

# An Innovative Method for the Determination of Normal Values in Hematology Using Peer Group Laboratories

JOHN A. KOEPKE, M.D.

*Department of Pathology, University of Iowa,  
Iowa City, Iowa*

Koepke, John A.: An innovative method for the determination of normal values in hematology using peer group laboratories. *Am J Clin Pathol* 70: 577-579, 1978. The author describes a proposed innovative method for the determination of normal values in hematology in which a large number of laboratories participating in the CAP proficiency testing program submit results of a previously performed, complete blood count. These data are compared with those derived from more formal studies of normal values. The advantages of this system, if validated, include its inexpensive simplicity and the possibility of determining normal values for relatively uncommon groups of patients such as neonates, infants, and small children. (Key words: Normal values; Hemoglobin; Leukocyte count; Differential leukocyte count.)

THE DETERMINATION of "normal values" in laboratory medicine has become somewhat of a pre-occupation of laboratory scientists in the last decade. The preliminary identification of the "normal" population to be studied poses the first significant hurdle in such a study. Variables that may affect the data include age, sex, pregnancy, fasting or non-fasting state, smoking, ambulatory or recumbent status, hospitalization, and medications.

In hematology, the problem is compounded by the vital nature of the so-called formed elements, which are variably affected by the anticoagulants used in the collection of the specimen. The life span of these cells is sometimes quite short, and degenerative changes in leukocytes can be seen on smears within a few hours after the specimen is drawn. The osmolality of the anticoagulant solution can affect the sizes of the cells, especially erythrocytes, and must be carefully controlled.

While this list of factors that conceivably could affect values derived from hematology measurements is not encyclopedic, it serves to alert those wishing to determine normal values to pay careful attention to the choice of "normal" subjects, as well as specimen handling.

However, once the raw data are gathered, there is yet another hurdle. This is the statistical manipulation of the data. While traditionally means  $\pm$  two standard deviations have been used for normal range determinations, more recently the central 95 percentile estimate of the group studied has become more popular.

Address reprint requests to Dr. Koepke: Department of Pathology, University of Iowa, Iowa City, Iowa 52242.

In view of the need for better normal-value data, for the newborn and early pediatric age groups in particular, one of the recommendations of a recent College of American Pathologists (CAP) Aspen Conference stated that special efforts should be devoted to this problem.<sup>6</sup>

In the spring of 1977, an innovative method for generating normal values in hematology was tested. The original idea is attributed to a member of the CAP Chemistry Resource Committee. Inserted with the current survey kit was a short questionnaire that requested the survey participant to return anonymously to the CAP Computer Center the results of the most recently performed "CBCs" for a male patient and a female patient between 30 and 39 years old, inclusive. The subject was to have a normal hemoglobin level and a normal total leukocyte count.

## Materials and Methods

The special questionnaire was sent to all laboratories participating in the Comprehensive Hematology (H), Basic (A), and Physician's Office or PEP (L) surveys. These are the surveys in which whole-blood specimens for hematology measurements were included. Each laboratory was asked to list all components of the complete blood count most recently done for a male patient between 30 and 39 years of age and the corresponding count for a female patient in the same age range. The values were to include hemoglobin, hematocrit, erythrocyte count, erythrocytic indices, and total and differential leukocyte counts. In the case of the female patient, the laboratory was to indicate whether or not she was pregnant or whether pregnancy status was unknown.

The data returned were analyzed by survey group and by sex (as well as by pregnancy status in the female group). The analysis included determinations of the means and standard deviations of all data submitted, as well as the same determinations after standard outlier exclusion (data more than three standard deviations

Table 1. Normal Ranges for Hemoglobin\* (Fourth Decade)

Age Group (Years)	Male			Female			Reference
	No.	g/dl		No.	g/dl		
		Mean	Range		Mean	Range	
30-35	55	16.0	14.2-17.8	40	13.9	12.0-15.8	Spaander and Helleman <sup>8</sup>
35-40	46	16.0	14.2-17.8	56	13.8	11.8-15.8	
18-44	1038	15.9	13.5-17.9	2406	13.9	11.5-15.9	HANES <sup>2</sup>
30-40	79	15.5	13.6-17.7	29	13.8	12.1-15.7	Klee <sup>4</sup>
30-39 H <sup>†</sup>	1198	15.4	12.9-17.9	546	13.6	11.8-15.4	This report
30-39 A <sup>†</sup>	2094	15.2	13.1-17.3	427	13.6	11.7-15.5	
30-39 L <sup>†</sup>	161	15.6	13.5-17.5	106	13.7	12.1-15.4	
25-34	183	15.3	13.4-17.2	218	13.3	11.3-15.3	Kelly and Munan <sup>3</sup>
35-44	134	15.3	13.2-17.4	167	13.3	11.0-15.7	
25-49	2711	14.6	12.4-16.9	4326 <sup>‡</sup>	13.0	11.0-15.0	Weatherburn <i>et al.</i> <sup>9</sup>
30-35	55	14.5	Not stated	112	12.6	Not stated	Cruikshank <sup>1</sup>
36-41	51	14.5	Not stated	103	12.7	Not stated	

\* All ranges given as central 95%.

<sup>†</sup> H = Comprehensive Hematology Survey; A = Basic Survey; L = Physician's Office Survey.

<sup>‡</sup> 19-44 years old.

from the mean are discarded and means and standard deviations recalculated). In addition, histograms were prepared so that percentile estimates for all modalities could be made.

For the hemoglobin values, a separate analysis was done to determine mean hemoglobin values for each study group by state. For the leukocyte data, in addition to the relative differential count, the absolute number of each normal leukocyte type was determined using the submitted differential percentages multiplied by the corresponding total leukocyte count.

### Results

The response to our inquiry was quite good: approximately 2,500 questionnaires were returned. When they were received the postmark location was transferred to the worksheets so that the hemoglobin data could be analyzed by location. Other than this, all laboratory data remained anonymous.

A voluminous amount of data was generated in this pilot study and only the highlights, in particular hemoglobin and the leukocyte count normal values, are presented in this paper. It should be noted that examination of the participant summary data from the contemporary surveys showed that they were within the usually acceptable limits. In other words, the participant laboratories seemed to be performing with the expected precision with regard to the hematologic modalities in question.

In Table 1 the means and normal ranges of hemoglobin values for the various study groups are presented. For comparative purposes, data from several normal-value studies are also given. All of these studies have reported normal-range data as the central 95 percentile estimates.

The data generated in the state-by-state analysis of hemoglobin values showed that several of the Rocky Mountain states had mean values significantly higher than corresponding values from the Plains states. This is intriguing in view of the differences in the altitudes of these states.

Table 2 summarizes the total absolute and differential leukocyte count data for the Comprehensive Hematology Survey group. Comparative data, where available from an independent study, are also given in parenthesis.

### Discussion

Age- and sex-adjusted normal values in hematology have been found to be fragmentary and determined in less than optimal fashion. For example, it is now generally accepted that they should be expressed as the mid-95% range rather than the previous practice of the mean  $\pm 2$  standard deviations.

The performance of laboratories in the United States in the measurement of erythrocytic and leukocytic modalities has been shown to be acceptable.<sup>5</sup> These laboratories therefore could be expected to make these measurements accurately in contemporary patient

specimens. When specific patient groups are defined these measurements can be thought to represent a normal population group.

This pilot study was undertaken to determine the feasibility of determining normal values for common hematologic modalities in an innovative way. The results indicate a reasonable correlation between results obtained by this method and several much more tedious methods. While this conclusion is based, in part, on intuitive judgement, the correspondence of the hemoglobin and leukocyte values is quite striking for the most part.

The biases in hemoglobin levels between this study and the studies of Weatherburn and associates<sup>9</sup> and Cruickshank<sup>1</sup> are not easily explained, however. Examination of these two studies indicates appropriate methods and standardization, but calibration errors conceivably could be responsible for the differences.

Comparisons of the normal values for leukocytes with those in the extensive studies of Munan and colleagues<sup>7</sup> show good correlation for lymphocytes, monocytes and eosinophils. For neutrophils this study reveals a positive bias of about a thousand cells. However, it is also of interest that we found very close agreement of the normal ranges for the total leukocyte count. The discrepancies found remain unexplained.

These data conceivably might be improved by eliminating values from laboratories shown to be outliers when their survey data are evaluated in the usual manner. Conversely, the choice of subject might be inappropriate, and larger numbers of abnormals may have been included in this pilot study.

Finally, if the proposed method can be validated, the generation of normal hematologic values for such patients as neonates, infants and other pediatric groups should be able to be done with accuracy. This is especially important in view of the inability of single institutions to acquire statistically significant sample sizes for age-adjusted normal values, especially in the perinatal period.

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Table 2. Normal Ranges for Leukocytes (Fourth Decade)\*

Cell Type	Male	Female	
		Non-pregnant	Pregnant
Segmented neutrophils			
Mean	4,790 (3,800)	4,670 (3,500)	6,200
Range	2,210-7,870 (2,000-6,400)	2,340-7,350 (2,000-6,100)	2,070-10,740
Band neutrophils			
Mean	170	150	210
Range	0-840	0-680	70-1,390
Lymphocytes, normal			
Mean	2,420 (2,600)	2,470 (2,400)	2,450
Range	770-4,030 (1,500-4,100)	900-4,030 (1,400-3,800)	0-4,410
Lymphocytes, reactive			
Mean	20	30	70
Range	0-200	0-220	0-590
Monocytes			
Mean	280 (360)	240 (290)	210
Range	0-910 (60-950)	0-760 (70-720)	0-740
Eosinophils			
Mean	170 (180)	140 (150)	140
Range	0-580 (60-540)	0-490 (50-510)	0-440
Basophils			
Mean	20	20	10
Range	0-160	0-160	0-100
Total leukocytes			
Mean	7,530 (7,100)	7,380 (6,600)	8,840
Range	4,900-10,700 (4,600-11,000)	4,800-10,100 (4,800-9,900)	5,800-12,700

\* Normal values per Munan *et al.*<sup>7</sup> in parentheses.

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