and monitoring depend on knowledge of reference ranges. This study was conducted to determine the reference intervals for CBC parameters measured by XN-2000, with emphasis on (ACP) and transference of ranges from XE-2100 to XN-2000 by the method of estimation of 60 samples. The ranges of commonly reported hematology parameters were found to be similar on both analyzers. However, range for IPF was significantly different and hence new range from XN-2000 was adopted.

Key Words

Transference, Reference Range, XN-2000, XE-2100, Advanced Clinical Parameters, CBC.

INTRODUCTION

The advent of automation and constant refinement of technology in the field of hematological cell counters has led to introduction of newer parameters on CBC analyzers. Many advanced ACP are being put into routine clinical use and their clinical evaluation and monitoring depend on knowledge of reference ranges. Establishment of reference ranges for any parameter requires careful planning, control and documentation of large data, thus making it difficult for labs to conduct these studies. Thus the focus is on "Transference of Ranges" from one lab to another or from one analyzer to another within same lab as this requires less effort and less data than establishment of reference ranges. The NCCLS approved guidelines, C28-A describes different ways to validate the "Transference of Ranges": divine judgment, verification with 20 samples, estimation with 60 samples, and calculation from comparative method.¹⁾

AIMS AND OBJECTIVES

The aim of the study was to determine the reference intervals for CBC parameters measured by XN-2000,

with emphasis on ACP and transference of ranges from XE-2100 to XN-2000 by the method of estimation of 60 samples.

MATERIAL AND METHODS

OPD patients under health check category having normal CBC, reticulocyte count, ESR, Serum Iron studies, Vitamin B12 and Folic acid levels were evaluated. Altogether 62 patients (39-M, 23-F) were included in study. The mean / median and ranges of all parameters on XN-2000 were evaluated. The method of "Estimation with 60 samples" was used to validate transference of ranges. Comparison of reportable ACP on XN-2000 and XE-2100 was done for transference of ranges. Statistical analysis was done using SPSS V20.0 software. The reference ranges of all parameter on XN-2000 were evaluated by statistical analysis as explained in **Fig. 1**. Comparison of reportable advanced clinical parameters was done using Z-statistic test (**Fig. 2**), for transference of ranges from XE-2100 to XN-2000. We adopted the reference intervals of XN-2000 if z-test value was more than critical Z value.

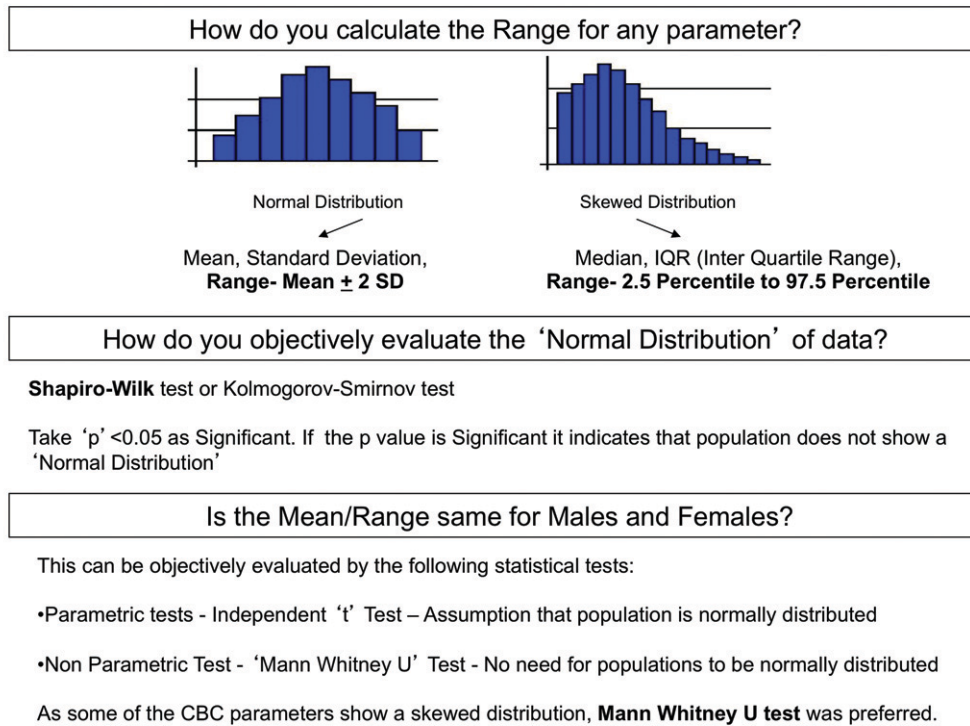


Fig. 1 Summary of statistical tests applied for evaluation of reference ranges

$$Z = \frac{|x_1 - x_2|}{\left[\left(\frac{S_1^2}{n_1} \right) + \left(\frac{S_2^2}{n_2} \right) \right]^{1/2}}$$

Compare the calculated z with a critical value z *

$$z^* = 3\{[(n_1 + n_2)/2]/120\}^{1/2}$$

Fig. 2 Z-test formula

RESULTS

Altogether 62 healthy adult volunteers were evaluated for calculating normal ranges of CBC parameters on XN-2000. The normal distribution for all parameters was analyzed using Shapiro-Wilk test and results were considered significant at $p < 0.05$. Statistical parameters such as Mean, SD, and Range (Mean + 2SD) were calculated when data was normally distributed ($p < 0.05$). Whereas for parameters with skewed distribution ($p > 0.05$), instead of Mean and SD; Median, IQR with percentile based Range (2.5-97.5 percentile) were considered. Normal range of all the XN-2000 parameters is shown in **Table 1 (a)**, **Table 1 (b)**, **Table 1 (c)** and **Table 1 (d)**.

The non-parametric Mann-Whitney U test was applied to see whether there was any significant difference between

male and female subgroups. Null hypothesis was that distribution of data is same in both the subgroups. Separate ranges for males and females for relevant parameters are shown in **Table 2**.

Comparison of means of parameters on XN-2000 was done with published data of parameters on XE-2100²⁾ using Z-statistic test. Z-test formula is shown in **Fig. 2** where X_1 and S_1 are mean and SD of parameters from XN-2000, X_2 and S_2 are mean and SD of parameters on XE-2100 from published data²⁾. Critical Z value = 2.46 was obtained using sample size of $n_1 = 62$ in data of XN-2000 and $n_2 = 100$ from published data of XE-2100. At Z value > 2.46 , the ranges of parameters from XN-2000 were adopted and for Z value < 2.46 the ranges of parameters from XE-2100 were retained. **Table 3** shows parameters for which ranges from XN-2000 were adopted along with Z value.

Table 1 (a) Reference ranges for parameters reported on Sysmex XN-2000

Parameters	N	Shapiro-Wilk test							
		P value	Variance	Mean	SD	Range (Mean ± 2SD)	Median	IQR	Range (2.5-97.5 percentiles)
<i>WBC(10³/μL)</i>	<i>62</i>	<i>0.018</i>	<i>1.384</i>				<i>6.99</i>	<i>1.8000</i>	<i>5.47-9.72</i>
RBC(10 ⁶ /μL)	62	0.828	0.165	4.99	0.40	5.80-4.17			
HGB(g/dL)	62	0.150	1.402	14.53	1.18	16.90-12.16			
HCT(%)	62	0.173	9.259	43.03	3.042	49.11-36.94			
MCV(fL)	62	0.537	9.583	86.34	3.095	92.53-80.15			
MCH(pg)	62	0.880	1.206	29.15	1.09	31.34-26.95			
<i>MCHC(g/dL)</i>	<i>62</i>	<i>0.001</i>	<i>0.676</i>				<i>33.60</i>	<i>1.1500</i>	<i>32.65-35.60</i>
PLT(10 ³ /μL)	62	0.839	3197.206	290.68	56.54	403.76-177.58			
RDW-SD(fL)	62	0.621	5.649	40.65	2.37	45.40-35.89			
RDW-CV(%)	62	0.354	0.218	12.92	0.46	13.86-11.99			
PDW(fL)	62	0.260	2.424	12.06	1.55	15.17-8.95			
MPV(fL)	62	0.381	0.616	10.36	0.78	11.93-8.79			
P-LCR(%)	62	0.351	42.953	27.91	6.55	41.02-14.80			
PCT(%)	62	0.660	0.002	0.28	0.04	0.38-0.18			
NEUT#(10 ³ /μL)	62	0.120	0.693	3.99	0.83	5.65-2.32			
LYMPH#(10 ³ /μL)	62	0.351	0.237	2.38	0.48	3.36-1.41			
MONO#(10 ³ /μL)	62	0.018	0.016	0.55	0.12	0.80-0.29			
EO#(10 ³ /μL)	62	0.213	0.012	0.25	0.11	0.47-0.03			
<i>BASO#(10³/μL)</i>	<i>62</i>	<i>0.010</i>	<i>0.000</i>				<i>0.05</i>	<i>.0300</i>	<i>0.01-0.10</i>
NEUT%(%)	62	0.617	25.635	54.93	5.06	65.06-44.81			
LYMPH%(%)	62	0.408	28.587	33.18	5.34	43.87-22.49			
MONO%(%)	62	0.734	1.932	7.64	1.38	10.42-4.86			
EO%(%)	62	0.354	2.308	3.56	1.51	6.60-0.53			
BASO%(%)	62	0.194	0.075	0.66	0.27	1.21-0.11			

Italic font highlights 2 things;

1. Those values are non-normal in distribution. Hence, median was provided.
2. Those parameters which values exceeded the critical z-score of 2.46. In which case, XN-2000 ranges were adopted.

Table 1 (b) Reference ranges for parameters reported on Sysmex XN-2000

Parameters	N	Shapiro-Wilk test							
		P value	Variance	Mean	SD	Range (Mean ± 2SD)	Median	IQR	Range (2.5-97.5 percentiles)
<i>IG#(10³/μL)</i>	<i>62</i>	<i>0.001</i>	<i>0.000</i>				<i>0.03</i>	<i>.0300</i>	<i>0.01-0.08</i>
IG%(%)	62	0.092	0.040	0.46	0.19	0.86-0.07			
RET%(%)	62	0.052	0.154	1.44	0.39	2.23-0.66			
<i>RET#(10⁶/μL)</i>	<i>62</i>	<i>0.036</i>	<i>0.000</i>				<i>0.06</i>	<i>.0287</i>	<i>0.03-0.12</i>
<i>IRF(%)</i>	<i>62</i>	<i>0.029</i>	<i>8.608</i>				<i>7.70</i>	<i>3.7500</i>	<i>2.67-14.11</i>
<i>LFR(%)</i>	<i>62</i>	<i>0.029</i>	<i>8.608</i>				<i>92.30</i>	<i>3.8</i>	<i>85.88-97.32</i>
MFR(%)	62	0.168	6.201	7.046	2.49	12.02-2.06			
<i>HFR(%)</i>	<i>62</i>	<i>0.000</i>	<i>0.312</i>				<i>0.70</i>	<i>.6250</i>	<i>0.00-2.28</i>
RET-He(pg)	62	0.650	1.277	30.58	1.12	32.84-28.32			
<i>IPF(%)</i>	<i>62</i>	<i>0.001</i>	<i>2.206</i>				<i>2.45</i>	<i>2.3250</i>	<i>0.75-6.75</i>

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Table 1 (c) Reference ranges for parameters reported on Sysmex XN-2000

Parameters	N	Shapiro-Wilk test				
		P value	Variance	Mean	SD	Range (Mean \pm 2SD)
[PLT-I($10^3/\mu\text{L}$)]	62	0.693	2882.064	281.34	53.68	388.70-173.96
[MicroR(%)]	62	0.000	1.124	2.32	1.06	4.44-0.20
[MacroR(%)]	62	0.048	0.130	4.04	0.36	4.76-3.32
[TNC($10^3/\mu\text{L}$)]	62	0.018	1.384	7.23	1.17	9.58-4.88
[WBC-N($10^3/\mu\text{L}$)]	62	0.018	1.384	7.23	1.17	9.58-4.88
[TNC-N($10^3/\mu\text{L}$)]	62	0.018	1.384	7.23	1.17	9.58-4.88
[BA-N#($10^3/\mu\text{L}$)]	62	0.010	0.000	0.048	0.02	0.09-0.00
[BA-N%(%)]	62	0.194	0.075	0.66	0.27	1.21-0.11
[WBC-D($10^3/\mu\text{L}$)]	62	0.057	1.369	7.277419	1.16	9.61-4.93
[TNC-D($10^3/\mu\text{L}$)]	62	0.057	1.369	7.27	1.16	9.61-4.93
[NEUT#($10^3/\mu\text{L}$)]	62	0.126	0.678	3.95	0.82	5.60-2.31
[NEUT%(%)]	62	0.589	25.184	54.46	5.01	64.50-44.43
[LYMP#($10^3/\mu\text{L}$)]	62	0.323	0.236	2.37	0.48	3.35-1.40
[LYMP%(%)]	62	0.495	28.389	33.05	5.32	43.71-22.39
[HFLC#($10^3/\mu\text{L}$)]	62	0.000	0.000	0.009	0.01	0.03-0.01
[HFLC%(%)]	62	0.000	0.033	0.12	0.18	0.49-0.23
[BA-D#($10^3/\mu\text{L}$)]	62	0.007	0.000	0.046	0.018	0.08-0.00
[BA-D%(%)]	62	0.213	0.060	0.63	0.247	1.12-0.14
[NE-SSC(ch)]	62	0.789	9.300	151.58	3.04	157.68-145.48
[NE-SFL(ch)]	62	0.397	4.227	44.80	2.05	48.91-40.69
[NE-FSC(ch)]	62	0.828	5.380	86.93	2.31	91.57-82.29

Table 1 (d) Reference ranges for parameters reported on Sysmex XN-2000

Parameters	N	Shapiro-Wilk test							
		P value	Variance	Mean	SD	Range (Mean ± 2SD)	Median	IQR	Range (2.5-97.5 percentiles)
<i>[LY-X(ch)]</i>	<i>62</i>	<i>0.005</i>	<i>1.909</i>				<i>79.25</i>	<i>2.1250</i>	<i>76.67-83.42</i>
[LY-Y(ch)]	62	0.283	6.500	64.61	2.54	69.71-59.52			
[LY-Z(ch)]	62	0.232	0.587	56.18	.7661	57.71-54.65			
[MO-X(ch)]	62	0.000	2.548	118.28	1.5962	121.47-115.08			
[MO-Y(ch)]	62	0.409	14.579	104.54	3.8183	112.18-96.90			
[MO-Z(ch)]	62	0.000	3.692	63.922	1.9213570	67.76-60.07			
[NE-WX]	62	0.734	144.965	310.77	12.040	334.85-286.69			
[NE-WY]	62	0.708	620.239	614.08	24.905	663.88-564.27			
[NE-WZ]	62	0.033	679.053	608.89	26.059	661.00-556.76			
[LY-WX]	62	0.538	1144.112	487.23	33.825	554.87-419.57			
[LY-WY]	62	0.381	2804.114	876.13	52.954	982.03-770.22			
[LY-WZ]	62	0.886	352.815	480.85	18.783	518.42-443.28			
[MO-WX]	62	0.288	301.045	261.94	17.351	296.63-227.23			
[MO-WY]	62	0.247	7119.195	696.60	84.375	865.34-527.84			
[MO-WZ]	62	0.000	3350.114	561.02	57.880	676.77-445.25			
[RBC-O(10 ⁶ /μL)]	62	0.932	0.147	4.90	0.3838543	5.67-4.14			
[PLT-O(10 ³ /μL)]	62	0.930	2548.069	267.89	50.478	368.84-166.93			
[RBC-He(pg)]	62	0.929	0.936	27.752	0.9675	29.68-25.81			
[Delta-He(pg)]	62	0.036	0.312	2.83	0.5584219	3.95-1.71			
[RET-Y(ch)]	62	0.691	14.367	172.98	3.7904	180.56-165.40			
[RET-RBC-Y(ch)]	62	0.887	12.834	163.08	3.5824640	170.25-155.92			
[IRF-Y(ch)]	62	0.000	29.147	172.64	5.3988384	183.44-161.85			
[FRC#(10 ⁶ /μL)]	62	0.000	0.000	0.00	0.0011166	0.00-0.00			

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Table 2 Ranges of parameters with difference in male and female subgroups

Mann Whitney U test					
Parameter	Significant p value	Males		Females	
		Mean or Median	Range	Mean or Median	Range
RBC($10^6/\mu\text{L}$)	0.000	5.2	5.82-4.58	4.62	5.14-4.10
HGB(g/dL)	0.000	15.22	16.98-13.46	13.36	14.40-12.32
HCT(%)	0.000	44.74	49.38-40.1	40.13	43.19-37.07
MCHC(g/dL)	0.000	34.04	35.72-32.36	33.29	34.31-32.27
PLT($10^3/\mu\text{L}$)	0.044	280.62	392.32-168.92	307.74	417.14-198.34
PCT(%)	0.003	0.27	0.35-0.19	0.31	0.41-0.21
RET-He(pg)	0.047	30.78	33.34-28.22	30.24	31.64-28.84
[MacroR(%)]	0.000	4.2	3.70-4.70	3.78	4.28-3.28
[LY-Y(ch)]	0.013	64.07	69.67-58.47	65.8	61.60-67.06
[LY-Z(ch)]	0.000	55.91	57.25-54.57	56.65	58.03-55.27
[MO-Z(ch)]	0.000	63.4	55.40-66.30	64.86	67.92-61.80
[NE-WX]	0.018	308.18	329.22-287.14	315.17	341.89-288.45
[MO-WX]	0.018	258.08	291.62-224.54	268.48	301.78-235.18
[RBC-O($10^6/\mu\text{L}$)]	0.000	5.1	5.7-4.5	4.57	5.05-4.09
[RET-Y(ch)]	0.046	173.64	182.26-165.02	171.86	176.60-167.12

Table 3 Comparison of parameters on XN-2000 and XE-2100 by Z-statistic test

Parameters	XN-2000 values		XE-2100 values		2-statistic test
	Mean	SD	Mean	SD	
WBC($10^3/\mu\text{L}$)	7.236613	1.1764561	6.447373737	1.445003062	3.797066
HCT(%)	43.032258	3.0428510	44.627	3.712519224	2.975939
MCH(pg)	29.150	1.0984	28.16	1.180566548	5.41731
MCHC(g/dL)	33.764516	0.8222514	32.318	0.820492584	10.89212
RDW-CV(%)	12.927	0.4670	13.46	0.690191014	5.852467
MPV(fL)	10.362903	0.7847384	10.66	0.67	2.473961
IG#($10^3/\mu\text{L}$)	0.035000	0.0177182	0.000416667	0.002477973	15.27657
IG%(%)	0.469	0.1997	0.00625	0.034981198	18.09192
RET%(%)	1.448387	0.3922996	0.944646465	0.312090754	8.568494
IPF(%)	2.722581	1.4852741	2.033928571	0.905794836	3.291036
[Delta-He(pg)]	2.835484	0.5584219	3.098958333	0.735061013	2.579522
[RET-Y(ch)]	172.987	3.7904	170.5541667	4.704262718	3.61466
[RET-RBC-Y(ch)]	163.085484	3.5824640	159.825	4.149698784	5.294774
[IRF-Y(ch)]	172.648387	5.3988384	175.8420455	6.824208456	3.301358

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DISCUSSION

A clinical evaluation and monitoring of ACP depends on knowledge of reference ranges. Present study was conducted to determine the reference intervals for CBC parameters measured by XN-2000, with emphasis on ACP and transference of ranges from XE-2100 to XN-2000 by the method of estimation of 60 samples. A broad spectrum of parameters available with the analyzer XN-2000 was assessed and reference ranges for the same evaluated in the Indian population. Transference of ranges was done from XE-2100 to XN-2000 in our hematology laboratory.

The ranges of commonly reported hematology parameters for which the principle and technology used were same on the both analyzers, showed no difference as expected. However, the range of IPF was significantly different and this is expected as the technology used for measuring IPF on both the machines is different with the XN-2000 using a newer specific dye for measuring fluorescent platelets and IPF. For routine reporting of cases on XN-2000 for the ACP, the newer reference range of IPF was adopted whereas for other parameters the older reference range values were retained.

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