Canine Hematology and Biochemistry Reference Values

J.H. Lumsden, K. Mullen and B.J. McSherry*

ABSTRACT

Reference hematology and biochemistry values for 53 variables are presented from 51 clinically healthy dogs, 26 female and 25 male, approximately six to 24 months of age and of mixed breed. These dogs were sampled because of their good health status and the opportunity to collect the volume of blood required to complete the variable analysis of interest. Collection of blood specimens and laboratory analysis was done in a standard described manner, the latter including a continuing quality control program.

For each variable the data were examined for homogeneity and when present, outliers (n=9) were excluded. Parametric analysis was used to calculate the reference interval for those variables which had a Gaussian distribution or could be changed to a Gaussian distribution by any of four transformations. For those variables in which Gaussian distribution was not present or attained, nonparametric analysis was used. Due to the small size of the population sample, the uncertainty of breed and the exact age of each dog, breed, age and sex effects were not examined.

Reference values should be used to assist interpretation of observations obtained from an animal or animals of comparable origin, i.e. similar subpopulation, and only if the same laboratory techniques are followed. Until each laboratory is able to generate reference values using adequate sample size and current methodology for the numerous subpopulations of interest, reference intervals such as these are useful to clinicians and researchers.

RÉSUMÉ

Cette expérience visait à colliger des valeurs de référence relatives à 53 paramètres hématologiques et biochimiques. On utilisa à cette fin 51 chiens croisés et cliniquement sains, 25 mâles et 26 femelles, dont l'âge variait de six mois à deux ans. On utilisa ces chiens parce qu'ils étaient en bonne santé et qu'on pouvait prélever la quantité de sang requise pour effectuer une étude exhaustive des paramètres choisis. Les prélèvements de sang et les analyses de laboratoire s'effectuèrent selon des données standards impliquant un programme continuel de vérification qualitative.

On porta une attention spéciale à l'homogénéité des données relatives à chacun des paramètres, en excluant les extrêmes (n=9). On utilisa l'analyse paramétrique pour calculer l'intervalle de référence relatif aux paramètres qui présentaient une distribution de Gauss ou qu'on pouvait ordonner selon cette distribution, par l'une ou l'autre de quatre transformations appropriées. On utilisa par ailleurs l'analyse non paramétrique pour les paramètres qui ne présentaient pas la distribution de Gauss ou qu'on ne pouvait ordonner selon cette distribution. Vu le nombre relativement peu élevé d'animaux utilisés, ainsi

*Department of Pathology (Lumsden and McSherry) and Department of Mathematics and Statistics (Mullen), University of Guelph, Guelph, Ontario N1G 2W1.

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que l'incertitude relative à la race et à l'âge de chacun d'eux, on ne s'attarda pas aux effets de la race, de l'âge ou du sexe.

Il convient d'utiliser des valeurs de référence, lors de l'interprétation des résultats obtenus chez un ou plusieurs animaux d'origine comparable, i.e. faisant partie d'une sous-population similaire, à condition d'utiliser cependant les mêmes techniques de laboratoire. D'ici à ce que chaque laboratoire dispose de valeurs de référence résultant de l'analyse d'un nombre adéquat d'échantillons et de l'utilisation d'une méthodologie courante, portant sur les nombreuses sous-populations qui présentent de l'intérêt, des valeurs de références telles que celles que les auteurs nous présentent revêtent une utilité pour les cliniciens et les chercheurs.

INTRODUCTION

Hematology and biochemistry observations from an individual or a group of animals are traditionally compared to reference intervals developed from a corresponding population of animals using similar laboratory techniques. The observations are considered normal if they fall within the quoted reference interval. This concept is theoretical and too simplistic for clinical diagnostic purposes for a variety of reasons. History, clinical signs, disease incidence, etc must be weighed together with the laboratory data (9, 31).

For a few diseases in man retrospective or prospective studies have generated information regarding both the sensitivity and specificity of a test which taken together with the prevalence of disease in the population allows calculation of the likelihood of a particular disease existing for various test results, i.e. predictive value (11). These predictive values will be generated even more slowly in veterinary medicine even though they may exist in a related form in the mind of the experienced clinician (31).

Although ideal, it is often not practical or even possible for each veterinary laboratory to develop reference values of adequate sample size for each subpopulation of interest. Reference values are influenced by changes in methodology such as time or temperature of reaction, substrate used, instrumentation, etc. The confidence in the lower and upper limits of the reference interval is influenced by the size of the population sample, the statistical analysis of the data and the variation inherent within each laboratory method monitored as part of the quality control program (9, 14,16,17,28,29).

These hematology and biochemistry reference values developed for use in a veterinary teaching hospital are presented for judicious use by clinicians and researchers interpreting canine data determined by similar laboratory methods.

MATERIALS AND METHODS

Blood samples were obtained from 26 female and 25 male, clinically healthy, six to 24 month old mongrel and purebred dogs which had been immunized against distemper and hepatitis, treated for parasites where indicated and fed a balanced diet in a preconditioning unit for four to six weeks prior to testing. Blood samples were taken from ten to 12 animals weekly between 0800 of 0900 hours at least 15 hours postprandially but while having free-choice water. Thirty ml of blood was collected from the jugular vein through a 20 gauge collection needle directly into vacuum tubes[1] with and without the following anticoagulants: potassium ethylenediaminetetraacetic acid (K EDTA), ammonium heparin, sodium oxalate or sodium fluoride with oxalate. The serum was allowed to separate up to two hours at room temperature. Serum and plasma were harvested by double centrifugation for 15 minutes each at 3000 rpm. The heparin plasma was separated immediately and an aliquot stored at —20°C until tested.

Leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using a Coulter Model S[2]. The packed cell volume was confirmed by a microhematocrit technique[3]. Air-dried

[1]Becton, Dickinson and Co., Clarkson, Ontario.

[2]Coulter Electronics of Canada Ltd., Mississauga, Ontario.

[3]Clay Adams Microhematocrit, Canlab Supplies, Toronto, Ontario.

smears were stained using an automated stainer[4]. Reticulocytes were vital stained for ten minutes using equal parts of K EDTA blood and 0.1% brilliant cresyl blue, then air-dried smears were counterstained using Wright's stain[4]. The number of reticulocytes for 1000 erythrocytes were counted using a 1:9 Miller type ocular disc (3). The rate of erythrocyte sedimentation (ESR) at one hour was observed for K EDTA blood in disposable Wintrobe tubes. Platelets were counted in K EDTA blood within two hours of sample collection using Unopette[1] diluting pipettes and Nebauer counting chambers[5]. Leukocytes were differentiated according to the description of Schalm (23).

Serum calcium and magnesium concentrations were determined in lanthanum chloride dilutions read at 422.7 nm and 285.2 nm respectively using an atomic absorption spectrophotometer[6] (26). Serum phosphorus content was estimated following the reduction of phosphomolybdate to stable molybdenum blue complex and read at 700 nm (13). Sodium and potassium concentration in heparinized plasma were measured by flame emission[7] using an internal lithium standard. Heparin plasma chloride content was determined by coulometric dilution[8]. Osmolality was determined by freezing point depression[9].

The o-toluidine method as described by Feteris (10) was used for determination of plasma glucose as preserved in sodium fluoride. Blood urea nitrogen (BUN) concentration was determined using the modified diacetyl monoxime thiosemicarbazide method at 520 nm (7). The modified Jaffe reaction was used to determine serum creatinine content (21). Total cholesterol[10] was determined according to the method of Wybenga *et al* (31). Direct, indirect and total bilirubin were determined according to the method of Jendrassik as modified by Nosslin[11] (19). Serum iron and iron binding capacity were determined using the Ferrochek II system[12].

Total protein was determined in K EDTA plasma using a hand refractometer[13] and in serum using the manual biuret reaction (27). The serum proteins were separated on agarose gel by electrophoresis at pH 8.6[14], stained with amido black 10 B and quantitated by transmission densitometry[15]. Fibrinogen was determined by measuring the thrombin time of oxalated plasma[16].

Cortisol was measured by fluorometery[17] according to the method of Breznock and McQueen (4), thyroxine (T_4) by competitive protein binding[18] and triiodothyronine % uptake (T_3 Uptake) by a resin uptake procedure[19].

Serum aspartate-aminotransferase (ASAT or SGOT formerly) and serum alanine-aminotransferase (ALAT or SGPT formerly) activity were determined according to the modified method of Karmen (16) and Wroblewski and LaDue (30) respectively using GOT or GPT-combination kits[20] at 30°C in an LKB Model 8600 Reaction Rate Analyzer[21]. Serum lactate dehydrogenase activity (LDH) was assayed using an LDH-L Test Combination kit[20] at 30°C as described by Gay (12). Serum alkaline phosphatase activity (AlkP) was determined according to Babson (2) using the Phosphatrate Alkaline kit[22] at 37°C measured at 550 nm. The starch iodine colorimetric amylase method of Caraway[23] (5) was used at 37°C and color change read at 660 nm.

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[4]Ames Hema-tek Slide Stainer, Fisher Scientific Co. Ltd., Toronto, Ontario.

[5]Spencer Bright-Line Improved, Canlab Supplies, Toronto, Ontario.

[6]Techtron AA5, Varion Assoc. of Canada Ltd., Georgetown, Ontario.

[7]Flame Photometer Model 143, Instrumentation Laboratory Incorp., Fort Lee, New Jersey.

[8]Buchler-Cotlove, Chloridometer, Buchler Instruments Incorp., Fort Lee, New Jersey.

[9]Advanced Osmometer Model 3D, Advanced Instruments Incorp., Needham Heights, New Jersey.

[10]Dow Chemical Co., Indianapolis, Indiana.

[11]Jendrassik Bilirubin Set, American Monitor Corp., Indianapolis, Indiana.

[12]Hyland Division, Travenol Lab. Incorp., Costa Mesa, California.

[13]American Optical Corp., Buffalo, New York.

[14]Corning ACI, Palo Alto, California.

[15]Clifford Electrophoresis Densitometer, Clifford Instruments Incorp., Natick, Massachusetts.

[16]Fibrometer Coagulation Timer, Becton, Dickinson and Co., Clarkson, Ontario.

[17]Turner Fluorometer, Model 111, G K Turner Assoc., Palo Alto, California.

[18]Tetrasorb-125, Abbott Laboratories, Chicago, Illinois.

[19]Trisorb-125, Abbott Laboratories, Chicago, Illinois.

[20]Boehringer Mannheim, Ville St. Laurent, Quebec.

[21]LKP Produkter AB, Brommal, Sweden.

[22]General Diagnostics, Toronto, Ontario.

[23]Harleco Amylase Reagent Set, Harleco, Gibbstown, New Jersey.

Serum lipase activity was determined using olive oil substrate incubation at 37°C[24] and measured titrimetrically (25). Creatine phosphokinase (CPK) activity was determined using a CPK activated UV kit[20] at 30°C according to Rosalki (22) with reaction rate measurement[25]. Gamma glutamyl transferase activity was determined using a kit method[20] according to the method described by Szasz (24).

The values obtained for each variable were examined for outliers and homogeneity. Parametric analysis was used to calculate the deciles and the 2.5 and 97.5 percentiles where data was initially or successfully transformed to Gaussian distribution, otherwise nonparametric analysis was used as previously described (18).

The reference values have been presented using the Système International Units expected to be initially adopted in 1979 by Canadian medical laboratories.

RESULTS

The original data was based on observations from 51 dogs except for some variables where insufficient sample volume or technical workload did not allow analysis within the predetermined time limits. The values obtained for nine variables, maximum of one in any dog, were classed as outliers and removed from subsequent analysis: platelet count of 830 x 10^9/l, ESR at 2, 3 and 5 mm/h, magnesium value of 1.1 mmol/l, plasma sodium of 164 mmol/l, blood urea nitrogen of 8.18 mmol/l, unconjugated bilirubin of 8.5 mmol/l and ASAT of 76 units/l.

The lower and upper limits of 95% of the population variables are presented as the 2.5 and 97.5 percentiles in Tables I, II and III. The tables also contain the mean values, standard deviations, the number of observations included and the distribution or transformation of the data for parametric analysis when the observations were initially observed to be, or transformed to Gaussian distribution, or the use of nonparametric analysis when the observations were nongaussian with the four transformations examined.

Daily internal and bimonthly external quality control samples were used routinely. The analytical bias for most methods is presented for ready clinical interpretation as the standard deviation observed for one level, generally the high normal range, within lot, day to day variation of one of the quality control samples ($n > 30, < 50$).

DISCUSSION

The hematology and biochemistry values presented include most of the tests of interest in a routine clinical pathology laboratory. The population sample is smaller than the minimum 80 required to calculate the 0.95 tolerance interval with confidence of 0.90 probability, capable with parametric analysis of data having gaussian distribution. The 2.5 and 97.5 percentile interval can be justified for the sample size presented (n=51), if parametric analysis can be used. If nonparametric analysis must be used, i.e. the data is nongaussian even after transformation, the population sample should be much larger and preferably greater than 120 (16), otherwise the 2.5 and 97.5 percentiles are essentially the lowest and highest values observed (18,20). The laboratory tests should be done in duplicate for these extreme observations (16), impossible if data are examined retrospectively as occurred with these samples.

A defined source of clinically healthy dogs from which the required volume of blood can be withdrawn and processed in a standard manner is not always readily available. The population sample used is as large or larger than for many reference values in current veterinary texts where gaussian distribution of the data is assumed or where lower and upper limits for the variables can not be readily calculated (6,23). With increasing use of automated instrumentation, decreased volume requirements and technical input, reference values from larger population samples are becoming available. Recommendations con-

[24]Sigma Lipase Kit, Sigma Chemical Co., St. Louis, Missouri.

[25]Unicam SP 500 2 UV-Visible Spectrophotometer with AR Linear/Log 0.5 Decade Recorder, Unicam Instruments Ltd., Cambridge, England.

TABLE I. Canine Hematology Reference Intervals[a]

		2.5	97.5[b]	$\bar{x}$	SD	n	d[c]	SD[d]
B-Hemoglobin.....	g/l	126	194	160	17	51	G	1
B-Hematocrit.....	%	36.9	55	46.0	4.6	51	NP	0.6
B-Erythrocytes....	$x10^{12}/l$	5.5	8.2	6.8	0.7	51	G	0.09
B-Mcv.....	fl	62	70	66	2	51	G	0.8
B-MCH.....	pg	22	25	23.2	0.8	51	NP	0.8
B-MCHC.....	g/l	330	360	348	8	51	NP	5
B-Reticulocytes...	%	0.1	2.4	0.6	0.6	42	$\sqrt{x}$	
B-ESR.....	mm/h	0	1	0.2	0.4	40	NP	
P-Protein.....	g/l	5.8	7.6	6.7	0.4	48	G	
B-Platelets.....	$x10^9/l$	80	560	320	120	50	G	
B-Leukocytes.....	$x10^9/l$	6.6	18.4	12.5	3.0	51	G	0.1
B-Neutrophils								
Segmented....	$x10^9/l$	3.9	12	7.4	0.1	51	G	
	%	44.5	76	60.2	8.0	51	G	
Band.....	$x10^9/l$	0	1	0.2	0.3	51	$\sqrt{x}$	
	%	0	5	1.5	1.6	51	$\sqrt{x}$	
B-Lymphocytes...	$x10^9/l$	0.8	5.6	3.2	1.2	51	G	
	%	10.4	40.6	25.5	7.7	51	G	
B-Monocytes.....	$x10^9/l$	0.1	1.8	0.9	0.5	51	G	
	%	1	14.3	7.6	3.4	51	G	
B-Eosinophils.....	$x10^9/l$	0	1.9	0.5	0.5	51	$\sqrt{x}$	
	%	0	14.5	3.6	3.7	51	$\sqrt{x}$	
B-Basophils.....	$x10^9/l$	0	0.2	0	0	49	NP	
	%	0	1	0.04	0.2	51	NP	

[a]Conditioned male and female dogs of age range six to 24 months
[b]Determined 2.5 and 97.5 percentile interval
[c]Distribution of data, transformation used or method of analysis:
G = gaussian; $\sqrt{x}$ = square root transformation; NP = nonparametric
[d]Analytical bias (1 SD) observed for one level of control sample, generally high normal range ($n > 30, < 50$)

cerning the collection of reference values (1), use of statistical terminology (9) and statistical analysis (16,18) should be given due consideration.

Reference intervals for specific tests become outdated with changes in laboratory methods. Within this laboratory, reticulocytes are now reported in absolute values instead of percentage of erythrocytes, triiodothyronine is not used and thyroxine and cortisol are determined by radioimmunossay techniques.

The reference intervals presented in the tables have been reported in the SI units expected to be adopted by Canadian medical laboratories during 1979, e.g. hemoglobin in g/l instead of mmol/l, enzymes in a modified international unit (units/l) retaining the same numerical values for IU instead of converting to ukat/l. These reference intervals are available from the authors reported in the traditional units and will be revised using additional SI recommendations as finally adopted by the medical laboratories.

There are reference intervals presented for some variables which may be questioned by the reader. Reexamination of the sample collection and handling, standards and control values during laboratory testing procedures and statistical analysis supports the values reported. Physiological effects such as severe dehydration could influence the plasma sodium and osmolality but should also be reflected in the hematocrit and plasma or serum proteins (6), unlikely with water supplies replenished in individual cage bowls two or three times a day. The low serum iron and high iron binding capacity in some individual dogs would indicate at least relative iron deficiency not manifest in the erythrocyte values, a condition not uncommon in growing animals.

For application in a small laboratory, reference values such as these should be compared with observations from a minimum number of clinically healthy animals (e.g. $n > 10$). If the mean and range are similar one may cautiously adopt the reference intervals published until variables from a larger number of animals can be determined and specific in-house reference intervals created.

TABLE II. Canine Biochemistry Reference Intervals[a]

		2.5	97.5[b]	$\bar{x}$	SD	n	d[c]	SD[d]
S-Calcium	mmol/1	2.52	3.00	2.75	0.13	51	G	0.05
S-Phosphorus	mmol/1	1.07	2.81	1.94	0.45	51	G	0.1
Ca/Phos		1.3	3.1	1.8	0.5	51	1/x	
S-Magnesium	mmol/1	0.79	1.06	0.93	0.09	50	G	0.02
P-Sodium	mmol/1	143	158	150	3.7	46	G	1.2
P-Potassium	mmol/1	3.7	4.7	4.2	0.2	46	G	0.1
Na/K		32	40	36	2.2	46	G	
P-Chloride	mmol/1	104	116	110	3.1	46	G	0.9
P-Osmolality	mmol/1	295	315	305	5	50	G	2.9
B-Urea Nitrogen	mmol/1	3.6	18.6	11.4	4.3	50	G	1.0
S-Creatinine	umol/1	44.2	97.2	70.7	17.7	51	G	5.3
BUN/Creatinine		9.0	34.3	19.2	6.6	51		
P-Glucose	mmol/1	3.66	5.66	4.66	0.5	51	G	0.21
S-Cholesterol	mmol/1	2.54	6.76	5.15	1.06	51	G	0.15
S-Bilirubin								
Total	umol/1	3.4	13	6.8	1.7	51	NP	1.7
Unconjugated	umol/1	0	5.1	1.7	1.7	50	NP	
Conjugated	umol/1	1.7	8.6	5.1	1.7	51	NP	
Unconj./Conj.		0	2.6	0.5	0.7	50	NP	
S-Iron	umol/1	3	64	34	15	51	G	1
S-Iron binding capacity	umol/1	55	84	69	8	51	G	2
S-Iron binding saturation	%	16	98	48	21	51	NP	
S-Triiodothyronine uptake	%	41	53	47	3.2	47	G	
S-Thyroxine	nmol/1	12	53	32	10	51	G	7
P-Cortisol	nmol/1	19	386	196	91	51	G	36

[a]Conditioned male and female dogs of age range six to 24 months
[b]Determined 2.5 and 97.5 percentile interval
[c]Distribution of data, transformation of data or method of analysis:
G = gaussian; 1/x = inverse transformation; NP = nonparametric
[d]Analytical bias (1 SD) observed for one level of control sample, generally high normal range ($n > 30, < 50$)

TABLE III. Canine Biochemistry Reference Intervals[a]

		2.5	97.5[b]	$\bar{x}$	SD	n	d[c]
S-Transaminases:							
Aspartate-amino (ASAT)	units/1(30C)	7	18	13	2.9	51	G
Alanine-amino (ALAT)	units/1(30C)	3	20	10	4.6	50	x
S-Alkaline phosphatase	units/1(37C)	9	56	32	11.7	51	G
S-Gamma glutamyl transferase	units/1(30C)	0.1	4.5	2.3	1.1	51	NP
S-Lactate dehydrogenase	units/1(30C)	8	139	54	35	51	$\sqrt{x}$
S-Amylase Caraway	units/1(37C)	38	77	56	10	51	G
S-Lipase Sigma Tietz	units/1(37C)	0.1	0.7	0.3	0.2	51	$\sqrt{x}$
S-Protein[d]	g/1	52	69	60	4	51	G
S-Albumin	g/1	24	36	30	3	51	G
S-Globulin	g/1	24	37	31	3	51	G
Albumin/globulin		7	13	10	2	51	G
S-Alpha 1 globulin	g/1	5	8	7	1	51	NP
S-Alpha 2 globulin	g/1	5	8	6	1	51	NP
S-Beta 1 globulin	g/1	5	11	8	2	51	G
S-Beta 2 globulin	g/1	3	7	5	1	51	NP
S-Gamma globulin	g/1	3	8	5	1	51	NP
P-Fibrinogen	umol/1						

[a]Conditioned male and female dogs of age range six to 24 months
[b]Determined 2.5 and 97.5 percentile intervals
[c]Distribution of data, transformation or method of analysis:
G = gaussian; $\sqrt{x}$ = square root transformation; NP = nonparametric
[d]Analytical bias (1 SD = 1.6 g/1) for protein method observed at the 65 g/1 control sample level ($n > 30, < 50$)

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REFERENCES

1. **ALSTROM, T., R. GRASBECK, M. HJELM and S. SKANDSEN.** Recommendations concerning the collection of reference values in clinical chemistry and activity report. Scand. J. clin. lab. Invest. **35**: 1-4. 1975.
2. **BABSON, A.L., S.J. GREELEY, C.M. COLEMAN and G.E. PHILLIPS.** Phenolphthalein monophosphate as a substitute for serum alkaline phosphatase. Clin. Chem. **12**: 482-490. 1966.
3. **BRECHER, G. and M. SCHNIEDERMAN.** A time-saving device for the counting of reticulocytes. Am. J. clin. Path. **20**: 1079-1083. 1950.
4. **BRESNOCK, E.M. and R.D. McQUEEN.** Rapid fluorometric analysis of plasma corticosteroids as a test of adrenocortical function in the dog. Am. J. vet. Res. **30**: 1523-1533. 1969.
5. **CARAWAY, W.T.** A stable starch substrate for the determination of amylase in serum and other body fluids. Am. J. clin. Path. **32**: 97-99. 1959.
6. **COLES, E.H.** Veterinary Clinical Pathology, 2nd Ed. Philadelphia: W.B. Saunders Company. 1974.
7. **CROCKER, C.** Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. med. Technol. **33**: 361-365. 1967.
8. **DYBKAER, R.** Concepts and nomenclature in theory of reference values. Scand. J. clin. lab. Invest. Suppl. **126**. 1972.
9. **DYBKAER, R., K. JORGENSENZ and J. NYBOE.** Statistical terminology in clinical chemistry reference values. Scand. J. clin. lab. Invest. Suppl. **144**. 1975.
10. **FETERIS, W.A.** A serum glucose method without protein precipitation. Am. J. med. Technol. **31**: 17-21. 1965.
11. **GALEN, R.S. and S.R. GAMBINO.** Beyond Normality: The Predictive Value and Efficiency of Medical Diagnosis. Toronto: John Wiley and Sons. 1975.
12. **GAY, R.J., R.B. McCOMB and G.N. BOWERS.** Optimum reaction conditions for the human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. Clin. Chem. **14**: 740-753. 1968.
13. **GOLDENBURG, H. and A. FERNANDEZ.** Simplified method for the estimation of inorganic phosphorus in body fluids. Clin. Chem. **12**: 871-882. 1966.
14. **GRAMS, R.R., A. JOHNSON and E.S. BENSON.** Laboratory data analysis system. Am. J. clin. Path. **58**: 177-219. 1972.
16. **KARMEN, A., F. WROBLEWSKI and J.S. LaDUE.** Transaminase activity in human blood. J. clin. Invest. **34**: 126-133. 1955.
17. **KRAUSE, R.D., V.D. ANAND, H.D. GRUEMER and T.A. WILLKE.** The impact of laboratory error on the normal range: a bayesian model. Clin. Chem. **21**: 321-324. 1975.
18. **LUMSDEN, J.H. and K. MULLEN.** On establishing reference values. Can. J. comp. Med. **42**: 293-301. 1978.
19. **MICHAELSSON, M.** Bilirubin determination in serum and urine. Scand. J. clin. lab. Invest. **13**: Suppl. 56, 1-79. 1961.
20. **MURPHY, R.B.** Non-parametric tolerance intervals. Ann. math. Statistics **19**: 88-94. 1948.
21. **OWEN, J.A., B. IGGO, F.J. SCANDVETT and C.P. STEWART.** Determination of creatinine in plasma or serum, and in urine: Critical examination. Biochem. J. **58**: 226-237. 1954.
22. **ROSALKIE, S.B.** An improved procedure for serum creatine phosphokinase determination. J. Lab. clin. Med. **69**: 696-705. 1967.
23. **SCHALM, O.W., N.C. JAIN and E.J. CARROLL.** Veterinary Hematology, 3rd. Ed. Philadelphia: Lea and Febiger. 1975.
24. **SZASZ, G.** A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clin. Chem. **15**: 124-136. 1969.
25. **TIETZ, N.W. and E.A. FIERECK.** A specific method for serum lipase determination. Clinica Chim. Acta **13**: 352-358. 1966.
26. **TRUDEAU, D.L. and E.F. FREIER.** Determination of calcium in urine and serum by atomic absorption spectrophotometry (AAS). Clin. Chem. **13**: 101-114. 1967.
27. **WEICHSELBAUM, T.E.** Accurate and rapid method for determination of proteins in small amounts of blood serum and plasma. Am. J. clin. Path. **16**: 40-49. 1946.
28. **WELDON, K.L. and J.S. MacKAY.** On improving the diagnostic value of quantitative medical laboratory tests in conventional practice. J. chron. Dis. **25**: 683-696. 1972.
29. **WERNER, M. and W.L. MARSH.** Normal values: theoretical and practical aspects. CRC Crit. Rev. Clin. Lab. Sci. **6**: 81-100. 1975.
30. **WROBLEWSKI, F. and J.S. LaDUE.** Serum glutamic pyruvic transaminase (SGP-T) in hepatic disease: preliminary report. Ann. intern. Med. **45**: 801-811. 1956.
31. **WYBENGA, D.R., V.J. PILEGGI, P.H. DIRSTINE and J. DiGIORGIO.** Direct manual determination of serum total cholesterol with a single stable reagent. Clin. Chem. **16**: 980-984. 1970.