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Reference Intervals of Common Clinical Chemistry Analytes for Adults in Hong Kong

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Key words

Reference Intervals, Reference Ranges, CLSI/IFCC C28.

List of Abbreviations:

PYNEH = Pamela Youde Nethersole Eastern Hospital
ALT = Alanine Transferase
AST = Aspartate Transferase
Alk Phos = Alkaline Phosphatase
Chol = Cholesterol
CK = Creatine Kinase
D-HDL = Direct-High Density Lipoprotein
GGT = Gamma-glutamyl Transferase
LD = Lactate Dehydrogenase
TIBC = Total Iron Binding Capacity

Declarations

Conflict of Interest Declaration: David A. Armbruster is an employee of Abbott Diagnostics
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Abstract

Background. Defining reference intervals is a major challenge because of the difficulty in recruiting volunteers to participate and testing samples from a significant number of healthy reference individuals. Historical literature citation intervals are often suboptimal because they're be based on obsolete methods and/or only a small number of poorly defined reference samples.
Methods. Blood donors in Hong Kong gave permission for additional blood to be collected for reference interval testing. The samples were tested for twenty-five routine analytes on the Abbott ARCHITECT clinical chemistry system. Results were analyzed using the Rhoads EP evaluator software program, which is based on the CLSI/IFCC C28-A guideline, and defines the reference interval as the 95% central range.
Results. Method specific reference intervals were established for twenty-five common clinical chemistry analytes for a Chinese ethnic population. The intervals were defined for each gender separately and for genders combined. Gender specific or combined gender intervals were adapted as appropriate for each analyte.
Conclusion. A large number of healthy, apparently normal blood donors from a local ethnic population were tested to provide current reference intervals for a new clinical chemistry system. Intervals were determined following an accepted international guideline. Laboratories using the same or similar methodologies may adapt these intervals if deemed validated and deemed suitable for their patient population. Laboratories using different methodologies may be able to successfully adapt the intervals for their facilities using the reference interval transference technique based on a method comparison study.

Introduction

The practice of clinical laboratory medicine requires that test results be assessed to determine if the patient is "normal" or is subject to some pathological condition. Thus it's imperative that laboratories report test results along with reference intervals, in the past, typically called "normal ranges." Establishing reference intervals has always been a challenge as significant differences may exist in disease frequencies; biological variation in analytes among ethnic groups, genders and ages; specimen collection techniques; test performance; test interpretation; and other factors. Physicians rely on the availability of appropriate and reliable reference intervals to accurately interpret laboratory test results combined with data collected during medical interview and clinical examination. Although health professionals understand the importance of reference intervals, many laboratories still do not have comprehensive data, especially ranges that are specific for their typical patient populations. There continues to be significant gaps in the available reference intervals as frequently intervals cited in the literature were obtained using older methodologies and instrumentation and cover a limited range of age groups or a relatively small number of samples.
Ferruccio Ceriotti, a former chair of the IFCC Committee for Reference Intervals and Decision Limits (C-RIDL), noted that "The theory of reference values was developed more than 30 years ago, but its application in most clinical laboratories is still incomplete today." and that "This is due to the fact that obtaining a 'good' reference interval is a very demanding activity, in terms of time, money and knowledge" (1). Cerriotti also noted that "The time, effort and money required to establish reference intervals are large and clinical laboratories are disinclined to modify reference intervals as this is a demanding task also requiring education of clinicians and patients. Large multicentre studies are needed … to make real progress in this field and bridge the large gap now existing between a very nice theory (IFCC and CLSI documents) and a very poor practice." Between 1987 – 1991, the IFCC published a series of 6 seminal papers providing detailed guidance for establishing reference intervals (2-7). The Clinical and Laboratory Standards Institute (CLSI, previously NCCLS) first published the C28 Guideline (Defining, Esishing, and verifying reference intervals in the clinical laboratory) in 1995 and the current edition of the guideline, C28-A3, in 2008 (8) The C28-A3 is a joint CLSI/IFCC document and incorporates the series of IFCC papers. C28 recommends the "direct approach" in which reference individuals are vetted to ensure that they are "healthy" and discourages the "indirect approach" in which existing data is used to establish ranges by retroactively identifying acceptable reference populations. Friedberg et al. noted that a College of American Pathologists (CAP) survey of 500 laboratories in 2001 found that 390 (78%) adopted manufacturers' values for reference intervals (9). In their own survey of 163 laboratories, about half reported conducting an internal study of healthy subjects to establish reference intervals. Of those laboratories, about one half tested between 21 – 50 samples and one quarter tested more than 100 samples. It is not clear how many of these labs followed an accepted guideline such as C28.
We report here reference intervals established for Hong Kong (Chinese) blood donors for twenty-five routine clinical chemistry analytes.

Materials and Methods

Assays

Table 1 lists the assays included in the study. All tests were performed following the manufacturer's package inserts for Abbott reagents on the Abbott ARCHITECT c16000 analyzer (Abbott Diagnostics, Chicago, IL, USA). Calibrator traceability and measurement uncertainty information for all assays were provided by the manufacturer (i.e., traceability to reference materials and/or methods as per ISO 17511). They represented routine clinical chemistry assays commonly performed at the Pamela Youde Nethersole Eastern Hospital (PYNEH). The purpose of this work was to develop reference intervals for these key analytes that reflected the hospital's patient population after the ARCHITECT became the test of record analytical system.

Table 1
Analytes included in the study. All assays were performed using reagents specific for the Abbott ARCHITECT c16000 analyzer

Albumin BCG	Alanine Transferas (ALT)	Alkaline Phosphatase (Alk Phos)	Amylase	Aspartate Transferase (AST)
Calcium (Ca)	Cholesterol (Chol)	Chloride (Cl)	Creatine Kinase (CK)	Creatine Kinase MB Isozyme(CKMB)
Creatinine (alkaline picrate Jaffe)	Direct High Density Lipoprotein (D-HDL)	Iron (Fe)	γ-Glutamyl Transferase (GGT)	Potassium (K)
Lactate Dehydrogenase (LD)	Magnesium (Mg)	Sodium (Na)	Phosphorus (Phos)	Total Iron Binding Capacity (TIBC)
Total Bilirubin (T-Bili)	Total Proteing (TP)	Triglycerides (Trig)	Urea (BUN)	Uric Acid

Specimens

Hong Kong blood donors, representing the local Chinese population, who passed the routine Red Cross blood donation screening criteria, were invited to participate in the reference interval study. The intent was to collect samples from about 300 female and 300 male donors. Donors signed a consent form allowing an extra blood sample to be drawn at the time of donation at the Hong Kong Red Cross Blood Transfusion Service. The consent form read: "The Chemical Pathology Laboratory of Pamela Youde Nethersole Eastern Hospital has recently changed its analyzers and has to re-establish the reference range for reporting purposes. Blood samples from the normal population have to be collected in sufficiently large numbers for testing by the new analyzers in order to establish the reference ranges and your help by contributing a few cc or our blood would be highly appreciated." Five milliliters of blood were collected into Beckton-Dickinson serum separator gel separator tubes (SST II). The blood samples were first collected in the empty sampling pouch of the blood bag and then drawn into the gel tube through a Luer adaptor. Tubes were labeled with the Red Cross identification number and the donor information included date of birth, gender, ethnicity (Chinese or non-Chinese) and the date of blood collection. The samples were collected over a period of twelve days, with 39 – 89 donors recruited per day. A messenger received the samples from the Red Cross at about 4 PM and delivered them to PYNEH in a specimen transportation box with an ice pack on the same day, arriving at the laboratory of PYNEH at about 5 PM. A total of 687 samples, 378 male and 309 female, were collected, with ages ranging between 16 – 62, and were labeled with PYNEH barcode labels after receipt. The samples were centrifuged, decapped, and assayed on the ARCHITECT c16000 on the same day as sample collection. Any excess samples were aliquoted and stored at – 50 C.

Statistical analysis

The data was analyzed using the Rhoads EP Evaluator (David G. Rhoads Associates, South Burlington, Vermont) software program. The reference intervals represent the central 95% range of the data set from a reference population consisting of apparently healthy individuals. EP Evaluator follows the recommendations of the CLSI C28-A guideline. It is intended to estimate intervals: (1) for endogenous analytes and not exogenous analytes (e.g., drugs), (2) for the patient population for which the intervals apply, (3) for a cohort of at least 120 reference specimens. The nonparametric method was chosen to calculate the reference intervals as it makes no assumptions about the shape of the population distribution. Although over 300 samples for both genders were available for each analyte, the age range was large and intervals can vary with age and lifestyle and the nonparametric approach is a conservative method. The confidence interval (CI) for the ranges is 90%. The confidence interval is a measure of the precision of the interval and indicates that for a repeat study using a different reference population, 90% of the values should fall within the CI. The confidence ratio is the ratio of the average CI width to the reference interval width. A value of 0.10 or less is desirable and values over 0.30 are indicated in Table 2. The confidence ratio is dependent on the sample size and improves with a larger number of samples.

Results

Table 2 summarizes the reference intervals for the twenty-five analytes. The statistical analysis provides intervals for each gender and for the combined genders. We have chosen to use gender specific intervals or the combined gender intervals as indicated in the table. The decision to adopt combined gender reference intervals is based on practical clinical considerations. Using Albumin as an example, the male interval is 38.1 – 49.4 g/L, the female interval is 37.6 – 48.6 g/L, and the combined interval is 37.9 – 48.9 g/L. While there may be a *statistically* significant difference between the male and female intervals, they are both so close to the combined interval that it is deemed to not to be a *clinically* significant difference. Using the electrolytes, Na, K, and Cl, as another example, the tight physiological control of these key analytes predicts that minimal gender difference would be observed. The observed data confirm that assumption, intervals differing by 1 (one) mmol/L or less and the gender combined intervals are therefore adopted.

Table 2
Analytes included in the study. All assays were performed using reagents specific for the Abbott ARCHITECT c16000 analyzer

Analyte	Male	Female	Male and Female
Albumin BCG (g/L) 1	38.1 – 49.4	37.6 – 48.6	37.9 – 48.9
ALT (U/L) 1	8 - 57	7 – 39*	7 - 49
Alk Phos (U/L) 2	47 – 168*	36 - 105	39 - 142
Amylase (U/L) 1	38.8 – 135	36.6 – 118	38.3 – 122
AST(U/L) 1	12 – 47*	11 – 26*	11 – 33*
Calcium (mmol/L) 1	2.19 – 2.49	2.19 – 2.51	2.19 – 2.50
Cholesterol (mmol/L) NA	2.99 – 6.69	3.04 – 6.44	3.01 – 6.61
Chloride (mmol/L) 1	102 – 109	103 – 109	102 – 109
CK (U/L) 2	65 – 270	37 – 173	44 - 253
CK-MB (U/L) 1	8 – 31*	7 - 23	7 – 28
Creatinine, alkaline picrate Jaffe (µmol/L) 2	69 - 110	55 - 83	57 - 105
D-HDL (mmol/L) NA	0.78 – 1.88	0.86 – 1.89	0.80 – 1.89
Iron (µmol/L) 2	8.5 – 37.8	5.9 – 31.1	7.1 – 35.6
GGT (U/L) 2	11 - 79	9 – 57*	9 - 76
K (mmol/L) 1	3.5 – 5.2	3.6 – 5.2	3.6 – 5.2
LD (U/L) 1	130 - 247	128 – 242*	128 - 245
Mg (mmol/L) 1	0.82 – 1.15	0.77 – 1.13*	0.80 – 1.14
Na (mmol/L) 1	137 - 144	137 - 143	137 - 143
Phosphorus (mmol/L) 1	0.75 – 1.36	0.77 – 1.41	0.76 – 1.40
T-Bili (µmol/L) 1	4.5 – 22.8	3.7 – 16.3	4.0 – 21.0
Total Protein (g/L) 1	62.9 – 79.3	63.5 – 79.0	63.0 – 79.1
Triglycerides (mmol/L) NA	0.52 – 4.96	0.48 – 3.14	0.50 – 4.29
TIBC (umol/L) 2	42.6 – 70.6	45.3 – 77.5	43.7 – 74.3
Urea (BUN) (mmol/L) [1]	3.2 – 7.5	2.3 – 6.4	2.7 – 7.2
Uric Acid (mmol/L) [2]	0.22 – 0.55	0.16 – 0.37	0.17 – 0.52

[1] Laboratory uses the combined male and female reference interval
[2] Laboratory uses the gender specific reference intervals for male and female
NA Reference intervals not applicable for Chol, D-HDL, and Trig as the laboratory uses the NCEP Adult Treatment Panel III Report cutoff values
** Confidence ratio exceeds 0.30*

Other analytes predictably demonstrate gender specific differences that are great enough to warrant maintaining separate gender intervals. Examples of these analytes include CK and creatinine for which the difference in muscle mass between males and females results in both *statistically* and *clinically* significant differences.

A third category of analytes are those for which reference intervals are typically used in clinical practice. This applies to cholesterol, D-HDL, and triglycerides for which the NCEP clinical cutoffs for assessing cardiovascular disease risk are used (10) Although the standard NCEP medical decision levels are used when reporting the results for these lipid assays, it is nevertheless instructive to know the distribution of these lipids in the local population.

Discussion and Conclusion

While the need for up to date, relevant reference intervals is undeniable, the task of generating them is daunting. Pertinent issues have been well reviewed by Horn and Pesce, who recognize the CLSI C28 guideline as a good source document (11). C28 recommends the direct method, either a priori or a posteriori, meaning that the reference individuals are pre-selected and judged to be "normal," or otherwise meet the desired criteria for inclusion in the study. The indirect method, in which existing data is used to determine reference intervals, variously known as "data mining" or the Hoffman approach, can be useful as an alternative, although debate about it's limitations continues in the current literature (12,13). An interesting paper by Seccombe describes application of the indirect approach for five analytes and reports intervals for them that are remarkably similar to intervals previously established using the direct approach (14). Limitations of this technique include the need for very large numbers of observations from multiple laboratories that are all using similar instrumentation and assay methods, i.e., an

analytically standardized peer group. While the indirect approach may be used successfully, as determined by intervals that are essentially equivalent to those based on the direct approach, the validity of the indirect intervals may be questioned unless the intervals from a proper direct approach study are available for comparison. Several recent pediatric reference interval studies using the direct approach and following C28 have been published, two of them having used the ARCHITECT c8000 analyzers and Abbott reagents, as in the work reported here (15-17).

We were able to cooperate with the local Red Cross to secure a large number of samples from "healthy" males and females representative of the indigenous Chinese ethnic population in Hong Kong. We used statistical analysis based on a well recognized guideline for determining reference intervals, CLSI/IFCC C28, to develop reference intervals for twenty-five common clinical chemistry analytes. We've adopted the intervals for result reporting purposes and believe that they are appropriate for the patient population served by the Pamela Youde Nethersole Eastern Hospital in Hong Kong.

Another reference interval study for the Hong Kong population has recently been reported (18). About half the number of samples (335 vs. 687) and consisted of 138 males and 197 females. The reference intervals were drawn from the staff of the Queen Elizabeth Hospital, Hong Kong, and their families, relatives, and friends. Subjects were excluded if glucose, cholesterol, and triglycerides were elevated and fasting specimens were collected. Sixteen analytes were tested using the Roche Modular system and the manufacturer's reagents. The intervals were general similar to those reported here except for phosphorus and alkaline phosphatase. Another recent Asian study included samples from cities in Japan, Korea, Taiwan, Indonesia, and Hong Kong.19 A total of 580 individuals, 279 males and 301 females, were tested, including 46 men and 74 women from Hong Kong ranging in age from 20 – 62. Twenty two clinical chemistry analytes (electrolytes, metabolites, enzymes, proteins) were tested using an Hitachi 7170 analyzer and a variety of different different methodologies. Because the data was pooled, it's difficult to directly compare the intervals from this work with those reported here for the Hong Kong population. Unexpectedly large between-city differences were noted for LD, total protein, globulin, Na, K, Cl, BUN, and phosphorus. This work was not intended to define reference intervals to demonstrate any differences in analyte intervals between samples from the six Asian cities included.

There are limitations of this work, hardly surprising given the inherent difficulties in conducting a full scale reference interval study. The blood donors who volunteered to participate were considered to be "normal," but that's a relative term. Blood donors underwent a screening process that included a limited medical history and cursory physical exam (e.g., blood pressure, hemoglobin, temperature) and had to meet certain predetermined criteria. It's reasonable to assume that all of the subjects were "healthy," at least healthy enough to donate a unit of blood. That's not a guarantee they were "normal" in the sense of being free of pathological conditions that could affect the values of various analytes measured in this study. More extensive vetting of the subjects for inclusion in the study would have been preferable, but the approach taken was practical. The age range of the subjects was large, 16 – 62 years of age. It's quite possible that the reference intervals for some the analytes study vary by age. Combining the data for this wide age range and using only gender as a partitioning factor does not allow for a nuanced interpretation. However, to apply age as another partitioning factor, it would have been necessary to collect many more reference specimens, theoretically, a minimum of 120 for each age category and gender. The protocol did not strictly follow the CLSI/IFCC C28 guideline, which would have been a daunting task, but attempted to conform to C28 as much as practical.

The work reported here allowed the Pamela Youde Nethersole Eastern Hospital to generate current reference intervals for the typical local patient population for a large number of routine analytes using a new analytical system (instrumentation and reagents). These intervals were more appropriate than literature citations or the intervals listed in manufacturer's package inserts. While the ranges reported here are by no means definitive, they represent a reasonably good start. Certainly this effort provided valuable experience. A future study, or even a series of studies, using the same protocol should be manageable and the results could be used to assess the appropriateness of the original intervals. If appropriate, additional data might be pooled with the current numbers, allowing for a more *statistically* robust estimate of the intervals and potentially allowing for the application of more partitioning factors.

References

1. Ceriotti F. Prerequisites for use of common reference intervals. Clin Biochem Rev 2007; 28:115-121.
2. Solberg HE. Approved recommendations on the theory of reference values. Part 1. The concept of reference values. Clin Chim Acta 1987; 167:111-118.
3. PetitClerc C, Solberg HE. Approved recommendation on the theory of reference values. Part 1. Selection of individuals for the production of reference values. J Clin Chem Clin Biochem 1987; 25:639-644.
4. Solberg HE, PetitClerc C. Approved recommendation on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. Clin Chim Acta 1988; 177:S1-S12.
5. Solberg HE, Stamm D. Approved recommendations on the theory of reference values. Part 4. Control of analytical variation in the production, transfer, and application of reference values. Eur J Clin Chem Clin Biochem 1991; 29:31-535.
6. Solberg HE. Approved recommendations on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987; 25:645-656.

7. Dybkaer R, Solberg HE. Approved recommendations on the theory of reference values. Part 6. Presentation of observed values related to reference values. J Clin Chem Clin Biochem 1987; 25:657-662.
8. CLSI/IFCC Guideline C28-A3. Defining, establishing, and verifying reference intervals in the clinical laboratory. CLSI Institute, Wayne, PA, 2008.
9. Friedberg RC, Souers R, Wagar EA, Stankovic AK, Valenstein PN. The origin of reference intervals. A College of American Pathologists Q-Probes study of "normal ranges" used in 163 clinical laboratories. Arch Pathol Lab Med 2007; 131:348-357.
10. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Executive summary of the third report on the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatmetn Panel IIII). JAMA 2001; 285:2486-2497.
11. Horn PS, Pesce AJ. Reference intervals: an update. Clin Chim Acta 2003; 334:5-23.
12. Roberts WL, Rockwood AL, Bunker AM, Kushnir MM, Meikle AW. Limitations of the Hoffman approach to determine pediatric reference intervals for two steroids. Clin Biochem 2010; 43:933-934.
13. Soldin SJ, Soldin OP. Reply to "limitations of the Hoffman approach to determine pediatric." Clin Biochem 2010; 43:935.
14. Katayev A, Balciza C, Seccombe DW. Establishing reference intervals for clinical laboratory test results. Is there a better way? Am J Clin Pathol 2010; 133:180-186.
15. Chan MK, Seiden-Long I, Aytekin M, Quinn F, Ravalico T, Armbruster D, Adeli K. Canadian Laboraotory Initiative on Pediatric Reference Interval Database (CALIPER): Pediatric reference intervals for an integrated clinical chemistry and immunoassay analyzer, Abbott ARCHITECT ci8200. Clin Biochem 2009; 42:885-891.
16. Kulasingam V, Jung BP, Blasutig IM, Baradaran S, Chan MK, Aytekin M, Colantino DA, Adeli K. Pediatric reference intervals for 28 chemistries and immunoassays on the Roche cobas 6000 analyzer- A CALIPER pilot study. Clin Biochem 2010; 43:1045-1050.
17. Southcott EK, Kerrigan JL, Potter JM, Telford RD, Waring P, Reynolds GJ, Lafferty ARA, Hickman PE. Establishment of pediatric reference intervals on a large cohort of healthy children. Clin Chim Acta 2010; 411:1421-1427.
18. Chan AOK, Lee KC, Leung JNS, Shek CC. Reference intervals of commone analytes of Hong Kong Chinese. J Clin Pathol 2008;61:632-636.